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Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.01/A1

Topic: A.04. Transplantation and Regeneration

Support: HHMI
NIH Grant R35NS097227
National Health Research Institutes Grant

Title: A photo-switchable assay system for dendrite degeneration and repair in Drosophila melanogaster

Authors: *H.-H. LIU[1,2], C.-H. HSU[3], L. JAN[4], Y. JAN[4];
[1]Ctr. for Neuropsychiatric Res., Natl. Hlth. Res. Inst., Miaoli, Taiwan; [2]Departments of Physiol., [3]Dept. of Pharmaceut. Chem., [4]Departments of Physiol. and Biochem., Univ. of California San Francisco, San Francisco, CA

Abstract: Neurodegeneration arising from aging, injury, or disease has devastating health consequences. Whereas neuronal survival and axon degeneration have been studied extensively, much less is known about how neurodegeneration impacts dendrites. To develop an assay for dendrite degeneration and repair, we used photo-switchable caspase-3 (caspase-LOV) in peripheral class 4 dendrite arborization (c4da) neurons to induce graded neurodegeneration during development and adulthood in *Drosophila melanogaster*. We found that both developing and mature c4da neurons were able to survive while sustaining mild neurodegeneration induced by moderate caspase-LOV activation. Further, we observed active dendrite addition and dendrite regeneration in developing and mature c4da neurons, respectively. Using this assay, we found that the mouse Wallerian degeneration slow (Wld^S) protein can protect c4da neurons from caspase-LOV-induced dendrite degeneration and cell death. Moreover, our data show that Wld^S can reduce dendrite elimination without affecting dendrite addition. In addition to these findings, we will also present our progress in searching for genes regulating dendrite degeneration and repair.

Disclosures: H. Liu: None. **C. Hsu:** None. **L. Jan:** None. **Y. Jan:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.02/A2

Topic: A.04. Transplantation and Regeneration

Support: CRSNG
Innovation
Université de Sherbooke

Title: Rodent sciatic nerves mechanical characterization: a path to better develop nerve guidance conduits

Authors: *E. PETIT[1], *E. PETIT[1], V. BAVYKINA[2], W. CHOINIÈRE[1], M. THIBAULT[3], A. BILODEAU[3], B. LAURENT[2], M.-A. LAUZON[3];
[1]Biotechnological engineering department, [2]Biochem. and functional genomics department, [3]Biotechnological engineering, Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Until now, it is still hard to recover from peripheral nerves injuries with complete transections. Treatments such as autografts, the current clinical gold standard, or nerve guidance conduits (NGCs) do not fully optimize the restoration of limb mobility and sensitivity in patients. Research on NGCs has increased in the past 10 years, and this therapeutic approach showed promising results on short-gap (< 30 mm) peripheral nerve injuries both in animal and human. However, the use of NGCs for large-gap (> 30 mm) peripheral nerve injuries remains a challenge. Beside their structures and compositions, NGCs should also be strong enough mechanically to resist body movements, to support properly nerve regeneration throughout the repair process. Ideally, NGCs should mimic the mechanical behaviour of native nerves. Literature about human nerves mechanical properties is scarce and there is no well-defined standardized procedure for performing the mechanical characterization. This can be attributed to challenges in acquiring human nerves and the absence of suitable devices for characterizing their mechanical behaviors in conditions close to the physiology. Studying the mechanical properties of rodent sciatic nerves is a first step towards a better understanding of native nerves mechanical properties, since NGCs are primarily tested on rodents. Therefore, we have developed a custom-made tensile device, specifically designed to characterize anisotropic biological tissues and biomaterials, to study rodent sciatic nerve mechanical properties. This tensile device allowed to conduct traction and cycling tests in pseudo-physiological conditions (controlled humidity and temperature) and is precise in force detection down to 1 mN. In this work, freshly collected sciatic nerves from both mice (9 females, 7 males from 3 to 6 months) and rats (10 males from 2 to 6 months) have been studied. For mice, statistical analysis revealed that there was no significant difference between male and female sciatic nerves mechanical properties. Mean elastic modulus was of 4.5 ± 1.8 MPa, mean maximum stress was of 1.3 ± 0.4 MPa and mean strain at break was of 54.9 ± 15.9 %. For male rats, mean elastic modulus was of 21.4 ± 9.1 MPa, mean maximum stress was of 3.0 ± 2.0 and mean strain at break was of 30.0 ± 9.7 %. Histological cuts of rats' sciatic nerves have been made to link mechanical results to the sciatic nerve physiology. The obtained results from rodent sciatic nerves' mechanical characterization provide valuable insights about the mechanical properties that should be targeted during the development of NGCs.

Disclosures: E. Petit: None. **E. Petit:** None. **V. Bavykina:** None. **W. Choinière:** None. **M. Thibault:** None. **A. Bilodeau:** None. **B. Laurent:** None. **M. Lauzon:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.03/A3

Topic: A.04. Transplantation and Regeneration

Title: Unravelling molecular underpinnings of cell behavior within engineered biomaterials: implications for nerve regeneration

Authors: *V. BAVYKINA, E. PETIT, M.-A. LAUZON, B. LAURENT;
Univ. de Sherbrooke, Sherbrooke, QC, Canada

Abstract: The peripheral nervous system has the capability for injured neurons to regenerate axons and rebuild functional connections. This unique potential relies on Schwann cells (SC) that support axon regeneration. However, axon growth is a slow process and is usually limited to a few millimeters in humans. To ensure a full functional nerve recovery, nerve guide conduits (NGC) are increasingly being considered as a potential alternative since they can bridge cut nerves and promote axonal regeneration. We developed a novel composite NGC featuring interconnected microchannels with a composition analogous to the extracellular matrix. Our overarching goal is to assess its potential for enhancing nerve regeneration by evaluating the impact of NGC composition and coating on cellular behavior and identity. To do so, we used human SC and induced pluripotent stem cells (iPSC) and cultured them in a 3D biomaterial microenvironment. We evaluated cell engraftment via immunohistochemistry and immunofluorescence assays on biomaterial cuts. Cell survival and proliferation were evaluated with a viability-cytotoxicity differential staining. An RNA-seq analysis was performed to determine the changes in cell identity and behavior within the biomaterial and identify a transcriptomic shift following cell contact with the NGC. Our findings give insights into the biological mechanisms by which cells sense and interact with their surrounding mechanical environment. Our results highlight how physical stimulation plays an important role in regulating cell behavior and will help developing personalized regenerative medicine approaches for effective treatment of peripheral nerve injuries.

Disclosures: **V. Bavykina:** None. **E. Petit:** None. **M. Lauzon:** None. **B. Laurent:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.04/A4

Topic: A.04. Transplantation and Regeneration

Support: the Samsung Research Funding & Incubation Center of Samsung Electronics SRFC-MA1802-07

Title: Gpr151 mRNA Enhances Ribosomal Association and Stability of RP-mRNAs to Promote Axon Regeneration after Injury via CSDE1-Mediated Regulation

Authors: *Y. CHO[1], J. SHIN[2];
[1]Dept of Brain Sci., DGIST, Daegu, Korea, Republic of; [2]Dept. of Mol. Neurosci., Dong-A Univ., Busan, Korea, Republic of

Abstract: New protein synthesis is crucial for axon regeneration after injury, which is mediated by mTOR pathway. However, the injury-related metabolism of ribosomes, the core complex of translation is not understood. We found that ribosome protein-coding mRNAs (RP-mRNAs) are a novel regulon under control of an RNA-binding protein, cold shock domain-containing E1 (CSDE1) that regulates translation and stability of its binding RNA. The binding between RP-mRNAs and CSDE1 protein become stabilized in mouse dorsal root ganglion neurons after sciatic nerve injury, which requires *Gpr151* mRNA that is identified by the analysis of differential gene expression plus ribosome association efficiency. *Gpr151* mRNA highly increases but does not direct to ribosomes for its translation. Instead, injury-induced *Gpr151* mRNA binds to CSDE1 via its 5'-untranslated region (5'UTR) and increases the stability of the interaction between CSDE1 and RP-mRNAs. *Gpr151*-mediated stabilization is required for directing RP-mRNAs regulon to ribosomes. This pathway promotes axon regeneration in vitro and in vivo in sciatic and optic nerve after injury, indicating that 5'UTR of *Gpr151* is a potential tool for developing therapeutic applications to improving axon regeneration via enhancing ribosomal association and stability of RP-mRNAs.

Disclosures: Y. Cho: None. **J. Shin:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.05/A5

Topic: A.04. Transplantation and Regeneration

Support: PVA22_R_00037

Title: Minimum therapeutically effective dose of conditioning electrical stimulation to enhance regeneration of sensory and motor axons in mice.

Authors: *M. SOLIMAN[1], J. JARA[2], N. CHEN[1], E. HOLLIS, II[1];
[1]Burke Neurolog. Inst., White Plains, NY; [2]Univ. of California San Diego, San Diego, CA

Abstract: While axons of the peripheral nervous system (PNS) exhibit an intrinsic regenerative capacity, this inherent ability is slow, and the return of function can be limited. In animal models,

conditioning electrical stimulation (CES) has been found to enhance peripheral regeneration similar to conditioning by nerve crush. In previous studies, 20 Hz biphasic CES for 1 hour has been proven effective when delivered at up to two times the motor threshold; however, the duration and intensity of such stimulation may not be well-tolerated by participants in clinical trials. Therefore, the objective of our study was to determine the minimum therapeutically effective dose and duration of CES that significantly enhances the regeneration of sensory and motor axons. We have used transgenic mice with fluorescently labeled motor neurons (ChAT::tdTomato) and proprioceptive sensory neurons (Pvalb::tdTomato). The regenerative responses of motor and sensory neurons were compared following biphasic CES (20 Hz, 0.2 ms pulses) with intensities ranging from 0.5x to 2x the motor threshold and durations ranging from 15 minutes to 1 hour. Unilateral stimulation of the sciatic nerve was conducted, with the contralateral control sciatic nerve exposed to sham electrode placement without current for the same duration. One week after CES, mice received bilateral sciatic nerve crush injury, followed by isolation and evaluation of nerves for regeneration at three days post crush. Preliminary results show a graded regenerative response to CES with an effect in as little as 15 minutes of stimulation at 0.5x motor threshold. Our results indicate that sub-threshold CES is a clinically applicable method to promote regeneration of motor and sensory neurons crucial for facilitating peripheral nerve repair.

Disclosures: **M. Soliman:** None. **J. Jara:** None. **N. Chen:** None. **E. Hollis:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.06/A6

Topic: A.04. Transplantation and Regeneration

Support: Merkin-PNNR-2022

Title: The Effect of Ketogenic Diet on Nerve Regeneration After Sciatic Nerve Crush Injury

Authors: *A. RAWAT[1], S. RASTOGI[1], K. URIARTE[2], F. YANG[1], B. MORRISON[1];
[1]Johns Hopkins Univ. - Main Campus, Baltimore, MD; [2]Neurol., Boston Col., Chestnut Hill, MA

Abstract: Peripheral nerve injuries are common and can lead to significant functional impairment. Despite this, there are no approved treatments to improve recovery. The regeneration of damaged peripheral nerves is a complex process influenced by various cell types and metabolic factors. In this study, we investigate the role of ketones in nerve regeneration, prompted by previous studies showing an impact on neurite outgrowth in vitro and our laboratory's publication demonstrating the critical role for monocarboxylate transporter 1 (MCT1), which is a major transporter for ketones, in macrophages function and nerve regeneration.. The ketogenic diet (KD), in which ketones are induced by high-fat, low-carbohydrate, and adequate protein diets, is used for treatment of epilepsy and has been proposed

for other neurologic diseases, as well. As such, we designed experiments to assess the role of ketones and the KD on macrophages in vitro and nerve regeneration, respectively. We first tested the effect of acetoacetate (AcAce), one of the primary ketones produced by KD, on bone marrow differentiated macrophages (BMDM). BMDMs were polarized into M1with LPS and IFN-γ or M2 with IL-4, and the cytokine profile evaluated after 24 hours of induction, with or without AcAce exposure. We then assessed the efficacy of the KD in a sciatic nerve crush injury (SNCI) animal model. We found that BMDMs cultured in 30 mM AcAce for both short (24 hours) and long (6 days) durations exhibited significantly reduced levels of pro-inflammatory (M1) genes (TNF-α, IL-1β, and IL-12) and increased levels of pro-regenerative (M2) genes (Arginase 1, CD206, and VEGF). In the SNCI animal model, animals on the KD diet showed improved functional recovery, as evaluated by toe spread index, particularly at earlier time points (n=9-10 per treatment group). Experiments are ongoing to assess recovery by nerve electrophysiology, muscle reinnervation, and nerve counts. In conclusion, our results suggest that the KD may have neuro-regenerative effects following peripheral nerve injury through modifications of macrophage phenotype and function. Further investigations are necessary to determine the translational potential and optimize the therapeutic application of the KD for patients with nerve injuries.

Disclosures: **A. Rawat:** None. **S. Rastogi:** None. **K. Uriarte:** None. **F. Yang:** None. **B. Morrison:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.07/A7

Topic: A.04. Transplantation and Regeneration

Title: Pten regulates microtubule polymerization through mtorc2, but not mtorc1, in peripheral sensory neurons

Authors: *S. EVANS[1], A. NAYAK[2], C. MINSKY[2], C. LEWIS[2], E. MCNEIL[3], W. WANG[2], M. LI[2], B. LUIKART[2], J. HONG[4];
[1]Integrative Neurosci., [2]Dartmouth Col., Hanover, NH; [3]Neurosurg., Beth Israel Deaconess Med. Ctr., Boston, MA; [4]Section of Neurosurg., Dartmouth-Hitchcock Med. Ctr., Lebanon, NH

Abstract: At least 20 million people in the U.S. are estimated to be living with peripheral neuropathy, but development of effective treatments is hindered by poor understanding of the genetic and molecular mechanisms underlying peripheral neuron regeneration. One gene of interest is phosphatase and tensin homolog (*Pten*), a tumor suppressor which negatively regulates many aspects of cell growth. Specifically, *Pten* knockout promotes dendritic and axonal growth in multiple neuronal subtypes. One mechanism by which *Pten*-KO drives this growth is by increasing microtubule (MT) polymerization rates. However, PTEN regulates a broad variety of cell growth signaling pathways, including the two mTOR complexes (mTORC1 and mTORC2).

The specific signaling pathway by which PTEN inhibits MT polymerization is unknown, and it is unclear if this mechanism could drive regeneration in peripheral neurons.
Here, we demonstrate PTEN regulates MT polymerization through mTORC2, but not mTORC1, in peripheral sensory neurons. Specifically, we use a primary culture system of adult murine dorsal root ganglia (DRGs) to measure MT polymerization and neuronal growth. The primary culture system provides tight control of experimental variables, and it accounts for individual animal differences through individual-matched controls. Using a Cre/lox system, we knock out both *Pten* and either *Rictor* (a necessary component of mTORC2) or *Raptor* (of mTORC1), and live-image these neurons to visualize MT polymerization. MT polymerization velocity is quantified using automated tracking of EB3-GFP puncta velocity.
We confirm *Pten*-KO significantly increases MT polymerization rates in DRG axons (*Pten*-WT 0.08717±0.01928um/s, *Pten*-KO 0.1166±0.02977um/s, n=34 cells each, $p<0.0001$). We then find *Rictor*-KO, but not *Raptor*-KO, rescues *Pten*-KO-accelerated MT polymerization rates (*Pten*x*Raptor* KOxKO 0.1179±0.02898um/s, *PtenxRictor* KOxKO 0.08313±0.01969um/s, n=19 cells each). Finally, we find evidence that this pathway drives cellular-level outgrowth by demonstrating *Rictor*-KO, but not *Raptor*-KO, rescues *Pten*-KO-driven axonal outgrowth (as determined by Sholl analysis) and somatic hypertrophy. This somewhat contrasts with the existing paradigm of CNS neuronal dendritic arborization, where both mTORC1 and mTORC2 are necessary for overgrowth after *Pten* loss.
Taken together, these results not only elucidate the downstream mechanisms of PTEN-modulated hypertrophy, but also support further investigation of the cytoskeleton's role in peripheral neuronal regeneration. Most importantly, we highlight the MT cytoskeleton as a novel therapeutic target for peripheral neuropathy.

Disclosures: S. Evans: None. **A. Nayak:** None. **C. Minsky:** None. **C. Lewis:** None. **E. McNeil:** None. **W. Wang:** None. **M. Li:** None. **B. Luikart:** None. **J. Hong:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.08/A8

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant 1R25NS127776
NIH Grant 1R15GM124595

Title: Characterization of the insulin-like growth factor system components in echinoderms during intestinal regeneration.

Authors: *E. J. AVILES-RIOS, M. GROSSO-GARCIA, J. E. GARCÍA-ARRARÁS;
Biol., Univ. de Puerto Rico, Rio Piedras, San Juan, Puerto Rico

Abstract: Insulin and insulin-related peptides such as Insulin-like Growth Factors (IGFs) are crucial in different biological processes including growth, cell differentiation and metabolism. Other important genes associated with the IGF system are the IGF-binding protein (IGFBP), the Acid Labile Subunit (IGFBP-ALS) and the IGF receptor (IGFR). The first two form a trimeric complex with the IGF and are involved in the regulation, transportation and stability of the peptide. Despite their significance, the understanding of the IGF system and its involvement in regeneration remains limited. In the present study, we have characterized the components of the IGF system in the echinoderm *Holothuria glaberrima* (sea cucumber), a marine organism known for its ability to regenerate lost body parts. We have then determined their expression profile during the process of intestinal regeneration. Bioinformatic analyses identified orthologs of IGF, IGFBP, IGFBP-ALS and IGFR in the *H.glaberrima* transcriptome, revealing that these genes are present in the holothurian digestive tract. HgIGF, HgIGFBP and HgIGFR show 46%, 53% and 63% similarity, respectively, when compared to the orthologs found in the sea urchin *Strongylocentrotus purpuratus*. We have also determined their expression profiles during intestinal regeneration. Surprisingly, each gene exhibited a distinct expression pattern. While IGF was upregulated during the first three weeks of regeneration, IGFBP-ALS was downregulated and IGFBP showed initial downregulation followed by over-expression in more advanced stages. Interestingly, the expression of the IGFR remained unchanged throughout the regeneration process. This study provides valuable insight into the presence and dynamics of the IGF system's molecular mechanisms in echinoderms and paves the way for future studies on its role in the regenerative process.

Disclosures: E.J. Aviles-Rios: None. **M. Grosso-Garcia:** None. **J.E. García-Arrarás:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

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Program #/Poster #: PSTR313.09/A9

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.: 07181356 and 08193956).

Title: Dissecting the roles of long non-coding RNAs in axon regeneration and function recovery after peripheral nerve injury

Authors: *Y. M. GAN, N. P. B. AU, C. H. E. MA;
Neurosci., City Univ. of Hong Kong, Kowloon, Hong Kong

Abstract: Injuries to the nervous system often result in irreversible sensory and motor function deficits which greatly impact the quality of life of patients. Mature neurons in the central nervous system (CNS) do not spontaneously regenerate their injured axons due to limited regenerative

capacity. The non-permissive extrinsic growth microenvironment in the CNS further hampers axon regeneration beyond the site of injury. In contrast, mature neurons in the peripheral nervous system (PNS) retain the ability to regenerate via the activation of intrinsic growth program after injury. In the past decades, the advance in high-throughput microarray and RNA sequencing technologies enabled the identification of thousands of regeneration-associated genes that are essential for axonal regrowth. A recent study revealed that up to 80% of the mammalian genome is actively transcribed and gives rise to long RNA transcripts (more than 200 nucleotides) without the potential to translate into functional proteins, namely long non-coding RNAs (lncRNAs). Growing evidence suggests that this highly heterogeneous collection of lncRNAs is important in various cellular functions, including the control of gene expression, mRNA stability, translation, and post-translational modifications. However, the functional roles of these lncRNAs in regulating axonal outgrowth remain largely unexplored. Here, we report several novel lncRNA candidates that promote long-distance axon regeneration after peripheral nerve injury (PNI). We hypothesize that lncRNAs which show differential expressions in axotomized peripheral neurons are required for successful axon regeneration after PNI. We first perform bioinformatics analysis on lncRNA microarray and RNA-seq datasets and identify 12 lncRNAs that are up-regulated after PNI. Loss-of-function assay reveals that *in vivo* axon regeneration is markedly reduced after gene silencing of 3 lncRNAs as assessed by sciatic nerve pinch test and immunohistochemistry. By using a neuronal-specific serotype of adeno-associated virus to overexpress lncRNAs, two of the lncRNAs greatly promote the extent of *in vivo* axonal regrowth 3 days after PNI. More importantly, these two lncRNAs markedly accelerate sensory and motor function recovery and promote the reformation of functional neuromuscular synapses at target muscles after sciatic nerve crush injury in mice. Taken together, our study uncovers the novel roles of lncRNAs in axon regeneration after PNI. Further investigation is therefore warranted to examine whether these lncRNAs promote axonal regrowth after CNS injury.

Disclosures: **Y.M. Gan:** None. **N.P. B. Au:** None. **C.H. E. Ma:** None.

Poster

PSTR313. Regeneration in the PNS

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR313.10/A10

Topic: A.04. Transplantation and Regeneration

Support: NIH R01-NS089633
Dr. Miriam & Sheldon G Adelson Medical Research Foundation
Univ SC Office of Research

Title: Testing an autocrine loop that slows axon regeneration using local translation

Authors: J. LEE[1], C. BUCHANAN[2], L. VAUGHN[2], M.-C. HONOREE[2], A. MACKAY[2], *J. TWISS[2];
[1]Biol. Sci., [2]Univ. of South Carolina, Columbia, SC

Abstract: Neurons are highly polarized cells with axons extending over a meter in larger mammals. Localized translation of mRNAs in axons and dendrites provides a means to rapidly modify their proteomes in response to physiological stimuli. Localization, translation, and stability of mRNAs in subcellular sites is driven by RNA binding proteins (RBP). In mature PNS axons, these locally synthesized proteins play critical roles in response to injury and after regeneration. We recently reported that RBP KHSRP slows axon regeneration by promoting mRNA decay in axons (Patel et al., 2022, Nucl Acids Res). Axonal *Khsrp* mRNA, which resides in PNS axons before injury, is rapidly translated after axotomy via Ca^{2+}◊PERK◊eIF2α^{PS51}; but axonal KHSRP consistently increases out to 28 d after axotomy, and it is not clear how axonal *Khsrp* mRNA translation could be sustained for this period. Here, we sought to determine the mechanism underlying this sustained elevation of axonal KHSRP. We had previously shown that *Reg3a* mRNA, which encodes a secreted lectin-like protein, is up-regulated after injury and transported into regenerating axons (Kalinski et al., 2015, J Neurosci). We find that REG3A protein increases in regenerating sciatic nerve, and exogenous REG3A increases translation of axonal *Khsrp* mRNA, elevates axonal [Ca^{2+}], and triggers axonal eIF2α phosphorylation. Conversely, shRNA-mediated depletion of *Reg3A* mRNA from mice increases nerve regeneration and the *Reg3a*-depleted axons show lower levels KHSRP. These initial observations suggest that axonal translation of *Reg3a* mRNA provides an autocrine loop to sustain translation of *Khsrp* mRNA in regenerating axons and slow axon regeneration.

Disclosures: J. Lee: None. **C. Buchanan:** None. **L. Vaughn:** None. **M. Honoree:** None. **A. MacKay:** None. **J. Twiss:** None.

Poster

PSTR313. Regeneration in the PNS

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Program #/Poster #: PSTR313.11/A11

Topic: A.04. Transplantation and Regeneration

Support: NIH R01-NS0117821
Adelson Medical Research Foundation

Title: Acetylation of axonal G3BP1 increases axonal protein synthesis and promotes axon growth

Authors: *I. DALLA COSTA[1], M. MCELVEEN[1], L. VAUGHN[1], P. K. SAHOO[1,2], H. ZHU[3], J. L. TWISS[1];
[1]Univ. of South Carolina, columbia, SC; [2]Dept. of Biol. Sci., Rutgers Univ. Newark, Newark, NJ; [3]Dept. of Pharmacol. and Toxicology, R. Ken Coit Col. of Pharmacy, Univ. of Arizona, Tucson, AZ

Abstract: Although adult PNS neurons can spontaneously regenerate injured axons, the rate of growth is often not sufficient for complete functional recovery in mammals larger than rats and

mice. Nerve injury activates translation of axonal mRNAs, with the protein products promoting axon regeneration. We previously showed that the stress granule (SG) protein G3BP1 stores axonal mRNAs, providing a source of mRNAs for intra-axonal translation (Sahoo et al., 2018, Nat Comm). G3BP1 forms SGs through liquid-liquid phase separation (LLPS), and its threshold for LLPS is regulated by post-translational modification, including phosphorylation on Ser 149 ($G3BP1^{PS149}$) that triggers SG disassembly in axons (Sahoo et al., 2020, Curr Biol). Acetylation of G3BP1 on Lys 376 ($G3BP1^{AcK376}$) has also been shown to trigger SG disassembly (Gal et al., 2019, Mol Cell Biol), raising the question of whether G3BP1 acetylation occurs in axons. Here, we show that axonal $G3BP1^{AcK376}$ level rapidly increases after axonal injury. In a nerve ligation set up where the nerve is ligated proximal to the injury site, axonal $G3BP1^{AcK376}$ accumulates distal to the ligation site suggesting retrograde movement of $G3BP1^{AcK376}$ from the injury site. Live imaging with G3BP1 acetylmimetic ($G3BP1^{K376Q}$) vs. non-acetylatable ($G3BP1^{K376R}$) mutants also show distinct motilities with significatively more movement both anterogradely and retrogradely for these proteins in axons compared to wild type (WT) G3BP1. Expression of $G3BP1^{K376Q}$ in cultured DRG neurons significantly increases axon growth compared to expression of WT G3BP1, $G3BP1^{K376R}$, and phosphomimetic ($G3BP1^{S149E}$) mutants; this growth is not further enhanced by the expression of the double mutant $G3BP1^{K376Q/S149E}$. Finally, expression of $G3BP1^{K376Q}$ also showed an increase in axonal protein synthesis, possibly of proteins promoting axon growth. Taken together, these data indicate that axonal G3BP1 undergoes both acetylation and phosphorylation after nerve injury, with both modifications leading to disassembly of SGs and increasing axon growth.

Disclosures: I. Dalla Costa: None. **M. McElveen:** None. **L. Vaughn:** None. **P.K. Sahoo:** None. **H. Zhu:** None. **J.L. Twiss:** None.

Poster

PSTR313. Regeneration in the PNS

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Program #/Poster #: PSTR313.12/A12

Topic: A.04. Transplantation and Regeneration

Support: Adelson Medical Research Foundation
NIH R01-NS089633

Title: Distinct 3'UTR motifs in axonal Prenyl-Cdc42 mRNA regulate axon growth

Authors: M. D. ZDRADZINSKI[1], ***L. S. VAUGHN**[1], C. N. BUCHANAN[1], A. LOOMIS[1], M. CONWAY[1], A. MCKAY[1], J. OSES-PRIETO[2], A. L. BURLINGAME[2], J. L. TWISS[1]; [1]Biol. Sci., Univ. of South Carolina, Columbia, SC; [2]UCSF, San Francisco, CA

Abstract: Activation of the small Rho-family GTPase CDC42 plays important roles in neurite growth and axon regeneration. We previously reported that local translation of the axon localized *Prenylated Cdc42* mRNA (*Prenyl-Cdc42*), but not the somatodendritic-restricted *Palmitoylated*

Cdc42 mRNA spliceoform, promotes axon growth. Here, we show that the *Prenyl-Cdc42* mRNA has two distinct motifs in its 3'UTR - nucleotides 764-800 regulate its transport and nucleotides 801-875 mediate its survival. An AU-rich region, commonly recognized by RNA binding proteins (RBP), is found within the 801-875 region and we show that KHSRP directly binds to this motif. Axons from adult *Khsrp*$^{-/-}$ mice show remarkably increased *Prenyl-Cdc42* mRNA and accelerated nerve regeneration, suggesting a negative role of KHSRP in *Prenyl-Cdc42* mRNA stability. RNA Affinity Mass Spectrometry was performed on crushed sciatic nerve axoplasm, showing enriched and differential binding of RBPs to oligonucleotides corresponding to the 764-800 and 801-875 regions of the *Prenyl-Cdc42* 3' UTR. Additionally, levels of *Prenyl-Cdc42* mRNA and protein are increased in the presence of growth-promoting neurotrophins but decreased with growth-inhibiting CSPG treatment. We show alteration of CDC42 levels by CSPG treatment is through a mechanism regulated by KHSRP-RNA binding and requires an increase in cytoplasmic calcium. Interestingly, while CDC42 has been shown to promote actin filament polymerization and growth, another Rho family GTPase RHOA promotes depolymerization, pointing to opposing roles in growth. Consistent with previous work, we find that treatment with CSPGs increases intra-axonal transport and translation of *RhoA* mRNA but NTs show no effect. Collectively, these data suggest that while growth-promoting cues may be enough to stimulate production of prenyl-CDC42, simultaneous inhibition of RHOA may be required for optimal axon growth.

Disclosures: M.D. Zdradzinski: None. **L.S. Vaughn:** None. **C.N. Buchanan:** None. **A. Loomis:** None. **M. Conway:** None. **A. McKay:** None. **J. Oses-Prieto:** None. **A.L. Burlingame:** None. **J.L. Twiss:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.01/A13

Topic: A.07. Developmental Disorders

Support: SFARI Grant
FamilieSCN2A Foundation Grant
DoD Grant AR220030
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Title: Exploring the effects of premature stop codons in mouse and human models of SCN2A related autism and intellectual disability

Authors: *K. E. J. SCOTT[1], A. AL SANEH[2], L. GISSOT[2], O. KLEIN[3], J. SIKORA[3], M. LAUFFER[4], K. KRUTH[3], C. A. AHERN[2], A. J. WILLIAMS[3];
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Abstract: SCN2A encodes the alpha subunit of the voltage gated sodium channel $Na_V1.2$, which is necessary for action potential generation and propagation. Nonsense mutations in SCN2A are strongly associated with autism spectrum disorders (ASD) and intellectual disability. These mutations create a premature termination codon (PTC) in the reading frame of $Na_V1.2$ and are hypothesized to result in functional haploinsufficiency. In patients, multiple SCN2A PTCs have been Identified and it is unclear if different PTCs have similar functional impacts on neurons. Therefore, we have generated two mouse models of SCN2A PTCs that directly mimic patient mutations: R1626X and Y84X. We conducted preliminary ultrasonic vocalization (USV) tests on post-natal day 6 (P6) pups from the F2 generation of Y84X mice. To record the USVs, pups were separated from dams at P6 and recorded for 5 minutes. Overall, *Scn2a*$^{Y84X/+}$ and WT P6 mice had similar duration, number, and mean frequency of USVs. However, when broken down by sex, P6 *Scn2a*$^{Y84X/+}$ and WT females had a significant difference (p= .036) in mean frequency; the P6 *Scn2a*$^{Y84X/+}$ and WT males did not share this difference. We also performed Erasmus ladder testing of the F2 generation of R1626X mice. Adult *Scn2a*$^{R1626X/+}$ mice showed behavior indicative of impaired cerebellum-dependent learning on the Erasmus ladder. These preliminary data suggest that our SCN2A PTC mouse models display altered communication and cerebellum-dependent behaviors, both of which are observed in human patients. We plan to repeat these experiments in fully backcrossed mice to confirm our results. We will also examine neuronal morphology and function in neurons from SCN2A PTC mice (Y84X and R1626X) as well as human neurons derived from SCN2A patient stem cells (Y84X and C959X). Previous studies have shown that $Na_V1.2^{+/-}$ neurons have abnormal dendritic excitability and synaptic function, and we anticipate observing this in both mouse and human neurons. Ultimately, we aim to develop SCN2A research models that are more representative of patient populations and identify the differences between effects of varying PTCs in SCN2A. We hope that these tools and data will facilitate the development of more precise and effective treatments for SCN2A mutations.

Disclosures: **K.E.J. Scott:** None. **A. Al Saneh:** None. **L. Gissot:** None. **O. Klein:** None. **J. Sikora:** None. **M. Lauffer:** None. **K. Kruth:** None. **C.A. Ahern:** Other; Scientific Co-Founder, hC Bioscience. **A.J. Williams:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.02/A14

Topic: A.07. Developmental Disorders

Support: NIH F32MH125536
SFARI 675594
NIH R01MH126960
R01MH125978
T32GM007315

Title: Physical and functional convergence of the autism risk genes Scn2a and Ank2 in neocortical pyramidal cell dendrites

Authors: *A. NELSON[1], A. CATALFIO[2], J. PHILIPPE[2], L. MIN[2], R. CABALLERO[2], K. DEAN[2], C. ELVIRA[2], K. DERDERIAN[1], H. KYOUNG[1], A. SAHAGUN[1], S. SANDERS[1], K. J. BENDER[1], P. M. JENKINS[2];
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Abstract: Dysfunction in sodium channels and their ankyrin scaffolding partners have both been implicated in neurodevelopmental disorders, including autism spectrum disorder (ASD). In particular, the genes *SCN2A*, which encodes the sodium channel Nav1.2, and *ANK2*, which encodes ankyrin-B, have strong ASD association. Recent studies indicate that haploinsufficiency of *Scn2a* impairs dendritic excitability and synaptic function in neocortical pyramidal cells, but how Nav1.2 is anchored within dendritic regions is unknown. Here, we hypothesized that ankyrin-B is the primary ankyrin that localizes Nav1.2 to dendrites and haploinsufficiency of *Ank2* phenocopies intrinsic dendritic excitability and synaptic deficits observed in *Scn2a*$^{+/-}$ conditions. Immunostaining cultured neocortical neurons showed ankyrin-B is highly localized throughout the dendrites, distinct from ankyrin-G at the axon initial segment (AIS), suggesting that ankyrin-B is well-positioned to scaffold dendritic Nav1.2. Knockout of endogenous ankyrin-B *in vitro* resulted in a significant reduction in Nav1.2 levels within the dendritic membrane and rescue with the canonical wild-type ankyrin-B restored dendritic Nav1.2 through direct interaction. We next evaluated the functional significance of their interaction on dendritic excitability in neocortical layer 5 pyramidal neurons, a cell class implicated in ASD. We found significant attenuation of backpropagating action potentials into the distal dendrites of layer 5 neurons in acute brain slices from *Ank2* haploinsufficient mice and deficits in excitatory postsynaptic function, both of which mimic physiological impairments previously observed in *Scn2a* haploinsufficient neurons. These findings establish a direct, convergent link between two major ASD risk genes and reinforce an emerging framework suggesting that neocortical pyramidal cell dendritic dysfunction can be etiological to neurodevelopmental disorder pathophysiology.

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Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.03/A15

Topic: A.07. Developmental Disorders

Support: SFARI

Title: Characterization and Therapeutic Restoration of Sodium Channel Models for Autism Spectrum Disorders

Authors: *A. AL SANEH, K. SCOTT, L. GISSOT, *A. AL SANEH, O. KLEIN, H. WEN, A. WILLIAMS, C. AHERN;
Univ. of Iowa, Iowa City, IA

Abstract: Autism spectrum disorder (ASD) is estimated to occur in one out of every 36 children. The understanding of the mechanism behind ASD remains limited largely due to the disorder's remarkably complex etiology. Hundreds of genes have been implicated in autism risk, in addition to factors such as parental age, environmental conditions, and even some medications. Nevertheless, a small percentage of ASD cases are monogenic in origin and can be traced to a single causal genetic variation. These ASD examples provide an invaluable, simplified model of ASD that can be used to better correlate molecular dysfunction to phenotype. One such monogenic form of autism is caused by variations in the *Scn2a* gene which lead to premature truncation of the encoded protein, the sodium channel NaV1.2. Nonsense mutations leading to the abortive translation of the *Scn2a* gene result in reduced social interactions and repetitive behaviors as well as ataxia and cerebellar atrophy, highlighting the important role of *Scn2a* in the cerebellar cortex. We have developed two *Scn2a* PTC mice (p.Tyr84X and p.Arg1626X), first PTC autism rodent models to date. These provide an opportunity to investigate the effects of *Scn2a* haploinsufficiency. In parallel. we have developed an approach using anticodon-edited transfer RNA (tRNA) to repair in-frame termination codons. This strategy is generally agnostic to the position of the variation and has the potential to be disease-modifying, that is, of the primary defect of the disease, not just symptomatic treatment. We have previously developed a platform of anti-codon modified human tRNAs to identify multiple tRNA types for every known human PTC disease variant. Using ribosomal profiling and mass spectrometry we have found that expressed suppressor tRNAs have minimal contact with native stop codons. Further, through quantitative RNA methods, we show that *Scn2a* PTC mRNAs display modest nonsense mediated decay. We will also present data on a new mouse model that stably expresses a codon-edited tRNA targeted to the "safe harbor" *Hipp11* mouse locus. This resource will allow us to investigate, *in vivo*, the possible interactions between suppressor tRNA and native stops, and ER stress/UPR pathway activation, thereby further validating our codon-edited tRNA as a viable therapeutic strategy. The project provides valuable new resources for therapy as we investigate the engineered tRNA's fidelity, interactions with native stop codons and its potential to activate the ER and UPR stress pathways. Such developments have significant implications for all diseases resulting from PTCs, well beyond monogenic autism.

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Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR314.04/A16

Topic: A.07. Developmental Disorders

Support: NIH R01 MH125978
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SFARI 629287
SFARI 513133

Title: Seizures induced from potassium channel block in Scn2a haploinsufficient mice

Authors: *E. HAMADA[1], A. NELSON[1], H. KYOUNG[2], X. ZHOU[2], S. TALOMA[1], P. W. SPRATT[3], N. AHITUV[2], K. J. BENDER[2];
[1]Univ. of California San Francisco, San Francisco, CA; [2]Univ. of California, San Francisco, San Francisco, CA; [3]Neurosci., Univ. Of California San Francisco, San Francisco, CA

Abstract: Genetic variation in *SCN2A* has been identified as a major risk factor for neurodevelopmental disorders, including developmental epilepsies, autism spectrum disorder (ASD), and intellectual disability. *SCN2A* encodes the voltage-gated sodium channel $Na_V1.2$, which, in mature neocortex, is primarily localized throughout the somatodendritic compartment of excitatory pyramidal neurons. Gain-of-function variants in *SCN2A* are associated with early infantile epileptic encephalopathy, whereas loss-of-function variants contribute to ASD and intellectual disability. Interestingly, an estimated 20-30% of patients with *SCN2A* haploinsufficiency also develop epileptic encephalopathies; however, the mechanisms that underlie seizures in loss-of-function cases are not well understood. Recent work from our lab leveraging cell-autonomous conditional *Scn2a* knockout showed that the complete loss of $Na_V1.2$ channels in neocortical pyramidal cells paradoxically increased their excitability, with an increase in action potential (AP) output and increase in bursts of APs at high-frequency. This hyperexcitability was due to interactions between $Na_V1.2$ and potassium channels in the somatodendritic domain. Loss of $Na_V1.2$-supported somatodendritic depolarization during APs reduced the driving force for potassium channel-supported AP repolarization. This lack of strong repolarization between APs made it easier for neurons to reach threshold for subsequent APs, thereby increasing overall AP excitability. Given these interactions between $Na_V1.2$ and potassium channels recruited during AP repolarization, we hypothesized that 4-AP, an antagonist of inward rectifier potassium channels, may reveal increased seizure susceptibility in *Scn2a* haploinsufficient mice compared to littermate controls. We tested this with a range of 4-AP doses. Preliminary analysis suggests that 4-AP is more likely to induce seizures in $Scn2a^{+/-}$ mice, as revealed by behavioral scoring and analysis of cortical electroencephalography. Remarkably, these seizures can be tempered in $Scn2a^{+/-}$ mice by administration of CRISPR-activator based therapeutics that increase expression of the residual, functional allele. CRISPR-activated based rescue was performed at postnatal day 30, suggesting that restoration of *Scn2a* function to wild type levels is an effective therapeutic approach for lowering seizure burden in cases of *Scn2a* haploinsufficiency, even when administered relatively late in development.

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Regal Therapeutics. **K.J. Bender:** 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.; BioMarin Phamaceutical. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Regal Therapeutics.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.05/A17

Topic: A.07. Developmental Disorders

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AMED Grant JP22km0405214
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KAKENHI Grant JP20H05777
KAKENHI Grant JP21H02855
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KAKENHI Grant JP22K20751

Title: Topologically associating domains define the impact of de novo promoter variants on autism spectrum disorder risk

Authors: *S. MIZUNO[1], T. NAKAMURA[1], J. UEDA[1], K. HONDA[1], A.-A. KAZUNO[1], H. YAMAMOTO[1,2], T. HARA[1,3], A. TAKATA[1,4];
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Abstract: Whole-genome sequencing (WGS) studies of autism spectrum disorder (ASD) have demonstrated contributions of non-coding *de novo* variants (DNVs), especially those hitting gene promoters, to the disease risk. However, a large part of the enrichment of promoter DNVs in ASD cannot be explained by those immediately upstream of known ASD genes. Recently, a phenomenon called enhancer release and retargeting, where disruption of a promoter induces activation of other genes in the same topologically associating domains (TADs) by enhancer retargeting, was reported. Based on this newly discovered gene regulatory mechanism, we performed an analysis of promoter DNVs considering gene contents within TADs in the human dorsolateral prefrontal cortex using two large-scale ASD WGS datasets from the Simons Simplex Collection and the Simons Foundation Powering Autism Research for Knowledge (5,044 ASD probands, 4,095 unaffected siblings, and their parents). By meta-analyzing the two datasets involving WGS data from a total of 19,227 individuals, we observed that promoter

DNVs within TADs containing known ASD genes (defined by the SFARI Gene database) among genes other than that immediately downstream of DNV (referred to as ASD gene TADs), but not those in non-ASD gene TADs, are significantly associated with ASD (P = 0.00292, OR = 1.096 for ASD gene TAD promoter DNVs and P = 0.643, OR = 1.009 for non-ASD gene TAD promoter DNVs). This pattern of association was largely replicated in analyses using other brain or stem cell-derived TADs. On the other hand, there was no or modestly significant association when TADs in peripheral tissues were used. When we performed a genome-wide exploration of TADs with enrichment of promoter DNVs in ASD, we identified two TADs, each spanning chr1:207.8-209.8 Mb or chr13:72.74-73.71 Mb, significant after Bonferroni multiple testing correction. Promoters in TADs with nominally significant (uncorrected P &lt 0.05), but not promoters of the other TADs, are significantly enriched for common variant heritability of ASD, providing convergent evidence that rare and common variants in these regions contribute to the genetic liability of ASD. Besides, an experimental analysis of human induced pluripotent stem cell (iPSC) harboring selected ASD TAD promoter DNVs showed that single promoter DNVs can influence multiple genes in the TAD containing the DNV, and result in overall dysregulation of genes associated with ASD and neurodevelopment. These results collectively indicate that the integration of information on TADs and gene regulation improves our understanding of the genetic architecture of ASD accounted for by rare non-coding variants.

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Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.06/A18

Topic: A.07. Developmental Disorders

Support: Simons Center for the Social Brain at MIT
Yang-Tan Autism Center
Yang-Tan Center for Molecular Therapeutics
McGovern Institute

Title: Comparative analysis of resting-state functional connectivity in wild type vs SHANK3-mutant marmoset monkeys

Authors: *F. A. C. AZEVEDO[1], J. SHARMA[2], W. MENEGAS[3], M. JIANG[3], A. TAKAHASHI[3], G. FENG[3], R. DESIMONE[3];
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Abstract: The SHANK3 (SH3 and ankyrin repeat domains 3) gene encodes scaffold proteins at excitatory synapses, which coordinates the assembly of signaling molecules and alignment of

glutamatergic neurotransmitter receptors. Disruption or mutation of this gene are linked with the Phelan-McDermid Syndrome (PMS) and with idiopathic variants of the Autistic Spectrum Disorder (ASD). Such disorders have been associated with differences in functional connectivity in resting-state fMRI data. Previous work from our group showed that SHANK3-mutant cynomolgus macaques presented long range hypo-connectivity especially in the default mode regions but hyper-connectivity in the somatosensory cortex, extrastriate cortex, and posterior cingulate cortex. Here we expanded the aforementioned analysis comparing resting-state functional connectivity using ultra-high field (9.4T) MR imaging in Wild Type vs Shank3-mutant marmosets. The marmoset monkey is an important emerging nonhuman primate model, especially for research related to neurodevelopmental disorders, such as PMS and ASD. These small bodied primates are easy to breed in captivity, have complex social structure, possess large vocal repertoire for social communication and emotional expression, and genetic modifiability. Our preliminary results suggest significant differences in the strength of local connectivity in areas including intraparietal (IP), somatosensory (S1), A23 and A24 (in the cingulate gyrus), as well as differences in the strength of global connectivity in the temporoparietal transitional area (Tpt), parainsular cortex (PaI) and belt areas. Besides shedding light on the understanding of the aberrant functional connectivity in neural circuits in PMS and ASD, our methodology and results can be used as a tool to diagnose and to evaluate the efficacy of therapies for such neurodevelopmental disorders.

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Poster

PSTR314. Autism: Genetic Models

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Program #/Poster #: PSTR314.07/A19

Topic: A.07. Developmental Disorders

Support: NINDS Grant R01NS091220

Title: Role of ARID1B in early social bonding behavior in the context of adversity

Authors: *T. L. FORD[1], B. JEON[2], W.-Y. KIM[2];
[1]Biol. Sci., Kent State Univ. - Biol. Sci., Kent, OH; [2]Biol. Sci., Kent State Univ., Kent, OH

Abstract: Adverse living environments can disrupt maternal behavior, leading to early life stress for infants. However, the molecular and cellular mechanism underlying adversity-induced behavioral changes in infants are unclear. AT-Rich Interactive Domain 1B (ARID1B) is a chromatin modifying protein and is involved in early cell development. Although *ARID1B* haploinsufficiency is known to cause autism spectrum disorder, nothing is known about whether ARID1B haploinsufficiency contributes to behavioral alteration after early life adversity. Using *Arid1b* heterozygous mice as a model of *ARID1B* haploinsufficiency, we created an early

scarcity-adverse (S-A) environment for pups for a few days by reducing wood chip bedding and nesting materials by 80% in the cage. Then we assessed social bonding behavior of the pups by a battery of behavioral tests including the rodent strange situation paradigm (rSSP). Results showed that the wild type pups that underwent the S-A condition displayed decreased social attachment to their mother compared to wild type non-S-A pups. Importantly, the *Arid1b* haploinsufficient S-A pups showed a moderate increase in attachment behavior compared to the non-S-A *Arid1b* group. We further examined adolescent mouse behavior after weaning. The open field and three-chamber tests revealed a decrease in social exploration and an increase in anxiety-like behavior in the wild type S-A group compared to non-S-A wild type mice. However, both *Arid1b* haploinsufficient non-S-A and S-A groups showed decreased anxiety, which was not reflective of adult *Arid1b* mutant behavior, suggesting a potential phenotypic change throughout development. Together, our data reveals an impact of early adverse environment on pup social bonding and further suggests ARID1B as a mechanistic factor for the development of early social behavior.

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Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.08/A20

Topic: A.07. Developmental Disorders

Title: Crispr screens in assembloids reveal autism spectrum disorder genes that interfere with human interneuron development

Authors: *X. MENG[1], D. YAO[1], K. IMAIZUMI[1], X. CHEN[1], K. KELLEY[1], N. REIS[1], M. THETE[1], A. MCKINNEY[3], S. KULKARNI[1], G. PANAGIOTAKOS[3], M. BASSIK[1], S. P. PASCA[2];
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Abstract: The assembly of cortical circuits involves the generation and migration of cortical interneurons from the ventral to the dorsal forebrain, which has been challenging to study in humans as these processes take place at inaccessible stages of late gestation and early postnatal development. Autism spectrum disorder (ASD) has been associated with abnormal Abnormal cortical interneuron development has been associated with aAutism spectrum disorder (ASD). However, it is still unknow and challenging to study which of the hundreds of ASD genes impact interneuron generation and migration into circuits and how they mediate these effects. We previously developed a stem cell-based forebrain assembloids platform resembling the ventral forebrain to study human cortical interneurons in organoids resembling the ventral forebrain and their migration into the cerebral cortex using forebrain assembloids. Here, we coupled forebrain assembloid technology with CRISPR screening to systematically investigate the involvement of 425 ASD related genes in human interneuron development. We revealed 13 candidate genes in

the interneuron generation screen, including the canonical TGFβ signaling activator *SMAD4, and the* RNA-binding protein *CSDE1*. Then, we ran an interneuron migration screen in ~1,000 forebrain assembloids that identified 33 candidate genes, including the endoplasmic reticulum (ER)-related gene *LNPK*. Interestingly, we discovered that, during interneuron migration, the ER is displaced along the leading neuronal branch prior to nuclear translocation. Deletion of *LNPK* interfered with this ER displacement and resulted in reduced interneuron saltation length and speed, indicating a critical role for the ER in this migratory process. We further confirmed that mouse *Lnpk* has a similar role in mouse interneuron migration. Taken together, these results highlight how this versatile CRISPR-assembloid platform can be used to systematically map disease genes onto early stages of human neural development and to reveal novel mechanisms regulating interneuron development.

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Poster

PSTR314. Autism: Genetic Models

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.09/A21

Topic: A.07. Developmental Disorders

Support: Max Planck Society

Title: Connectomic screening of genetic mouse models of autism spectrum disorder.

Authors: *A. M. KHALIFA, S. LOOMBA, V. GANGADHARAN, M. HELMSTAEDTER; Max Planck Inst. For Brain Res., Frankfurt Am Main, Germany

Abstract: One leading hypothesis in autism research is to consider the disease as a connectopathy where different causes converge on a small number of neuronal circuit elements that lead eventually to behavioral phenotypes. We approached this question using 3D EM, applied to cortical tissue of 4 genetic mouse models of autism (Shank3 KO, CNTNAP2 KO, Fmr1 KO and NLGR451c) to study neuronal circuits at synaptic level. We analyzed the balance of inhibition and excitation at the level of neurons, synaptic input to pyramidal cells, and axonal output properties. While we find convergent effects on I/E balance, the underlying circuit alterations differed between the models. Synapse-level alterations were found in both the excitatory and inhibitory subcircuits. Systematic connectomic screening of disease related mouse models may contribute to a quantitative definition of connectopathies as circuit diseases in the brain.

Disclosures: **A.M. Khalifa:** None. **S. Loomba:** None. **V. Gangadharan:** None. **M. Helmstaedter:** None.

Poster

PSTR314. Autism: Genetic Models

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Program #/Poster #: PSTR314.10/A22

Topic: A.07. Developmental Disorders

Support: CIHR
NSERC-USRA

Title: Can early postnatal environment rescue impaired auditory processing and sensorimotor gating in a genetic rat model for autism spectrum disorder?

Authors: *E. E. DOORNAERT, D. MÖHRLE, A. EL-CHEIKH MOHAMAD,, G. JOHAL, B. ALLMAN, S. SCHMID;
Univ. of Western Ontario, London, ON, Canada

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental condition affecting one in 160 children worldwide. The homozygous *Cntnap2*-knockout (*Cntnap2*-/-) rat is a preclinical genetic model for studying ASD-related phenotypes. Previous work has demonstrated that there are greater ASD-like deficits in the *Cntnap2*-/- rat when bred and reared by a *Cntnap2*-/- compared to a heterozygous (*Cntnap2*+/-) dam. Considering that these *Cntnap2*-/- offspring have the same genetic mutation, it suggests that environmental factors are influencing their development. This exploratory project investigated if these environmental effects occur pre- or postnatally. To do this, we conducted a cross-fostering paradigm in which *Cntnap2*-/- offspring were bred from a *Cntnap2*-/- dam and transferred to be reared by a *Cntnap2*+/- dam. These cross-fostered animals were compared to *Cntnap2*-/- animals bred and reared by a *Cntnap2*-/- dam as well as *Cntnap2*-/- and wildtype animals bred and reared by a *Cntnap2*+/- dam. All animal groups contained both sexes and met adequate sample sizes. Throughout development, we examined ASD-like deficits in auditory processing and sensorimotor gating. All *Cntnap2*-/- regardless of parental genotype and cross-fostering showed impaired neural responsiveness in the auditory brainstem response (the neural activity in the brainstem in response to auditory input), the acoustic startle response (the whole-body contraction reflex elicited by the sudden presentation of a loud auditory stimulus), and prepulse inhibition (the reduction in the startle response if the startling stimulus is preceded by a low-intensity prepulse). However, cross-fostering restored a deficit in the maturation of hearing sensitivity for *Cntnap2*-/- rats bred from a *Cntnap2*-/- dam. Together, this research provides evidence that some ASD characteristics observed in the *Cntnap2*-/- are not fixed by the genetic mutation but can be malleable by early postnatal environmental conditions. Furthermore, the results have implications for how all researchers conduct breeding when using genetic animal models to study neurodevelopmental conditions.

Disclosures: E.E. Doornaert: None. **D. Möhrle:** None. **A. El-Cheikh Mohamad,:** None. **G. Johal:** None. **B. Allman:** None. **S. Schmid:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.11/A23

Topic: A.07. Developmental Disorders

Title: Effects of Deletion of Autism-related Gene Gigyf2 in Mice

Authors: *Q. XIA[1], W.-C. CHEN[1], A. SINGH[1], C. SONG[1], J. WANG[1], Z. XUAN[1], C. M. POWELL[1], A. SINGH[2];
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Abstract: Autism Spectrum Disorders (ASD) are neurodevelopmental disorders in which children display differences in social interaction/communication and repetitive stereotyped behaviors along with variable associated features. *GIGYF2* (Grb10 Interacting GYF (glycine-tyrosine-phenylalanine) Protein 2) is a protein-coding gene associated with neurodevelopmental disorders. GIGYF2 interacts with the Grb10 (growth factor receptor bound protein 10), which is an adaptor protein that known to interact with insulin or insulin-like growth-factor receptors and signaling molecules. This interaction suggests that *GIGYF2* is involved in signal transduction pathways and cellular signaling processes. The exact function of *GIGYF2* is not fully understood, but several sequencing studies have implicated its involvement in ASD. Previous studies suggest that *GIGYF2* mutations disrupt 4EHP function to increase protein translation. Although complete knockout of *Gigyf2* is early post-natal lethal, our *Gigyf2* conditional knockout heterozygous (HET) mice are still viable and have a 40% reduction in *Gigyf2* expression and demonstrate modest behavioral differences including decreased locomotor activity in open field, increased repetitive grooming behavior, decreased rearing behavior, normal sociability (social preference) but impaired social novelty preference (social novelty) in 3-chamber test of sociability. Both frequency and amplitude of mEPSC and mIPSC are unchanged in *Gigyf2* HET mice hippocampus. Heterozygous deletion of *Gigyf2* also did not affect baseline synaptic transmission, presynaptic transmission, or LTP in hippocampus except a slight increase in Paired Pulse Ratio (PPR) in hippocampus. AAV-Cre infection reduced Gigyf2 protein by 30% and 78% expression in HET and homozygous (HOM) neurons in culture, respectively. Both CaMKII-Cre mediated heterozygous deletion of *Gigyf2 in vivo* and AAV-Cre mediated homozygous deletion of *Gigyf2 in vitro* did not significantly impair the phosphorylation of AKT, ERK and S6K in mouse cortical neurons. Frequency and amplitude of mEPSC recordings were comparable between AAV-Cre infected *Gigyf2* WT, HET and HOM neurons in culture. Taken together, these findings indicate that *Gigyf2* regulates some behaviors without significantly affecting synaptic transmission in hippocampus or cortical neurons in culture.

Disclosures: Q. Xia: None. **W. Chen:** None. **A. Singh:** None. **C. Song:** None. **J. Wang:** None. **Z. Xuan:** None. **C.M. Powell:** None. **A. Singh:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.12/B1

Topic: A.07. Developmental Disorders

Support: Brain Canada Foundation
Scottish Charitable Rite Foundation

Title: Altered cortical activity and connectivity and associated behavioral abnormalities in Shank3B$^{-/-}$ mouse model of autism spectrum disorder

Authors: *Z. YU, *Z. YU, R. ZAHACY, Y. MA, I. WINSHIP, A. CHAN;
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Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder, which may manifestas difficulties in speech, nonverbal communication, and social interactions, along with restricted and repetitive patterns of behaviour, interests, or activities. Altered brainactivity and connectivity were observed in children with ASD but how these alterations relate to diagnostic criteria is unclear. B6.129-Shank3<tm2Gfng>/J mice (Shank3B$^{+/+}$(WT), Shank3B$^{+/-}$ (HET), Shank3B$^{-/-}$ (KO)) , aged 3-8 months, were used in this study as a mouse model of autism spectrum disorder to investigate cortical activity and connectivity through intrinsic signal optical imaging and calcium imaging of the dorsal neocortex. Through 30-minute home-cage recordings, we measured the grooming time of KO mice, which demonstrated hyper-grooming — a behavior indicative of their repetitive tendencies ($F(2, 18) = 15.37$, $P=0.0001$). We used open field test to assessthe anxiety level in KO mice. These mice exhibited reduced movement in the open field test, which could indicate heightened anxiety ($F(2, 61) = 11.17$, $P<0.0001$). Furthermore, KO mice show social deficits which was measured by the Three Chamber test. There is no significant difference between time spent with the object and social mouse ($N=9$, $P=0.9982$) while time spent with social mouse is significantly higher than with the object in WT group ($N=13$, $P=0.0015$). These results are consistent with animal cognates of altered behaviours, such as repetitive behaviours, difficulties in social interaction and anxiety in autism spectrum disorder. We next employed intrinsic signal imaging to assess resting-state global cortical activity across the neocortex and processed the data dependent on the regions of interest (ROI), there is no significant difference in different ROIs among three groups ($F(2, 30) = 0.1059$, $P=0.8999$). However, by using direct and high-resolution measure, calcium imaging, we found hyperconnectivity and heightened cortical activity in KO mice, compared to WT mice. By revealing hyperconnectivity and heightened cortical activity, in addition to observing corresponding impaired behaviours, we have established a foundation for delvingdeeper into the possible mechanistic pathophysiology of Shank3B$^{-/-}$ and potentially identifying new therapeutic targets.

Disclosures: **Z. Yu:** None. **Z. Yu:** None. **R. Zahacy:** None. **Y. Ma:** None. **I. Winship:** None. **A. Chan:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.13/B2

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS123263
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NIH Grant R01MH085953
Brain Canada Multi-Investigator Initiative
The Canadian Institutes of Health Research
Canada First Research Excellence Fund
Institute of Data Valorization
Healthy Brain Healthy Lives

Title: Using organoids to model thalamocortical pathway development in 22q11 Deletion Syndrome

Authors: *D. SHIN[1], C. N. KIM[2], K. M. HENNICK[2], J. M. ROSS[2], N. PARANJAPE[2], M. G. KEEFE[2], B. PAVLOVIC[2], K. C. DONOHUE[2], C. MOREAU[4,5,7], D. E. ALLEN[2], G. POPOVA[1], A. BHADURI[8], C. E. BEARDEN[9], A. A. POLLEN[2], S. JACQUEMONT[6], D. HAUSSLER[10], A. WIITA[2], N. A. FROST[11], V. S. SOHAL[3], T. J. NOWAKOWSKI[2];
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Abstract: Thalamocortical pathway dysfunction is implicated in several psychiatric disorders, including schizophrenia and autism. However, the mechanisms by which these defects emerge remain poorly understood. To study if early steps in thalamocortical pathway development may be disrupted in psychiatric disorders, we studied thalamic and cortical axonogenesis in

thalamocortical organoids generated from pluripotent stem cells carrying the 22q11.2 microdeletion, which represents a strong risk factor for psychiatric disorders. We show that the 22q11.2 microdeletion leads to exuberant axonogenesis of thalamic neurons, and leads to early disruption of network activity in an organoid model of thalamocortical pathway development. Furthermore, we identify transcriptomic changes in thalamic neurons with the 22q11.2 microdeletion, which implicate several molecular candidates that could underlie axonogenesis defects. Together, our study suggests that early steps in thalamocortical pathway development may be vulnerable to perturbations to genetic risk factors for psychiatric disorders.

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Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.14/B3

Topic: A.07. Developmental Disorders

Title: Assessment of SLC6A1 knockout zebrafish in order to develop drug screening pipelines to identify treatments for SLC6A1 neurodevelopmental disorders

Authors: *G. SMITH[1], C. CROWDER[2], A. P. GLAZE[2];
[1]Neurobio., Univ. of Alabama, Birmingham, AL; [2]Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: *Solute carrier family 6 member 1* (*SLC6A1*) neurodevelopmental disorder (*SLC6A1*-NDD) is a rare condition resulting in epilepsy, autism spectrum disorder (ASD), and severe developmental, motor, behavioral, and intellectual disabilities. The *SLC6A1* gene encodes for the protein GABA transporter 1 (GAT1). GAT1 is required for reuptake of the inhibitory neurotransmitter, GABA, into the pre-synaptic nerve terminal. Despite the severe symptoms associated with *SLC6A1*-NDD, non-seizure and behavioral symptoms are not well characterized. Thus, *SLC6A1* knock-out (KO) zebrafish models were generated to model non-seizure and behavioral symptoms associated with SLC6A1-NDD. Phenotyping assays conducted include: motility (velocity and locomotive activity), swim patterns, and response to stimuli. After complete characterization of *SLC6A1*-NDD related phenotypes, artificial intelligence tool, mediKanren, was used to identify FDA-approved therapies hypothesized to upregulate and/or compensate for loss of GAT1 and treat behavioral symptoms. Identified therapies were used to treat larval *SLC6A1* KO models. Phenotypic expression of non-treated and treated SLC6A1 KO zebrafish larvae were compared to determine the impact of treatments. Based on this, possible therapies can be identified to improve quality of life for *SLC6A1*-NDD patients.

Disclosures: **G. Smith:** None. **C. crowder:** None. **A.P. Glaze:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.15/B4

Topic: A.07. Developmental Disorders

Support: NIH/NIMH R01MH111464
NIH/NICHD F30 HD098893

Title: Role of microglial MEF2C in brain development and its contribution to autism-like behaviors

Authors: *A. GREIGE, C. M. BRIDGES, Y. CHO, E. TSVETKOV, S. BERTO, C. COWAN; Med. Univ. of South Carolina Neurosci. Grad. Program, Charleston, SC

Abstract: Introduction: MEF2C Haploinsufficiency Syndrome (MCHS) is a neurodevelopmental disorder caused by loss-of-function mutations or microdeletions in one allele of the gene encoding the transcription factor MEF2C (Myocyte Enhancer Factor 2C). Common symptoms include autism spectrum disorder, intellectual disability, limited or absent speech, movement difficulties, epilepsy, and sensory differences. Many basic research studies have revealed important roles for MEF2C in the differentiation, maturation, and function of various cell-types and organ systems, including brain development, where it is enriched in many populations of developing and mature neurons and microglia, the brain's resident phagocytes that play key roles in neural circuit maturation. However, the impact of MEF2C hypofunction in microglia, and its contributions to symptoms of MCHS, remains unclear.
Methods: We utilized both global *Mef2c* loss-of-function mutant mice (*Mef2c*$^{+/Dexon2}$) and microglia-selective *Mef2c* heterozygous mutant mice. Using these mouse lines, we employed RNA-sequencing, immunohistochemistry-based analysis, patch-clamp electrophysiology, and behavior testing to analyze microglial contributions to microglial development and function, neuroimmune states, and developmental synaptic pruning.
Results: Our findings suggest that developing microglia in the MCHS mouse model exhibit an activated morphology, abnormal phagocytic activity, altered expression of neuroinflammatory genes, and structural and functional synapse pruning deficits in the medial prefrontal cortex, and these effects appear to be strongest in males. These changes correlate with deficits in social preference in the MCHS mice, and selective *Mef2c* heterozygosity in developing microglia is sufficient to produce social deficits.
Conclusion: These results suggest that MEF2C is important for the normal physiological functions of developing microglia, cortical circuit development, and social behavior, and that Mef2c heterozygosity in developing microglia might contribute in a cell-autonomous manner, at least in part, to the autistic features of MCHS.

Disclosures: **A. Greige:** None. **C.M. Bridges:** None. **Y. Cho:** None. **E. Tsvetkov:** None. **S. Berto:** None. **C. Cowan:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.16/B5

Topic: A.07. Developmental Disorders

Support: R21MH118685
NSF PRFB DBI 2011039
Saban Research Institute Research Career Development Fellowship
Simms/Mann Chair in Developmental Neurogenetics

Title: Determining the impact of offspring genetic diversity on early life experiences and differential susceptibility to Chd8 haploinsufficiency

Authors: *M. TABBAA[1], P. R. LEVITT[2];
[1]Neurol., Children's Hosp. Los Angeles, Los Angeles, CA; [2]Children's Hosp. Los Angeles, Children\'s Hosp. Los Angeles and Univ. of Southern California, Los Angeles, CA

Abstract: Maternal-offspring interactions are dyadic and influenced by the genetics and behavior of the mother and offspring. The genetic background of offspring may contribute to variability in these interactions and contribute to differential susceptibility to neurodevelopmental disorder risk. However, the significance of maternal-offspring interactions in neurodevelopmental disorders are typically studied in single inbred strains, or one genetic background. This experimental approach does not incorporate heterogeneity in the early social environment that exists in genetically diverse populations. The impact of genetic diversity of offspring on maternal-offspring interactions were examined in a mouse model of autism spectrum disorder. C57B/6J (B6) dams, heterozygous for the high-confidence autism risk gene, *Chd8* (*Chd8*$^{+/-}$), were paired with sires from 15 collaborative cross (CC) genetic reference panel strains to produce genetically diverse F1 B6-CC male and female wild-type or *Chd8*$^{+/-}$ littermates. Sires were removed prior to litter births to control for differences in paternal care. Litters (N=5-11 litters/strain) were observed for maternal and pup behaviors from the day after birth to weaning by a researcher blind to strain. A subset of mice from each strain were then tested for social, anxiety-like, and cognitive traits in adulthood. Body weights and brain weights were also recorded. Maternal care differed across offspring strains depending on the trait measured. The timing of pup developmental milestones, including eye opening and eating solid food, also varied across strains. In adulthood, genetic background regulated differential susceptibility to trait disruptions due to *Chd8*$^{+/-}$, and litter variables were correlated with the impact of *Chd8*$^{+/-}$ on adult trait disruptions. Determining the impact of offspring genetic background on early life experiences provides an important foundation for interpreting adult outcomes and determining biological mechanisms that underlie altered phenotypes.

Disclosures: **M. Tabbaa:** None. **P.R. Levitt:** None.

Poster

PSTR314. Autism: Genetic Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR314.17/B6

Topic: A.07. Developmental Disorders

Support: NIH Grant AG005214 (J.E.)
Center for Drug Design and Development, University of Toledo
deArce-Koch Memorial Endowment

Title: Phenotype-based screening for compounds that modulate repetitive behaviors in wild-type and Shank3B mutant zebrafish (Danio rerio)

Authors: *J. DIETRICH[1,2], C. WIDMAN[3], S. VENTRESCA[2], F. E. WILLIAMS[2], I. T. SCHIEFER[3], J. ELLIS[4], W. S. MESSER, Jr.[2];
[2]Pharmacol. and Exptl. Therapeut., [3]Medicinal and Biol. Chem., [1]Univ. of Toledo, Toledo, OH; [4]Psychiatry, Penn State Univ., Lebanon, PA

Abstract: Autism spectrum disorder (ASD) is a set of neurodevelopmental disorders characterized by early-onset social, motor, and cognitive deficits. ASD has two main categories of core symptoms - 1) communication difficulties and social challenges and 2) restricted and repetitive behaviors. *There are currently no pharmacological treatments approved by the Food and Drug Administration (FDA) that target the core symptoms.* Phelan-McDermid syndrome, a genetic disorder that stems from the deletion of a segment of chromosome 22 in the 22q13 region or a defect in the *Shank3* gene, is one of the most common genetic causes of ASD. A behavioral screen was developed to monitor repetitive behaviors in 5 days post fertilization (dpf) wild-type (WT) and *Shank3B* mutant zebrafish and identify compounds that modulate levels of repetitive behaviors. Zebrafish were exposed to various concentrations of compounds in a behavioral assay in alternating light and dark periods for 90 minutes. Video tracking software (Noldus DanioVision) was used to measure locomotor activity and other parameters including angular velocity and variance of turn angle - measures of repetitive behaviors. Preliminary studies examined the effects of BQCA, a positive allosteric modulator (PAM) of acetylcholine (ACh) potency at M_1 muscarinic receptors (M_1 potency PAM), on WT larval zebrafish. At doses of 3 and 10 μM, BQCA significantly decreased locomotor activity, while increasing angular velocity and turn angle. Several novel PAMs of ACh efficacy at M_1 muscarinic receptors (M_1 efficacy PAMs), including CW-6-65 and CW-6-77, also significantly decreased swimming, and increased turning behaviors and variance of turn angle. *Shank3B*$^{+/-}$ zebrafish displayed lower levels of locomotor activity during the initial spontaneous swimming period and the first dark period compared to WT zebrafish. Angular velocity and turn angle were decreased for all periods in *Shank3B*$^{+/-}$ larvae. The M_1 potency PAM, BQCA, had no effect on swimming or turning behaviors during the spontaneous light period. The M_1 efficacy PAMs, CW-6-65 and CW-6-77,

significantly decreased swimming, while increasing angular velocity and turn angle. These data suggest that M_1 efficacy PAMs might be useful in reducing repetitive behaviors associated with ASD.

Disclosures: **J. Dietrich:** None. **C. Widman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application holder. **S. Ventresca:** None. **F.E. Williams:** None. **I.T. Schiefer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application holder. **J. Ellis:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application holder. **W.S. Messer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent application holder; owner of Psyneurgy Pharmaceuticals LLC.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.01/B7

Topic: A.07. Developmental Disorders

Support: NAF Grant 918037
NIH R01DK120047
NIH R01DK120330
NIH R35GM130292
Michigan Protein Folding Disease Initiative

Title: Sel1l-hrd1 er-associated degradation is indispensable for hippocampal synaptic plasticity and neurodevelopment

Authors: *H. WANG[1,2], L. LIN[1,2], H. LEE[3], B. DUAN[3], L. QI[1,2,4];
[1]Mol. Physiol. and Biol. Physics, Univ. of Virginia, Charlottesville, VA; [2]Mol. and Integrative Physiol., [3]Molecular, Cellular, and Developmental Biol., [4]Div. of Metabolism, Endocrinol. & Diabetes, Dept. of Intrnl. Med., Univ. of Michigan, Ann Arbor, MI

Abstract: SEL1L-HRD1 complex, the most conserved Endoplasmic Reticulum (ER)-Associated Degradation (ERAD) machinery, is critical for disposing of misfolded proteins from the ER for cytosolic proteasomal degradation. We recently identified a novel group of inherited disorder in humans, presenting developmental delay, intellectual disability, microcephaly, and hypotonia/ataxia, as ERAD-associated neurodevelopmental disorder with onset in infancy (ENDI, *Wang et al., J Clin Invest, under revision;* ENDI-Agammaglobulinemia, *Weis, Lin, Wang et al., J Clin Invest, under revision*); however, the underlying molecular mechanism remains largely unclear. To this end, we recently generated a knockin (KI) mouse model carrying

a hypomorphic SEL1L p.S658P variant (*SEL1L*^{S658P} mouse), a variant previously identified in Finnish Hounds suffering cerebellar ataxia. Homozygous *SEL1L*^{S658P} KI mice showed mild growth retardation during development and smaller brain volume measured by magnetic resonance imaging (MRI), recapitulation developmental delay and microcephaly observed in human ENDI patients. At 5 weeks of age, KI mice also showed hindlimb clasping and abnormal gaits without impairment in muscle strength measured by grip strength test. Using patch clamp, we found that hippocampal long-term potentiation (LTP), which reflects hippocampal synaptic plasticity, was completely abolished in KI mice, while basal neural transmission was unaffected. Similarly, deletion of SEL1L in CamKII-expressing neurons (*SEL1L*^{CamKII} mouse) also abolished LTP generation in the hippocampus, indicating that SEL1L function in CamKII-expressing cells is necessary for maintaining hippocampal synaptic plasticity. Intriguingly, our preliminary data showed that the loss of LTP function is not associated with the defects in synaptic structure or the altered expression of synaptic AMPA and NMDA receptors in the hippocampus. In conclusion, our study demonstrated a critical role of SEL1L-HRD1 ERAD in neurodevelopment and synaptic plasticity *in vivo*.

Disclosures: **H. Wang:** None. **L. Lin:** None. **H. Lee:** None. **B. Duan:** None. **L. Qi:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.02/B8

Topic: A.07. Developmental Disorders

Support: Cystinosis Research Foundation

Title: Development and Characterization of a Cystinosis Knock-Out Mouse Model Using CRISPR/Cas9

Authors: ***H. CHANG**[1], M. C. MASTEN[2], Y. DING[3], J. FENG[2], L. PRIFTI[2], A. G. SOLORZANO[2], K. PADMANABHAN[2], K. WANG[2], E. G. FREEDMAN[2], J. FOXE[2]; [2]Neurosci., [1]Univ. of Rochester, Rochester, NY; [3]Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Cystinosis is a rare, childhood, lysosomal storage disorder (LSD) that is caused by mutations to the *Ctns* gene that results in a defective lysosomal transport protein called cystinosin. Cystinosin is responsible for the export of cystine - the oxidized form of cysteine - from the lysosome to the cytosol. Without a functioning transporter, cystine crystals accumulate in lysosomes and cause cell death. Typically, cystinosis presents early in childhood with failure to thrive and renal Fanconi syndrome, eventually progressing to end-stage renal disease and death. With certain medications, patients with cystinosis can better manage their disease and live longer. These treatments are not curative, though, and the disease will continue to progress over time. As they age, patients experience symptoms across multiple body systems. Cognitive

impairments are particularly problematic for patients and have been historically understudied until now. To understand the long-term effects of cystine accumulation in the brain, we have developed a *Ctns* knockout (*Ctns* -/-) mouse model using CRISPR/Cas9. This mouse model targets exon three of *Ctns*. Exon three is downstream of the start codon in *Ctns*. This allows for transcription to begin normally before quickly being terminated due to the introduction of a frameshift mutation and premature stop codon. Using both Sanger Sequencing and whole genome sequencing at 30X coverage, we see the complete deletion of our expected 428 base pair sequence in our *Ctns* -/- mice. Full-length CTNS is 367 amino acids (AA) long, analysis of our sequencing data predicts that our design will produce a truncated, 24 AA protein. We hypothesized that the premature stop codon would cause nonsense-mediated decay (NMD) of our mRNA transcript. To confirm this, we utilized two qPCR Taqman assays and analyzed four separate wild-type (WT) and knockout (KO) mice. Probes were directed toward both our deletion and a downstream exon to confirm that our transcript was undergoing NMD. Upon performing a relative quantification of transcript expression between WT and KO, it was clear that while mRNA is not being transcribed that contains our deletion, transcription continues through the remainder of the transcript and is not undergoing NMD as we had expected. Limitations of available antibodies have prevented protein-specific data from being presented. Future uses for this model will be to collect data on the neuropathology and neurophysiology seen in our mice and relate these to the symptomology patients experience.

Disclosures: **H. Chang:** None. **M.C. Masten:** None. **Y. Ding:** None. **J. Feng:** None. **L. Prifti:** None. **A.G. Solorzano:** None. **K. Padmanabhan:** None. **K. Wang:** None. **E.G. Freedman:** None. **J. Foxe:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.03/B9

Topic: A.07. Developmental Disorders

Support: NIH/NEI Grant EY012716
NIH/NEI Grant EY021580
Jewish Heritage Fund

Title: Establishing a mouse model of cortical/cerebral visual impairment (CVI)

Authors: *D. K. OAKES[1], J. M. CAI[2], A. W. MCGEE[1], W. GUIDO[1];
[1]Anatom. Sci. and Neurobio., [2]Pediatrics, Univ. of Louisville, Louisville, KY

Abstract: Cortical/cerebral visual impairment (CVI) is a disorder most often caused by perinatal hypoxic injury to the developing visual system in the brain. CVI is the leading cause of visual impairment in developed nations, encompassing deficits in many aspects of vision including visual acuity, contrast sensitivity, and object recognition. This disorder is not well understood, as

no animal model currently exists to study CVI. Here, we have developed a murine early postnatal hypoxic model of CVI by exposing mice to 9.5% O_2 at postnatal day (P) 3 for 7, 14, or 30 days. As adults (P40), we test their motor function with the rotarod, visual acuity with the visual water task, and binocular depth perception with the pole descent task. Next, we use anterograde tracing of retinal afferents to study the pattern of eye specific terminations in the dorsal lateral geniculate nucleus (dLGN), the exclusive relay of retinal information in route to cortex. Motor performance was normal and indistinguishable between normoxic and early postnatal hypoxic mice. However, average visual acuity was reduced, and the range of acuity was broader for groups of mice receiving early postnatal hypoxia for 7, 14, or 30 days. Binocular depth perception was also impaired for each early postnatal hypoxia group relative to normoxic controls. The pattern of eye specific segregation was disrupted, with early postnatal hypoxic mice showing a higher degree of overlap between ipsilateral and contralateral projections. Mice receiving early postnatal hypoxia have normal motor performance, but impaired visually guided behavior. These mice also display a pattern of immature retinogeniculate projections, similar to overlapping projections seen in early postnatal development. These visual deficits resemble facets of human CVI. Disruption in retinogeniculate axon segregation suggests, in addition to cortex, thalamus may be implicated in CVI. The establishment of a hypoxic mouse model of CVI is vital to progress in understanding the disorder and developing rational treatment options.

Disclosures: D.K. Oakes: None. **J.M. Cai:** None. **A.W. McGee:** None. **W. Guido:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.04/B10

Topic: A.07. Developmental Disorders

Title: Alterations in metabotropic mGluR1α and ionotropic AMPA receptors following status epilepticus during postnatal development with prior chronic anticonvulsant treatment

Authors: J. R. HOFFMAN, R. R. PRASAD, ***L. K. FRIEDMAN**;
Cell Biol. and Anat., New York Med. Col., Valhalla, NY

Abstract: Chronic exposure of anticonvulsant drugs (ADs) during the 2^{nd}-3^{rd} postnatal week failed to attenuate electrographic seizures and behavioral pathology induced by kainic acid (KA)-induced status epilepticus (SE). However, several ADs exerted neuroprotection. Glutamatergic Group I mGluR1α and AMPA receptor distributions were examined in the hippocampus for their underlying neuroprotective roles. Lamotrigine (LTG), carbamazepine (CBZ), phenytoin (PHT), and valproate (VPA) were administered on postnatal day 14 for 7 days. Half of the pups were injected with KA after the last AD treatment to trigger SE on P20, when the CA1 subregion becomes vulnerable to SE-induced injury. mGluR1α, GluR1, and GluR2 subunits were immunohistochemically analyzed. In controls, mGluR1α and GluR1 subunit expression was uniform with intense neuropillar staining throughout the hippocampus. GluR2 predominated in

pyramidal and granule cells. mGluR1α preferentially labeled interneurons of the stratum oriens/alveus (SO/A INs) and hilus. Following KA-SE, mGluR1α was elevated by ~2-fold in CA3 and reduced in SO/A INs. GluR1 levels were reduced in the CA1 and upper blade of the dentate gyrus (DG). GluR2 was decreased or relatively steady in the SO/A INs and stratum radiatum (SR), regions involved in synaptic plasticity. With prior AD treatment followed by KA-SE, mGluR1α expression remained elevated in CA3, the highest being after VPA. Marked increases by ~30% were observed in SO/A INs with VPA and PHT; LTG pups had near control levels. In the absence of seizures, VPA-treated pups had marked increases in GluR1 expression throughout the hippocampus, particularly within the pyramidal cell layers, whereas control GluR1 levels were observed in response to LTG, CBZ, or PHT. After KA, GluR1 was further intensified in VPA pretreated pups. In the LTG group, both GluR1 and GluR2 levels were stable, but GluR1 reductions persisted in the SR and DG. Except for CBZ treated pups, GluR2 expression was steady with other ADs. Increases in mGluR1α expression in principal neurons may facilitate endocytosis of AMPA GluR1 receptors after SE to reduce fast synaptic neurotransmission causing neuroprotection. Elevations within SO/A INs that receive excitatory inputs from CA1 neurons may increase synaptic strength with SR interneurons. Enhanced GABAergic output via these mGluR1α positive cells can also reduce glutamate excitotoxicity and may partially explain why learning acquisition in the VPA treated group exceeded that of the other ADs. LTG and VPA reduced dysregulation of AMPA receptor composition to balance ion permeability and fast synaptic conductance to prevent CA1 injury without fully blocking KA-SE.

Disclosures: **J.R. Hoffman:** None. **R.R. Prasad:** None. **L.K. Friedman:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.05/B11

Topic: A.07. Developmental Disorders

Support: NIH Grant R21 NS108722

Title: Early-life depletion of striatal cholinergic interneurons leads to tic-related behaviors in mice: new insights into the pathophysiology of Tourette syndrome.

Authors: *R. CADEDDU[1], G. BRACCAGNI[2], E. VANLUIK[2], M. BORTOLATO[3];
[1]Pharmacol. and Toxicology, [2]Univ. of Utah, Salt Lake City, UT; [3]Univ. of Utah, SALT LAKE CITY, UT

Abstract: Early-life depletion of striatal cholinergic interneurons leads to tic-related behaviors in mice: new insights into the pathophysiology of Tourette syndrome.*** R. Cadeddu**[1] , M. van Zandt[2], C. Pittenger[2-5], M. Bortolato[1]
Tourette syndrome (TS) is a neurodevelopmental disorder characterized by motor and phonic tics. Available treatment strategies remain unsatisfactory due to our limited knowledge of the

biological foundations of this disorder. Previous postmortem studies have shown a significant loss of cholinergic interneurons (CINs) in the dorsal striatum of individuals with high-severity TS. Previous research showed that CIN depletion in adult mice predisposes to tic-related responses in the presence of environmental triggers, such as stress. To test whether the same manipulation in early life may lead to behavioral aberrances akin to TS, we induced a ~50% depletion of striatal CINs in 18-day-old mouse pups. Like in adults, early-life CIN depletion did not result in significant alterations in spontaneous behavior but led to a greater predisposition to tic-related manifestations and stereotypies in response to acute stress. Strikingly, these responses were observed in male, but not female, mice. The development of CIN-depleted mice allowed us to explore several pathophysiological mechanisms related to TS. First, our data showed that acute stress led to tic-related behaviors via increased synthesis of the neurosteroid allopregnanolone in the prefrontal cortex. Indeed, countering allopregnanolone synthesis and signaling reduced the intensity of stress-induced tic-like behaviors. Second, we found that the steroid dehydroepiandrosterone (DHEA) sulfate and the cytokine tumor necrosis factor (TNF), whose levels are higher in male TS-affected children, elicited tic-like responses in CIN-depleted mice in a fashion similar to environmental stress. Finally, we validated that tic-like responses in CIN-depleted mice are underpinned by insufficient activation of M4 receptors in the striatum. Accordingly, positive allosteric modulation of these receptors elicited a marked amelioration of all abnormal phenotypes in CIN-depleted mice in a fashion similar to benchmark TS therapies. Taken together, these data highlight the high validity of CIN-depleted mice as a model of tic pathophysiology, and their high potential to identify novel therapies for TS.

Disclosures: R. Cadeddu: None. **G. Braccagni:** None. **E. Vanluik:** None. **M. Bortolato:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.06/B12

Topic: A.07. Developmental Disorders

Title: Behavioral evaluation for a mouse model of Tourette's syndrome - the C57BL/6-Slitrk1$^{tm1b(GEMMS)Narl}$/Narl mouse

Authors: *Y.-Y. MEI, H.-C. PAN, M.-C. SHIH, J.-J. LIN, H. WANG;
Natl. Lab. Animal Center, Natl. Applied Res. Labs., TAIPEI, Taiwan

Abstract: Mutations of the gene *Slitrk1*, related to synapse formation and neurite outgrowth in the mature neurons, are found in patients with Tourette's syndrome (TS). In the present study, we aim to assess the behavioral phenotype of the C57BL/6-*Slitrk1$^{tm1b(GEMMS)Narl}$/Narl* mouse to clarify whether it is an appropriate mouse model for TS. To observe repetitive behaviors and the stress-enhanced or -suppressed TS-like deficits, half of *Slitrk1$^{+/+}$* and *Slitrk1$^{-/-}$* mice (each group n=4-5) were given repeated 30 min spatial confinement (SC) before the locomotor activity test

during the transition from 7 weeks to 13 weeks of age in experiment 1. We found both the WT-SC group and the KO-control group showed significant stress responses such as less locomotion and impaired habituation, whereas the KO-SC group performed a stress-induced tic suppression such as normal habituation in the open field. To establish the face and predictive validity for our mouse model for TS, in experiment 2, *Slitrk1*$^{+/+}$ mice and *Slitrk1*$^{-/-}$ mice were divided into four groups: the WT-Saline group, the WT-SKF 82958 group, the KO-Saline group, and the KO-SCH 23390 group (each group n=4). A full D1 agonist SKF 82958 (1 mg/kg, ip) was used to mimic TS-like symptoms including eye blinks and PPI deficits in *Slitrk1*$^{+/+}$ mice. By contrast, a D1 antagonist SCH 23390 (1 mg/kg, ip) was used to rescue the potential TS-like symptoms in *Slitrk1*$^{-/-}$ mice. Prepulse inhibition (PPI) is a well-proven task to examine tic-related deficits in sensorimotor gating processes. Significantly increased startle responses at 120 dB imply sensory hypersensitivity in 5-week-old *Slitrk1*$^{-/-}$ mice. With repeated SC and drug treatments at 5 weeks, 7 weeks, 9 weeks, and 12 weeks of age, the sensitization and habituation of the basal startle reactivity were observed in the KO-Saline group and the WT-Saline group, respectively. Interestingly, mice in the WT-SKF 82958 group and the KO-Saline group showed significant SC-improved PPI performance at 5 weeks and 7 weeks of age but got worse at 12 weeks of age, in contrast with mice in the WT-Saline group. On the other hand, mice in the KO-SCH23390 group significantly showed delayed but well-stable PPI performance from 7 weeks to 12 weeks of age. In conclusion, our model shows an early-onset sensory hypersensitivity and a developmental time course of stress-induced tic suppressions on locomotor habituation, PPI performance, and persistent high anxiety during the transition from adolescence to adulthood, consistent with TS symptoms occurring in childhood, reaching the worst-ever severity in adolescence, and achieving partial or complete remission in adulthood.

Disclosures: **Y. Mei:** None. **H. Pan:** None. **M. Shih:** None. **J. Lin:** None. **H. Wang:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.07/B13

Topic: A.07. Developmental Disorders

Title: Behavioral and Motility Phenotyping and Drug Screening Pipelines in JIP3 Knock-out Zebrafish to Identify Targeted Treatments for MAPK8IP3-related Disorders

Authors: *C. M. CROWDER[1], K. TRAVIS[1], G. SMITH[2];
[1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Univ. of Alabama, Birmingham, AL

Abstract: MAPK8IP3 related disorders are caused by variants in the gene Mitogen activated protein kinase 8 interacting protein 3 that encodes the axonal transport protein JIP3. These disorders are characterized by motility issues, intellectual disability, brain abnormalities and global developmental delays and impact pediatric patients. In order to efficiently screen drugs to identify treatment options for patients, we conducted behavioral and motility phenotyping on a

JIP3 knockout (KO) zebrafish to establish a drug screening assay. JIP3 KO larval zebrafish displayed decreased locomotor activity and adults have decreased fecundity, body length and weight, compared to wild-type clutch mates. We used the artificial intelligence tool, mediKanren, and physician recommendations to prioritize a drug candidate list that we began screening at various concentrations in larval zebrafish to ameliorate the locomotor phenotype. We identified amantadine, levodopa, ketoconazole and resveratrol as promising drug candidates that rescued the locomotor phenotype in JIP3 KO zebrafish. We also demonstrated that overexpression of Spag9/JIP4 rescued locomotor phenotypes in larval zebrafish, indicating an alternative compensatory approach for treatment. We are working with physicians to screen the drug candidates in patients to determine the efficacy of these therapies to treat MAPK8IP3 related disorders hypothesized to result in JIP3 loss of function.

Disclosures: C.M. crowder: None. **K. Travis:** None. **G. Smith:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.08/B14

Topic: A.07. Developmental Disorders

Support: JST JPMJFR2061

Title: Network centralities of prefrontal and somatosensory cortices are decreased in a mouse model of developmental disorders

Authors: *H. UENO[1], Y. IYANAGA[1], Y. HARA[2], J. OHKUBO[1], Y. NAKAI[1], K. SEIRIKI[1], S. YAMAGUCHI[3], Y. AGO[4], K. TAKUMA[1], H. HASHIMOTO[1], A. KASAI[1];
[1]Osaka Univ., Suita, Osaka, Japan; [2]Kinki Univ., Higashiosaka, Osaka, Japan; [3]Gifu Univ., Gifu, Gifu, Japan; [4]Hiroshima Univ., Hiroshima, Hiroshima, Japan

Abstract: Developmental disorders are characterized by deficits in social interaction, and their pathogenesis is not fully understood. Previous studies using mouse models have identified the activity of specific brain regions associated with social behavior. However, the detailed alterations in functional neural networks related to social behavioral disorders in mice remain unclear. In this study, we analyzed the neural network related to social deficits in prenatal valproic acid (VPA)-exposed mice as a model for developmental disorders using behavioral data analysis with machine learning and brain-wide immediate early gene (IEG) mapping in Arc-dVenus reporter male mice. We found that behaviors during social interaction test for 20 minutes in mice, analyzed using DeepLabCut and SimBA, could be divided into 15 clusters based on Uniform Manifold Approximation and Projection (UMAP) dimensionality reduction and watershed segmentation. The time spent in the clusters including sniffing behaviors was significantly decreased in VPA-exposed mice. We classified the clusters commonly observed in control mice as social behavior clusters and those commonly observed in VPA-exposed mice as

non-social behavior clusters. The time spent in these clusters correlated with the number of Arc-dVenus-positive cells in each brain region. In addition, we employed graph theoretical analysis and observed a decrease in the PageRank, a parameter of centrality, in the prefrontal and somatosensory cortices in VPA-exposed mice. Furthermore, antiepileptic drugs improved the decreased centralities of the functional network and ameliorated the decreased time spent in the social behavior clusters in VPA-exposed mice. These findings suggest that the centralities of the prefrontal and somatosensory cortices are crucial for proper social behavior, serving as potential therapeutic targets and neural markers for social deficits in developmental disorders.

Disclosures: **H. Ueno:** None. **Y. Iyanaga:** None. **Y. Hara:** None. **J. Ohkubo:** None. **Y. Nakai:** None. **K. Seiriki:** None. **S. Yamaguchi:** None. **Y. Ago:** None. **K. Takuma:** None. **H. Hashimoto:** None. **A. Kasai:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.09/B15

Topic: A.07. Developmental Disorders

Support: ANR SYNDEV R22093AA

Title: Early network activity in a mouse model of STXBP1 disorder

Authors: ***D. SUCHKOV**[1], P.-P. LENCK-SANTINI[2], M. MILH[3], M. MINLEBAEV[4]; [1]INMED, Aix-Marseille Univ., Marseille Cedex 9, France; [2]INMED, [3]Aix-Marseille Univ., Marseille, France; [4]INMED INSERM U901, Marseille, France

Abstract: Mutations of the Syntaxin-Binding Protein1 (STXBP1 or Munc18-1) gene are one of the prevalent causes of developmental neurological disorders, including early onset encephalopathies with epilepsy (EEE). Most patients have severe to profound intellectual disability, motor disorders and seizures during infancy. Despite the early onset of epileptiform activity (spasms, suppression bursts, hypsarrhythmia or seizures), it is observed considerably later than the expression of the STXBP1 protein in the developing brain. Therefore, we hypothesize that epilepsy is the consequence of earlier disorders and, in particular, of abnormalities in the morphological and functional development of the brain neuronal networks.To test this hypothesis we investigated the electrophysiological correlates of neonatal network function in a mouse model of STXBP1 haploinsufficiency. Intracranial recordings using laminar extracellular electrodes have been performed in vivo in head-fixed mouse pups. Spontaneous and evoked activity has been recorded in the barrel cortex and the hippocampus. Ages of the first to 3rd postnatal week was used as a period of transition for the network activity both for the barrel cortex and hippocampus. We observed that in the beginning and middle of the second postnatal week animals with mutated STXBP1 gene showed abnormally large hippocampal activity patterns followed by periods of silence. The beginning of the second

postnatal week was also associated with significant decrease of the sensory evoked response duration in the barrel cortex. Both hippocampal and neocortical patterns normalized by the second postnatal week.We suggested that the presence of abnormal activity patterns during the first postnatal week has dramatic consequences on the later functional development of neuronal networks.

Disclosures: D. Suchkov: None. **P. Lenck-Santini:** None. **M. Milh:** None. **M. Minlebaev:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.10/B16

Topic: A.07. Developmental Disorders

Support: NC 123240.1

Title: Olanzapine modifies the cortical oscillatory activity in a neurodevelopmental model of schizophrenia

Authors: *S. REYES-CONTRERAS[1], A. SALMERON[2], L. BECERRIL[3], G. FLORES ALVARES[4], C. A. GONZÁLEZ-CASTAÑEDA[5], M. A. AGUILLÓN-PANTALEÓN[3], V. M. MAGDALENO-MADRIGAL[6];
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Abstract: Olanzapine modifies the cortical oscillatory activity in a neurodevelopmental model of schizophrenia
Reyes-Contreras Santiago, Salmeron Azul, Becerril Lizbeth, González Carlos, Aguillón Miguel, Flores Gonzalo, Magdaleno-Madrigal Víctor M
Aberrant synchronization and sleep spindle abnormalities are implicated in schizophrenia. In the neonatal ventral hippocampal lesion (NVHL), a heuristic animal model of schizophrenia, brain oscillation changes similar to schizophrenic patients have been reported. On the other hand, prolonged treatment with olanzapine provokes changes in the sleep-wake cycle in patients. The aim of this study was to analyze the effect of olanzapine administration on the cortical oscillatory activity in NVHL. Male Sprague-Dawley rats, and the model was prepared by excitotoxicity damage of the ventral hippocampus on postnatal day 7 (PD-7). Four chronic epidural EEG screws in both motor cortices were placed (2 mm posterior to the coronal suture, 2 mm from the midline on both sides) at PD-90-110. Rats were classified as follows: sham group and NVHL group, both received olanzapine (0.25 mg/kg) or vehicle for 21 days. EEG recordings for one

hour after olanzapine or vehicle administration were done. All animals showed a sudden behavioral arrest accompanied by widespread symmetric bilateral spike-wave discharges, this kind of activity was affected by olanzapine. Also, circular explorative behavior, burying behavior, mastication and neck jerk were observed. A significant increase of total number and duration of sleep spindle were observed. In addition, a significant decrease of SWD induced by olanzapine was noted. The influence of the treatment on these oscillatory activities suggest that olanzapine may regulate the aberrant oscillatory activity via the reticular-thalamic-cortical pathway.

Disclosures: S. Reyes-Contreras: None. **A. Salmeron:** None. **L. Becerril:** None. **G. Flores Alvares:** None. **C.A. González-Castañeda:** None. **M.A. Aguillón-Pantaleón:** None. **V.M. Magdaleno-Madrigal:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.11/B17

Topic: A.07. Developmental Disorders

Support: MOST 110-2410-H-002-235-MY3
MOST 111-2423-H-002-009
NSTC 112-2321-B-002-022

Title: Investigation of possible epistatic interactions between Poly(I:C)-induced maternal immune activation & serine racemase mutations in mouse model of schizophrenia

Authors: *Y. CHEN[1], W.-S. LAI[1,2];
[1]Grad. Inst. of Brain and Mind Sci., [2]Dept. of Psychology, Natl. Taiwan Univ., Taipei City, Taiwan

Abstract: Schizophrenia is a serious mental disorder affecting about 1% of the world'spopulation, but its pathogenesis remains unclear. Previous studies have identifiedmultiple factors, including genetic and environmental, that contribute to the pathologyof schizophrenia. The two-hit hypothesis of schizophrenia suggests that geneticpredisposition combined with overt developmental insults can prime individuals forlater events that eventually lead to onset of schizophrenic symptoms. Accordingly, theaim of this study is to examine possible epistatic interactions between serine racemase(i.e., SR, a schizophrenia candidate gene) mutations and poly(I:C)-induced maternalimmune activation (i.e., gestational infections during early development) using miceas a model. Based on previous studies, SR heterozygous pregnant dams receivedeither a single injection of poly(I:C) (5 mg/kg) or vehicle on gestation day 17.5. Abattery of behavioral tests was conducted in offspring in the postnatal period and earlyadulthood. In the postnatal period, a set of behavioral tests was performed to evaluatedevelopmental milestones in these mice, including righting reflex, geotaxis reflex

andgrasping reflex from postnatal day 3 to 7 and open field task on postnatal day 26. Ourresults show that maternal poly(I:C) injections lead to developmental delays inneuromotor functions compared to vehicle controls. In early adulthood, the mice weretested again to assess their behavioral and cognitive functions. Another set ofbehavioral tests was carried out in adult mice, including open field, spontaneousalternation Y-maze, novel object recognition task, object-based attention task,3-chamber social task, and trace fear conditioning. Our results revealed that maternalpoly(I:C) injections lead to hyperlocomotion and memory impairment in adultwild-type mice, but not in SR heterozygous and homozygous mutant mice. To furtherexamine neural mechanisms underlying behavioral alterations, brain alterations at thebiochemical level are currently being investigated using ELISA and Western blotting.Further investigation is needed.

Disclosures: **Y. Chen:** 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.; MOST 110-2410-H-002-235-MY3, MOST 111-2423-H-002-009, NSTC 112-2321-B-002-022. **W. Lai:** 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.; MOST 110-2410-H-002-235-MY3, MOST 111-2423-H-002-009, NSTC 112-2321-B-002-022.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.12/B18

Topic: A.07. Developmental Disorders

Support: Northwestern University Summer Undergraduate Research Grant

Title: Characterizing febrile seizure susceptibility in Pcdh19 mice

Authors: *A. C. BLASZKIEWICZ[1], J. RAKOTOMAMONJY[3], F. E. FAGBEMI[4], A. D. GUEMEZ-GAMBOA[2];
[1]Feinberg Sch. of Med., [2]Physiol., Northwestern Univ., Chicago, IL; [3]Northwestern University, Feinberg Sch. of Medici, Chicago, IL; [4]Northwestern University, Feinberg Sch. of Med., Chicago, IL

Abstract: PCDH19-related epilepsy is one of the most common forms of early-onset epilepsy in females, affecting about 30,000 people in the U.S. alone. This disorder, also known as PCDH19-CE, causes short and repeated seizure clusters that are often followed by developmental decline. PCDH19-CE is caused by pathogenic variants in the PCDH19 gene, which is predominantly expressed in the central nervous system during early development. PCDH19 codes for protocadherin-19, a cell-adhesion protein that is important for the formation of neural circuits. The PCDH19 gene is located on the X-chromosome; thus, PCDH19-CE is an X-linked disorder.

However, hemizygous males are unaffected, while heterozygous females experience severe symptoms. This unusual expression pattern is thought to be caused by a mechanism of cellular interference in which affected individuals have two different cell populations, caused by X-inactivation. This hypothesis is supported by symptomatic males presenting with mosaic pathogenic variants in PCDH19. Here, we established a mouse model to visualize the presence of two cell populations (WT/GFP-expressing and Pcdh19-KO) by breeding wild-type Pcdh19 males carrying XeGFP with heterozygous Pcdh19 females. We tested susceptibility to hyperthermia-induced seizures in our model, as patients with PCDH19-CE often experience uncontrollable seizures, initially triggered by fever. Then we aimed to determine if the presence of these two populations impacted the severity of febrile seizures. We found no significant difference in febrile seizure susceptibility between WT, $Pcdh19^{+/-,}$ and $Pcdh19^{-/y}$ mice. However, $Pcdh19^{+/-}$ mice consistently reached a higher seizure severity score compared to $Pcdh19^{+/+}$ mice. Additionally, $Pcdh19^{+/-}$ mouse brains exhibited segregation between Pcdh19 WT and KO cells, which supports the cellular interference hypothesis. The KO neurons were also more active than the WT cells, as shown by the presence of phospho-p42/44 MAPK. We are currently using tissue clearing methods to visualize Pcdh19 cell sorting in the brain, as well as areas of increased neural activity following hyperthermia. We expect to see increased segregation in the limbic system, since it has been shown that PCDH19 variants impair activity in this region. We also expect to see increased activity in KO cells, in line with our prior findings. Results from this project provide insight into the pathophysiology of PCDH19-CE and contribute to the growing efforts to find novel therapeutic approaches for this devastating disorder.

Disclosures: A.C. Blaszkiewicz: None. **J. Rakotomamonjy:** None. **F.E. Fagbemi:** None. **A.D. Guemez-Gamboa:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.13/B19

Topic: A.07. Developmental Disorders

Title: Genotype to phenotype assessment of five patient variants associated with MAPK8ip3 related disorders

Authors: *K. C. TRAVIS[1], C. CROWDER[2];
[1]Neurobio., [2]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: *MAPK8IP3*-related disorder is a rare neurodevelopmental disorder that leads to a range of phenotypes, including intellectual disability, global developmental delay, and movement disorders. This disorder is caused by *de-novo* mutations in the mitogen activated kinase 8 interacting protein 3 (*MAPK8IP3)* gene, which codes for JNK-interacting protein 3 (JIP3). To date, there have been 10 individual patient variants identified, the majority of which are missense *de-novo,* and it remains unclear the impact these variants have on JIP3 function.

This study aims to investigate genotype-phenotype correlations by overexpressing mRNA containing patient variants in zebrafish lacking wild-type JIP3 protein. JIP3 KO zebrafish display decreased motility, compared to wild-type fish. Patient variant mRNA, from 5 individual variants, was microinjected into zebrafish larvae at the single cell stage, and then larvae were assessed for movement phenotypes using the DanioVision and the MicroTracker locomotive assays. One-way ANOVA with Tukey's test with multiple comparisons was used to compare mRNA over expression in JIP3 KO, compared to wild-type clutch mates. Two variants, p.A816D and p.R1146C, rescued decreased locomotor activity in JIP3 KO fish, while 3 other variants, p.R521H, p.M543del, p.R578C, located in or in close proximity to the Rab-interacting protein domain, did not rescue and potentially worsened the motility phenotype. Amantadine, a drug used to treat Parkinson's Disease, was tested in JIP3 KO, and the 3 variants that did not rescue locomotive phenotypes. Amantadine ameliorated phenotypes in JIP3 KO and zebrafish injected with p.R578C mRNA, but did not impact the other p.R521H and p.M543del variants. This suggests altered pathophysiological mechanisms at play across individual variants, with more severe outcomes in variants located in the Rab-interacting domain, compared to other protein domains and amantadine as a potential therapeutic to treat patients with hypothesized JIP3 loss-of-function.

Disclosures: K.C. Travis: None. **C. crowder:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.14/B20

Topic: A.07. Developmental Disorders

Support: AHA 20POST35210428
NIH Medical Scientist Training Program T32GM007205
MH115939
NS105640
NS089662

Title: Heterozygosity for neurodevelopmental disorder-associated TRIO variants lead to distinct deficits in neuronal development and function

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Abstract: *TRIO* is a high-risk gene for schizophrenia (SCZ) and several neurodevelopmental disorders (NDD) including autism spectrum disorder (ASD) and bipolar disorder (BPD). TRIO is a member of Dbl family of Rho guanine nucleotide exchange factors (Rho GEFs) with two GEF

domains, that each activate specific Rho GTPases. In neurons, TRIO regulates Rac1, RhoG, and RhoA small-GTPases to control neuronal migration, morphogenesis, synapse development and function. However, it remains unclear whether and how discrete *TRIO* variants differentially impact these neurodevelopmental events. We previously demonstrated that disease-associated *TRIO* variants have discrete impacts on TRIO biochemical functions. Here, we investigated the impact of disease-associated *TRIO* variants associated with ASD, SCZ and BPD: (*TRIO) +/K1431M*, *+/K1918X*, *+/M2145T*, respectively. Heterozygosity for *TRIO* variants impacted behaviors in different ways and in some cases are selective to one sex. Sholl analysis revealed a significant reduction in basal arbor complexity and decrease in apical area in *+/K1918X* motor cortex (M1) layer 5 (L5) pyramidal neurons (L5-PNs), consistent with the smaller brain phenotype. *+/K1431M* M1 L5-PNs show increase in basal and apical tuft arbor complexity, but no difference in total dendrite length compared to WT despite the smaller brains of *+/K1431M* mice. Neither brain size nor dendritic arbors of *+/M2145T* neurons differ from WT. Structural analysis using electron microscopy revealed an increase to synaptic vesicle distribution in asymmetric synapses of the motor cortex for both docked and tethered vesicles in *+/M2145T*, while *+/K1431M* had an increase only in docked vesicles compared to WT. Mass spectrometry proteomic analysis revealed changes in the levels of the presynaptic regulatory proteins in cortex of *+/M2145T* and *+/K1431M* mice. Our single-cell recordings in acute slices indicate that all *TRIO* heterozygous mutants have deficiency in both excitatory and inhibitory signaling in M1 L5-PNs. Additionally, we find distinct changes in glutamate synaptic release in *+/K1431M* and *+/M2145T M1* L5-PNs vs WT, that is in correlation with synaptic vesicle distribution deficiency. We also found that *+/K1431M* and *+/K1918X* L5-PNs are unable to undergo long term potentiation compared to WT. Together, our data show that heterozygosity for distinct TRIO variants causes distinct anatomical, physiological, and behavioral phenotypes.

Disclosures: Y. Ishchenko: None. **A. Jeng:** None. **S. Feng:** None. **K. Nguyen:** None. **S. Myers:** None. **A. Koleske:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.15/B21

Topic: A.07. Developmental Disorders

Support: Intellectual and Developmental Disabilities Research Center - NIH/NICHD P50 HD105354

Title: Loss of Dot1L and H3K79me Impacts Memory, Sociability, and Synaptic Gene Expression in Mice

Authors: M. J. MARONI[1], M. BARTON[1], S. THUDIUM[1], K. LYNCH[1], R. LEE[3], A. DESHWAR[4], S. MEHTA[1], C. QUAYE[1], K.-J. ARMACHE[3], G. COSTAIN[4], E. KORB[2];

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Abstract: Neurodevelopmental disorders (NDDs) include a spectrum of highly prevalent conditions that manifest during development and can cause intellectual disability, developmental delays, and autism spectrum disorder. Recent work demonstrated that many chromatin regulators are mutated in NDDs, including the histone methyltransferase Dot1L. Dot1L methylates histone 3 of lysine 79 (H3K79me) which is associated with active transcription. In the context of the brain, Dot1L has a role in neural progenitor proliferation and mediating transcriptional changes in early life stress. However, the role of Dot1L in neurons and how its disruption contributes to NDDs remain unclear. We found that H3K79me is highly abundant and dynamically regulated in postmitotic neurons. Our data also indicate that H3K79me is critical for neuronal function. We found that a patient mutation results in a loss of Dot1L methyltransferase activity indicating that depletion of H3K79me can cause NDDs. Further, we found that Dot1L depletion alters transcription of synaptic genes and bidirectionally regulates GluA2, an AMPA receptor subunit. Finally, we found sex-specific memory and sociability deficits in Dot1L conditional knockout mice. Cumulatively, this work contributes to our understanding of the role of chromatin regulators in brain function and how changes to the chromatin landscape can contribute to NDDs.

Disclosures: M.J. Maroni: None. **M. Barton:** None. **S. Thudium:** None. **K. Lynch:** None. **R. Lee:** None. **A. Deshwar:** None. **S. Mehta:** None. **C. Quaye:** None. **K. Armache:** None. **G. Costain:** None. **E. Korb:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.16/Web Only

Topic: A.07. Developmental Disorders

Support: NRF-2019R1A6A3A01090600
NRF-2021R1A2C100447112

Title: Early Developmental Changes in a Rat Model of Malformations of Cortical Development: Insights into Abnormal Neuronal Migration and Altered Response to NMDA-Induced Excitotoxic Injury

Authors: *M. LEE[1,2], E.-J. KIM[2], M.-S. YUM[3];
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Abstract: Malformations of cortical development (MCDs) are caused by abnormal neuronal migration processes during the fetal period and are a leading cause of intractable epilepsy in infants. However, the exact timing of hyperexcitability or epileptogenesis in MCDs remains

unclear. To identify the earlier developmental changes in the brain of the MCD rat model, which exhibits increased seizure susceptibility during infancy (P12-15), we analyzed pathological changes in the brain and tested NMDA-induced seizure susceptibility during the neonatal period.Pregnant rats were administered two dosages of MAM (15mg/kg, i.p.) to induce MCD, while controls received normal saline. The cortical development of the offspring was measured by performing magnetic resonance imaging at postnatal day (P) 1, 5, and 8. At P8, some rats were sacrificed for immunofluorescence, Golgi staining, and western analysis. In another sets of rats, the number and latency to onset of spasms were monitored for 90 minutes after the NMDA (5mg/kg i.p.) injection at P8.In MCD rats, in vivo MR imaging showed smaller brain volume and thinner cortices from day 1 after birth ($p < 0.001$). Golgi staining and immunofluorescence revealed abnormal neuronal migration with a reduced number of neuronal cell populations and fewer dendritic arborization at P8. Furthermore, MCD rats exhibited significantly reduced expression of NMDA receptors and AMPAR4, along with increased AMPAR3 expression ($p < 0.05$). Although there was no difference in latency to seizure onset between MCD rats and controls, the MCD rats survived significantly longer than controls.These results provide insights into the early developmental changes in the cortex of a MCD rat model and suggest that delayed and abnormal neuronal development at the immature brain is associated with blunted response to NMDA-induced excitotoxic injury. These developmental changes may be implicated in the sudden onset of epilepsy in patients MCD or prenatal brain injury.

Disclosures: **M. Lee:** None. **E. Kim:** None. **M. Yum:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.17/B22

Topic: A.07. Developmental Disorders

Support: CIHR Project Award
CureGRIN Foundation

Title: Analysis of hematologic NMDA receptors in mouse models of GRIN1 disorder

Authors: *S. OKAFOR[1], W. HORSFALL[1], Z. LI[2], C. HOLICKA[4], B. KHATIR[3], K. GOLOVIN[3], G. SCOTT[4], T. MALLEVAEY[2], L. EDGAR[1], A. J. RAMSEY[1];
[1]Pharmacol. and Toxicology, [2]Immunol., [3]Dept. of Mechanical and Industrial Engin., Univ. of Toronto St. George Campus, Toronto, ON, Canada; [4]Biol., McMaster Univ., Hamilton, ON, Canada

Abstract: GRIN1 disorder is caused by a de novo mutation in the GRIN1 gene, which encodes the essential subunit of the NMDA receptor. Pathogenic variants of GRIN1 can confer a gain (GOF) or loss (LOF) of function on the biophysical and trafficking properties of the NMDA receptor but the clinical presentation for variants is similar. For example, patients with either

GOF or LOF variants can have symptoms of: intellectual disability, developmental delay, epilepsy, aphasia or limited speech, cortical visual impairment, and hypotonia or dystonia. To study phenotypic differences between GOF and LOF variants, we compared three mouse models: GRIN1 Q536R (LOF), GRIN1 Y647S (GOF), and GRIN1 knockdown mice (haploinsufficiency LOF). Specifically, we studied how these mutations affect blood cells and whether there were hematological biomarkers for loss- or gain-of function. Through this study we uncovered hematological differences between GOF and LOF variants. The hematology and histopathology of these variants suggest that GRIN1 LOF variants display iron dyshomeostasis, while the GRIN1 GOF variant displays polycythemia. Our study highlights the importance of hematologic research in GRIN disorder and the promise of biomarkers for differential diagnosis and clinical trial design. Future studies investigating patient hematology are warranted.

Disclosures: **S. Okafor:** None. **W. Horsfall:** None. **Z. Li:** None. **C. Holicka:** None. **B. Khatir:** None. **K. Golovin:** None. **G. Scott:** None. **T. Mallevaey:** None. **L. Edgar:** None. **A.J. Ramsey:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

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Program #/Poster #: PSTR315.18/B23

Topic: A.07. Developmental Disorders

Support: NIH Grant DC015000
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Title: Multiomic changes in sensory hair cells after ablation of Chromatin remodeling protein CHD4

Authors: J. KIM[1], E. MARTINEZ[2], A. JADALI[3], ***K. Y. KWAN**[1];
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Abstract: The organs of the inner ear are responsible for our ability to hear and balance. The cochlea is responsible for our ability to discriminate and hear complex sounds. The sensory cells that underlie these processes are the hair cells. Hair cells convert sound into neural signals to initiate hearing. Built for exquisite sensitivity, hair cells have a high metabolic demand and delicate mechanosensory structures that make them prime targets for ototoxic damage. Many insults can cause hair cell death, including genetic mutations, loud noises and ototoxic drugs. Over an individual's lifetime, hair cells continue to die, resulting in hearing loss. The chromodomain helicase binding protein 4 (CHD4) is an ATP-dependent chromatin remodeler and a core component of the nucleosome remodeling and deacetylase complex (NuRD). Pathogenic variants of the *CHD4* gene cause Sifrim-Hitz-Weiss (SIHIWES) disease. Patients with SIHIWES show delayed development, intellectual disability, facial dysmorphism, ear abnormalities, and hearing loss. Hair cells express CHD4 along with multiple inner ear cell

types. To determine whether altered CHD4 function in hair cells causes hearing loss, we generated a conditional knockout mouse that ablates exons coding the ATPase domain of *Chd4* at the early stages of hair cell development. Cochleae from *Chd4* cKO animals showed no noticeable differences compared to controls. However, hair cell loss becomes obvious approximately two weeks after birth. Hair cells start to degenerate and display abnormal chromatin structures. Using a single-cell multiomic approach, we interrogated the gene expression and epigenetic changes in hair cells after the ablation of CHD4. We discovered alterations in chromatin accessibility and changes in gene expression that may affect the maintenance of cell identity. We hypothesize that these changes are the underlying cause of cellular degeneration.

Disclosures: **J. Kim:** A. Employment/Salary (full or part-time):; Keck Center for Collaborative Neuroscience. **E. Martinez:** A. Employment/Salary (full or part-time):; Keck Center for Collaborative Neuroscience. **A. Jadali:** A. Employment/Salary (full or part-time):; Sampled. **K.Y. Kwan:** A. Employment/Salary (full or part-time):; Stem Cell Research Center, Keck Center for Collaborative Neuroscience.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.19/B24

Topic: A.07. Developmental Disorders

Support: NIH Grant R01HD104609
Fundación Alfonso Martín Escudero

Title: Cortical development is altered in a mouse model of DDX3X syndrome

Authors: *M. GARCIA-FORN[1,2,3,4,5], M. FLORES[2,1,3,4,5], P. OLA[2,1,3,4,5], A. VON MUEFFLING[1,2,3,4,5], S. DE RUBEIS[1,2,3,4,5];
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Abstract: DDX3X syndrome, characterized by intellectual disability (ID), behavioral problems, motor impairments and congenital brain malformations, accounts for approximately 1-3% of unexplained ID cases in females. While the genetic cause of the syndrome is known to be mutations in the *DDX3X* gene, the specific cellular and molecular mechanisms underlying it are still not fully understood. *DDX3X* is an X-linked gene that plays a role in regulating mRNA translation and has recently been implicated in corticogenesis and synaptogenesis. Our lab generated the first mouse model for this syndrome (*Ddx3x*$^{+/-}$) that has construct validity for *DDX3X* loss-of-function mutations. This model exhibits developmental and behavioral abnormalities, along with defective cortical lamination, providing evidence of face validity. Here, our goal is to understand the cellular and molecular mechanisms underlying DDX3X

syndrome during development. To investigate the impact of *Ddx3x* haploinsufficiency on corticogenesis, we used our *Ddx3x*$^{+/-}$ mice and examined cortical progenitors at different stages of embryogenesis using cell-specific markers. Additionally, we employed *in utero* electroporation of a GFP plasmid to assess the birthdate and migration of cortical glutamatergic neurons. Our findings revealed that *Ddx3x*$^{+/-}$ mice displayed an increase and altered distribution of cortical progenitor cells during early embryogenesis, which came at the expense of postmitotic neurons when compared to their *Ddx3x*$^{+/+}$ littermates. Collectively, these results provide novel insights into the cellular mechanisms that drive DDX3X syndrome.

Disclosures: M. Garcia-Forn: None. **M. Flores:** None. **P. Ola:** None. **A. Von Mueffling:** None. **S. De Rubeis:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.20/B25

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS131620

Title: Investigating neuronal-specific functions of the RNA exosome during aging through study of Pontocerebellar Hypoplasia Type 1b in Drosophila

Authors: *M. TORSTRICK, L. HIGGINSON, J. BURFORD, D. J. MORTON;
USC, Los Angeles, CA

Abstract: Eukaryotic genomes are complex and provide the blueprint for the diversity of cell types in the human body. The genetic information in our genome is differentially regulated to confer distinct functional properties to each cell type. Much of this regulation occurs through strict control of gene-specific transcription, which includes post-transcriptional RNA processing. A key post-transcriptional regulatory machine, the RNA exosome complex, is essential for mediating RNA metabolism within the cell. The RNA exosome is an evolutionarily-conserved 3'-5' ribonuclease complex responsible for precise processing and degradation of a variety of cellular RNAs. Recessive mutations encoding single amino acid substitutions in EXOSC3, a structural subunit of the RNA exosome, cause a neurodevelopmental disorder, Pontocerebellar Hypoplasia Type 1b (PCH1b). PCH1b is characterized by defective development and atrophy of the cerebellum and brainstem structures. To understand the impact of these disease-linked amino acid substitutions on RNA exosome activity and neuronal function, we created a *Drosophila* model of PCH1b via CRISPR/Cas9 technology. Initial analysis of PCH1b-engineered flies revealed an increased requirement for EXOSC3 (termed Rrp40 in flies) in aging neurons. The goal of this study is to now investigate how PCH1b-linked amino acid changes alter RNA exosome function and consequently cause age-related neuronal phenotypes. Previous RNA-seq analysis of brain enriched tissue from *Drosophila Rrp40* mutants revealed increases in the

steady-state levels of specific mRNA and ncRNA including synaptic regulator *Arc1*. Now, to understand how disease-causing defects in the RNA exosome impact fly brain development and homeostasis with age *in vivo*, I performed aging experiments in adult flies and evaluated organismal and molecular phenotypes. My preliminary results reveal age-dependent morphological and behavior defects including mushroom body defects and reduced locomotor activity. In addition, qRT-PCR experiments show age-dependent accumulation of *Arc1* mRNA expression in *Rrp40* mutant fly brains compared to wildtype control flies. To complement qRT-PCR experiments, I employed *in vivo* quantitative imaging (HCR RNA-FISH) to characterize the spatial gene expression of *Arc1* during aging in *Rrp40* mutant flies compared to wildtype controls. My data show increased expression and localization of *Arc1* in the central brain region of the *Rrp40* mutant flies compared to wildtype controls. Together, these data provide insight into how disease-linked changes in Rrp40 alter the RNA exosome complex's activity and contribute to age-related neuronal dysfunction.

Disclosures: M. Torstrick: None. **L. Higginson:** None. **J. Burford:** None. **D.J. Morton:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.21/B26

Topic: A.07. Developmental Disorders

Title: Role of the protocadherin FAT1 in cortical development and related disorders

Authors: *L. CORBIERES[1], V. GINAUX[2], F. HELMBACHER[3], C. CARDOSO[4];
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Abstract: Alteration of cortical development, due to genetic mutation, can result in malformation of cortical development (MCD) associated with intellectual disability, autistic features, and seizures. The most common form of MCD, Periventricular Nodular Heterotopia (PH), is characterized by ectopic neurons lining the ventricle. Using whole exome sequencing, we identified a biallelic mutation in the protocadherin FAT1 in a PH patient. A link between protocadherins and PH has already been reported (Capello & al, 2013). In addition, several FAT1 variants have been found in patients with bipolar affective or autism spectrum disorders (Grant & al, 2022; Hernando-Davalillo & al, 2022) and more recently with focal epilepsy (Zou & al, 2023). Protocadherins are involved in polarity of neural progenitors, migration processes and in cell-cell interaction. However, the function of FAT1 in brain development and the final outcomes of loss of FAT1 in the physiopathology of MCD remains poorly understood. To address this issue, we investigated the consequences of FAT1 loss of function, induced by in utero RNA interference, on neuronal proliferation and migration. We found that FAT1 depletion increases the number of apical progenitors and delays radial migration in the cortical plate. At

postnatal stages, our results showed that abnormal neuronal migration arrest gives rise to overmigration of neurons. In other hand, by using genetic model mice, we identify alteration on the cortical lamination due to depletion of FAT1 in different subtypes of neurons. Our findings demonstrate crucial roles of FAT1 in neocortical development, whose alterations can cause neurodevelopmental disorders.

Disclosures: L. Corbieres: None. **V. Ginaux:** None. **F. Helmbacher:** None. **C. Cardoso:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.22/B27

Topic: A.07. Developmental Disorders

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The Brain and Behavior Research Foundation (2015 NARSAD n. 23234)
Jerome Lejune Foundation ("Role of neuropeptides on Blood Brain Barrier formation and maintenance in a 22q11.2DS mouse model")
Istituto Italiano di Tecnologia

Title: Perinatal Oxytocin Closes the Brain Barriers Ameliorating Behavioral and Immunological Trajectories in 22q11.2 Deletion Syndrome Mice

Authors: G. CASTELLANI[1,2], M. CIAMPOLI[1], V. FERRETTI[1], ***A. BENEDETTI**[1], G. TRIGILIO[1], W. BARCIK[1], M. BUSNELLI[3,4], C. PAOLINI[3,4], C. DEVROYE[1], F. MALTESE[1], S. SANNINO[1], E. ALBANESI[1], M. NIGRO[1], C. BRACCIA[1], A. ARMIROTTI[1], S. DE MARTIN[2], B. CHINI[3,4], F. PAPALEO[1];
[1]Italian Inst. of Technol., Genova, Italy; [2]Dept. of Pharmaceut. and Pharmacol. Sciences, Univ. of Padova, Padova, Italy; [3]Natl. Res. Council (CNR), Inst. of Neurosci., Vedano al Lambro, Italy; [4]NeuroMI Milan Ctr. for Neuroscience, Univ. of Milano-Bicocca,, Milan, Italy

Abstract: Genetic vulnerability and immunological alterations are associated with the development of psychiatric disorders. However, little is known about how risk genes impact the immunological profile, and if their interconnections can be targeted to improve aberrant behavioral trajectories. Here, we focused on the 22q11.2 hemideletion, a well-established genetic predisposition factor to psychiatric disorders. By developmentally characterizing 22q11.2 hemideleted mice (LgDel/$^{+}$), we revealed adolescence as a turning point for behavioral and cortical abnormalities, associated with immunological alterations. Oxytocin (OXT) perinatal intranasal supplementation led to long-lasting behavioral and immunological ameliorations in LgDel/$^{+}$ mice, which have reduced endogenous OXT. These effects were primarily related to

OXT-dependent long-lasting closure of the blood-brain and the blood-cerebrospinal fluid barriers, independently on microglia and regulatory T cells contribution. Our findings shed new light on the complex interaction between genetic risk, altered immune system and consequent abnormal behavioral development, providing a roadmap for therapeutic approaches.

Disclosures: G. Castellani: None. **M. Ciampoli:** None. **V. Ferretti:** None. **A. Benedetti:** None. **G. Trigilio:** None. **W. Barcik:** None. **M. Busnelli:** None. **C. Paolini:** None. **C. Devroye:** None. **F. Maltese:** None. **S. Sannino:** None. **E. Albanesi:** None. **M. Nigro:** None. **C. Braccia:** None. **A. Armirotti:** None. **S. De Martin:** None. **B. Chini:** None. **F. Papaleo:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.23/B28

Topic: A.07. Developmental Disorders

Title: Exploring Concordance: High Throughput Planarian Behavioral Assays with Predictive Value for Translational Studies

Authors: *S. GUARIGLIA;
New York State Inst. for Basic Res., Staten Island, NY

Abstract: Traditional rodent behavioral assays have long been used to gain insights into various behaviors, including those relevant to neurodevelopmental disabilities. However, these assays may also be successfully adapted for planarians, providing a novel high throughput model with value as a screening model for rapidly selecting pharmacological, genetic, or toxicant agents that impact human health. We have modified the rodent open field test for planarians using AnyMaze, which is conventionally used for mouse behavioral tracking. AnyMaze permits automated quantification of important endpoints such as locomotor activity, exploratory behavior, avoidance behavior, and stereotypies. Our modifications can be applied to other open-source tracking software, allowing any interested lab to conduct such investigations cost-effectively. In our work, we demonstrate that a variety of pharmacological and toxicological agents (i.e., nicotine, Donepezil (Aricept), and lead (Pb^{2+}) increase locomotor activity, as they do in vertebrates. Furthermore, we use AnyMaze software to detect stereotypies (i.e., rotation on the center of mass) and demonstrate that Pb^{2+} and PFOA, two environmental toxicants suspected to contribute to neurodevelopmental pathologies, contribute to such stereotypes. Secondly, clustering may be used as a proxy to study social behavior typically. We have found that social clustering can be enhanced by specific pharmacological agents, such as MDMA (ecstasy), or diminished by microbiological factors (gut dysbiosis and antibiotic use). We have designed an assay that is simple to use and only requires using freely available FIJI plugins for analysis. Finally, we designed passive avoidance paradigms to assess learning and memory in planarians. In experiments involving scopolamine and MK-801, we observed impaired learning, whereas the administration of donepezil enhanced learning and memory. We are currently developing spatial

learning and reversal learning assays to further investigate cognitive abilities in planarians further. Our research demonstrates that our planarian behavioral assays are useful for studying various behavioral phenomena, offering solid predictive value for translational work. By replicating key findings from traditional rodent assays, our work suggests that high throughput planarian behavioral modeling is valuable for understanding behavioral responses to genetic manipulations, pharmacological agents, and toxicants that are relevant to human health.

Disclosures: S. Guariglia: None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.24/B29

Topic: B.06. Intrinsic Membrane Properties, Electrical Synapses, and Signal Integration

Support: NIH Grant R21

Title: Altered cortical connectivity in a 22q11 deletion syndrome mouse model leads to sensory processing deficits in V1 reflected by reduced MMN

Authors: *A. S. VÁZQUEZ, S. KIM, L. SJULSON, R. BATISTA-BRITO;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Schizophrenia (SZ) is a complex neuropsychiatric disease with a worldwide prevalence of 5%. SZ is associated with behavioral and cognitive deficits, such as attention deficits and alterations in basic sensory perception. Nevertheless, the exact pathological mechanisms and circuits involved in SZ remain elusive. 22q11.2 deletion syndrome, also known as DiGeorge syndrome, is a genetic disorder caused by a microdeletion of 30-50 genes in the 22q11.2 region. This syndrome affects about 1 in every 4,000 people and represents the highest known individual genetic risk factor for the emergence of SZ.
Cortico-cortical under-connectivity, as shown in functional imaging studies, may underlie the behavioral deficits registered in several neurological diseases, including SZ. It has been shown that 22q11.2 deletion leads to deficits in GABAergic control of network activity and changes inhibitory and excitatory synapses in layers II/III of the prefrontal cortex. However, the specific contribution of each interneuron class towards SZ circuit dysfunction remains largely unknown. Here we will combine *in vivo* dense extracellular recordings with optogenetic modulation in the primary visual system (V1) to study how altered bottom-up, top-down, and local cortical connectivity in a 22q11 deletion syndrome mouse model might lead to sensory processing deficits typical of SZ patients.
Overall, we demonstrate that both bottom-up and top-down connectivity are altered in 22q11DS mice, and that reduced bottom-up and top-up connectivity underlie deficits in MMN in SZ. This allows us to understand the role of cortical inhibition towards SZ, having the potential to identify

new disease-relevant biomarkers associated with inhibitory deficits and reveal circuit targets for treatment in SZ

Disclosures: **A.S. Vázquez:** None. **S. Kim:** None. **L. Sjulson:** None. **R. Batista-Brito:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.25/B30

Topic: C.05. Tauopathies, Synucleinopathies, and Other Related Diseases

Support: NIAID Grant 1 R15 AI156879
NIGMS Grant 5P20GM103427

Title: Fishing Out the Potential Link Between CPT2 Deficiency and the Development of Neurological Disorders

Authors: *A. MARTA[1], C. BAKER[2], N. ZIMMERMAN[3], A. SHIBATA[3];
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Abstract: Beta-oxidation of long-chain fatty acids (LCFAs) depends upon the carnitine transferase system. In normal LCFA catabolism, the carnitine shuttle protein carnitine palmitoyltransferase II (CPTII) facilitates the conversion of palmitoylcarnitine to palmitoyl-CoA. CPTII deficiencies are linked to neurodevelopmental abnormalities and potential neurocognitive disorders. A zebrafish model system was designed to investigate CPTII function during early vertebrate body an brain development. Translation and splice-blocking morpholinos were used to knockdown CPTII expression in wildtype TuAB zebrafish. Scrambled morpholino-injected and uninjected TuAB zebrafish served as controls. Whole body and brain morphology, lipid deposition, and behaviors assays of 5 days post injection (dpi) knockdown larvae were significantly different than wild type and control injected groups. Immunofluorescent and western blot experiments show significant differences the development of brain regions, neuronal projection and presence of catecholaminergic cells in CPTII knockdown compared control larvae. Tyrosine hydroxylase immunoreactivity increased in knockdown larvae brain ~7 fold compared to control ($p<0.0001$, $n=9$). RT-qPCR for mitochondrial, dopaminergic, and disease related genes were significantly altered in CPTII knockdown compared to control larvae. For example, CPTII knockdown results in a 4.7 ± 0.6fold (P-value: 0.0004; $n=4$) increase α-synuclein expression compared to control. These experiments suggest that CPTII function is necessary at early stages of brain development and that loss of CPTII and normal lipid metabolism influences the expression of genes associated with neurocognitive disorders.

Disclosures: **A. Marta:** None. **C. Baker:** None. **N. Zimmerman:** None. **A. Shibata:** A. Employment/Salary (full or part-time):; Creighton University.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.26/B31

Topic: A.07. Developmental Disorders

Support: NACC1 Sundry Fund

Title: A mouse model of the NACC1 neurodevelopmental disorder recapitulates patient symptoms and highlights synaptic dysfunction.

Authors: *M. DEEHAN[1], J. KOTHUIS[1], E. SAPP[1], K. CHASE[2], Y. KE[1], C. SEELEY[1], M. IULIANO[1], L. KENNINGTON[2], R. MILLER[2], X. LI[1], A. BOUDI[1], K. SHING[1], E. PFISTER[2], C. ANACLET[3], M. BRODSKY[2], K. KEGEL-GLEASON[1], N. ARONIN[2], M. DIFIGLIA[1]; [1]Neurol., Massachusetts Gen. Hosp., Charlestown, MA; [2]Med., UMass Med. Sch., Worcester, MA; [3]Dept. of Neurolog. Surgery, Univ. of California Davis Sch. of Med., Davis, CA

Abstract: There are over 7,000 rare diseases with approximately 45% of those being neurological disorders, many of which have major neurological effects. In 2017, whole exome sequencing identified a recurrent arginine to tryptophan (R>W) missense mutation in the gene *Nucleus Accumbens Associated 1* at amino acid 298 (*Nacc1,* c.892 C>T, p. R298W) in seven patients (now 40) displaying profound developmental delay (IQ<25), severe epilepsy, infantile spasms, postnatal microcephaly, delayed myelination, irritability, failure to thrive, and stereotypic hand movement. The *Nacc1* gene ranks in the highest categorical score a risk gene can have (S1) on the autism gene database Sfari. NACC1 localization is largely nuclear, and evidence supports its role as a transcriptional repressor and involvement in embryonic stem cell differentiation, behavioral sensitization to cocaine in the nucleus accumbens, and proteasome translocation and synaptic plasticity in cultured neurons. The R>W missense mutation in Nacc1 is located outside of its BTB/POZ domain at the N-terminal which facilitates protein-protein interactions and a BEN domain at the C-terminal which can directly bind DNA. *Nacc1's* roles in neurodevelopment and neuronal function remain unknown. To understand mechanisms underlying the NACC1 mutation, we developed a knock-in mouse model with the synonymous NACC1 mutation (R284W). NACC1 R284W mice displayed anxiety and motor deficits. Electroencephalogram (EEG) of NACC1 R284W mice found cortical absence seizure-like epileptiform discharges occurring 20-25 times per hour. Immunofluorescence analysis revealed NACC1 is expressed in excitatory and inhibitory neurons with increased protein expression in nuclei. Bulk RNA-sequencing of postnatal day 14 (P14) wildtype (WT), heterozygous (Het), and homozygous (Ho) mice revealed over 1,000 genes dysregulated in each genotype including upregulated genes enriched for gene ontology terms related to the post synapse, ion transport and glutamatergic receptor signaling. Western blotting of pre and post synaptic proteins revealed altered expression at the protein level. These data characterize the first knock-in mouse model of the NACC1 disorder and suggest the NACC1 R>W mutation may drive synaptic dysfunction at the mRNA and protein level.

Disclosures: **M. Deehan:** None. **J. Kothuis:** None. **E. Sapp:** None. **K. Chase:** None. **Y. Ke:** None. **C. Seeley:** None. **M. Iuliano:** None. **L. Kennington:** None. **R. Miller:** None. **X. Li:** None. **A. Boudi:** None. **K. Shing:** None. **E. Pfister:** None. **C. Anaclet:** None. **M. Brodsky:** None. **K. Kegel-Gleason:** None. **N. Aronin:** None. **M. DiFiglia:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.27/B32

Topic: A.07. Developmental Disorders

Support: Science Foundation of Oujiang Laboratory to Tao Tan (OJQD2022002)

Title: Behavioral deficits induced by high-risk NDDs gene CTNNB1

Authors: W. ZHUANG[1], P. LIN[1], T. YE[1], J. GAO[1], C. SUN[1], J. YANG[1], S. WU[1], W. WANG[2], ***T. TAN**[1];
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Abstract: CTNNB1 is the gene responsible for encoding β-catenin, a critical component of the Wnt signaling pathway that regulates cellular homeostasis. It has been implicated in neurodevelopmental disorders (NDDs), although the precise mechanisms involved remain unclear. In this study, we employed Ctnnb1-siRNA transfection into N2a cells to knockdown Ctnnb1, which suppressed cell proliferation and differentiation. Analysis of bulk RNA-seq data revealed that Ctnnb1 deficiency disrupted multiple cellular pathways. Furthermore, in vivo knockdown of Ctnnb1 in the medial prefrontal cortex (mPFC) using Ctnnb1-shRNA resulted in social and cognitive deficits, accompanied by decreased neuronal activity. To further investigate the effects of Ctnnb1 knockdown, we utilized AAV-Cre injection into the mPFC of Ctnnb1f/+ mice, which induced repetitive behaviors, along with symptoms of anxiety and depression. Similar behavioral deficits were observed in transgenic mice (Emx1-Cre; Ctnnb1f/+) with Ctnnb1 haploinsufficiency specifically in forebrain excitatory neurons. Collectively, our findings demonstrate behavioral abnormalities across multiple animal models following Ctnnb1 knockdown or knockout. These abnormalities may arise due to disrupted neuronal activity, as well as impairments in neuron proliferation and differentiation.

Disclosures: **W. Zhuang:** None. **P. Lin:** None. **T. Ye:** None. **J. Gao:** None. **C. Sun:** None. **J. Yang:** None. **S. Wu:** None. **W. Wang:** None. **T. Tan:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.28/B33

Topic: A.07. Developmental Disorders

Support: NIH Grant NS125519

Title: Novel genetic models of Tourette syndrome reveal alterations in striatal interneuron migration

Authors: *M. BORTOLATO, R. CADEDDU, G. BRACCAGNI, P. J. MOOS;
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Abstract: Tics are repetitive movements or vocalizations with variable intensity and complexity. The most debilitating tic disorder, Tourette syndrome (TS), is characterized by chronic motor and vocal tics and a strong male preponderance. Tic pathophysiology is rooted in genetic alterations, which lead to functional alterations in the striatum, including abnormalities of the dopaminergic pathways and interneuron deficits. Recent genetic studies have identified the *CELSR3* gene as one of the first high-confidence genes for TS risk. *CELSR3* encodes cadherin epidermal-growth-factor laminin-G seven-pass-G-type receptor 3, a protein implicated in neuron migration. Although different CELSR3 de novo mutations have been associated with increased TS risk, no direct evidence has shown a causal link between CELSR3 deficiency and tics. To address this gap, we characterized the behavioral responses in CELSR3 heterozygous mice (homozygous mutants are not viable after birth). Our results showed that CELSR3 mutants exhibit spontaneous tic-like behaviors, hyperactivity, and cognitive deficits. Spatial and single-nucleus transcriptomic analyses revealed that these behavioral alterations were associated with deficits in the gene-ontogeny profile of parvalbuminergic neurons and alterations of dopaminergic receptor genes in the striatum. Further studies are necessary to understand the mechanisms whereby CELSR3 deficiency leads to these neurobehavioral aberrances.

Disclosures: **M. Bortolato:** 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 R21NS125519. **R. Cadeddu:** None. **G. Braccagni:** None. **P.J. Moos:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.29/B34

Topic: A.07. Developmental Disorders

Support: P20GM103620
USD Graduate Student and Creative Scholarship Grant

Title: Using CRISPR in the embryonic mouse brain to model patient variants associated with malformations of cortical development

Authors: *C. M. KITTOCK[1,2], L.-J. PILAZ[3,1];
[1]Univ. of South Dakota, Sioux Falls, SD; [3]Sanford Res., [2]Sanford Res., Sioux Falls, SD

Abstract: Malformations of cortical development (MCDs) are a group of neurodevelopmental disorders characterized by abnormal prenatal cortical development, including defects in migration or proliferation. MCDs are a major cause of treatment resistant epilepsy in childhood, and with improved accessibility of genetic testing, increasing numbers of MCD-associated genes are being identified. Tools to analyze the role of these genes in MCD pathogenesis and screen for the pathogenicity of individual variants are valuable to understand these diseases. To model MCDs *in vivo* in mice, we developed Breasi-CRISPR to efficiently edit the genome of neural progenitor cells in the embryonic mouse cortex. Breasi-CRISPR is a combination of *in utero* electroporation and CRISPR-CAS9 genome editing that enables us to insert specific patient variants into the genome and allow the mouse embryos to continue developing *in utero*. In this way, we can model patient variants without relying on overexpression or RNAi mediated knockdowns, which may not accurately model the pathogenic mechanism occurring in the patient. Initial studies model *MAP1B*-associated periventricular nodular heterotopia (PVNH). Introduction of a frameshift *MAP1B*-associated PVNH variant via Breasi-CRISPR results in abnormal neuronal accumulation in the embryonic mouse cortex, thus partially recapitulating the patient phenotype. Live imaging in brain slices revealed that these neurons migrated slower and traveled shorter distances. *MAP1B*-associated PVNH variants demonstrated robust protein expression via immunofluorescence and Western blotting. This suggests that *MAP1B* variants escape nonsense-mediated decay and that the truncated protein is stable. Due to the expression of truncated MAP1B, we hypothesized that the pathogenic mechanism underlying PVNH could be the truncated protein acting as a dominant negative. However, overexpression of the same *MAP1B*-associated PVNH variant did not recapitulate the migration delay seen when this same variant was introduced via Breasi-CRISPR. Further experiments will include using Breasi-CRISPR to model other MCDs including lissencephaly and megaloencephaly to see the breadth of phenotypes that can be studied via Breasi-CRISPR. We will also use Breasi-CRISPR to model other types of genetic variants such as missense variants. In conclusion, these data indicate that introduction of *MAP1B*-associated PVNH variants in the endogenous *MAP1B* locus results in a neuronal migration delay in the embryonic mouse brain, but a dominant negative mechanism is unlikely to underlie this phenotype. More broadly, our results highlight how Breasi-CRISPR can be used to model MCDs.

Disclosures: C.M. Kittock: None. **L. Pilaz:** None.

Poster

PSTR315. Neurodevelopmental Disorders, Genetics and Gene Modifiers

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR315.30/B35

Topic: A.07. Developmental Disorders

Support: HFH grant A10263

Title: Deletion of AMP-Activated Protein Kinase Modulates Spontaneous Myelin Loss Mouse Model of X-Linked Adrenoleukodystrophy

Authors: N. KAUR[1], *J. SINGH[2];
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Abstract: Background: X-linked adrenoleukodystrophy (X-ALD) is a neurodegenerative disease characterized by progressive demyelination of the central nervous system and elevated levels of very long chain fatty acids (VLCFAs). It is caused by mutations in ABCD1 gene that encode peroxisomal membrane transporter protein, adrenoleukodystrophy protein (ALDP). X-ALD exhibits remarkable phenotypic variability is ranging from fatal cerebral adrenoleukodystrophy (cALD) to largely pre-symptomatic adrenomyeloneurpathy (AMN). Interestingly, all clinical phenotypes can occur within the same family, that is, there is no phenotype-genotype correlation. The mouse model of X-ALD (Abcd1-KO) does not reproduce the human severe phenotypic (cALD) and only exhibits phenotype resembling AMN. We recently reported a novel loss of AMP-activated protein kinase α1 (AMPKα1) in cALD patient-derived cells and postmortem brain tissue. AMPKα1 is the principal regulator of mitochondrial function and suppressor of inflammatory response. **Objective:** We hypothesized that down regulation of AMPKα1 may be a physiopathogenic factor leading to mitochondrial dysfunction and inflammatory response in cALD. To test this hypothesis, we generated a novel Abcd1 and AMPKα1 double knock-out mice (D-KO). **Methods:** D-KO mice was genotyped by tail PCR. Brain and spinal cord proinflammatory gene expression was compared by PCR arrays (Qiagen). Brain and spinal cord sections were stained with Luxol Fast Blue to detect demyelination. Seahorse Extracellular Flux analyzer was used for measuring mitochondrial dysfunction in brain and spinal cord isolated mitochondria. **Results:** Heterozygous Abcd1-KO female (*Abcd1-KO*) and *AMPKα1-KO male* mice were crossbred to generate dual-heterozygote-deficient progeny (F1). The F1 progeny were crossbred, to obtain double knockout F2 progeny, which were homozygous for Abcd1/AMPKα1 deficiency (D-KO). Gene expression and histological studies provide evidence of significantly higher proinflammatory genes expression and loss of myelin in the brain and spinal cord of the D-KO mice. Electron micrograph studies highlight the significant myelin degeneration in the spinal cord of D-KO mice. Mitochondrial dysfunction was measured as oxygen consumption rate (OCR) in mice brain-isolated mitochondria. Mitochondrial OCR was significantly reduced in the isolated brain mitochondria of D-KO mice compared to wild type and Abcd1-KO. **Conclusions:** The novel D-KO mice, represents the inflammatory severe phenotype of human X-linked adrenoleukodystrophy disease. This novel mouse model will be useful in deciphering the mechanistic underpinnings of disease progression in cALD.

Disclosures: N. Kaur: None. **J. Singh:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.01/B36

Topic: A.01. Neurogenesis and Gliogenesis

Support: NIH
The Zvi and Ofra Meitar Family Fund
Knowledge Foundation
Arsenov Foundation

Title: Expression of calcium-binding proteins in the embryonic human ectoderm

Authors: *I. BYSTRON;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Stem cell expansion and differentiation are regulated in vivo by environmental factors encountered in the stem cell niche. We report here a previously unknown components in the neural stem cell assembly of the embryonic human retina, thalamus, olfactory placode and the cerebral wall. The calcium-binding proteins calbindin (CB) and calretinin (CR) are localized in sub-populations of interneurons in the adult brain. During development they may play special roles in the regulation of calcium during neuronal differentiation, growth and migration. We used antibodies to calretinin (CR), calbindin (CB), neuron-specific microtubule-associated protein (MAP2) and neuron-specific βIII tubulin (Tu-20) to reveal the phenotypic characteristics of early stem cell niches. Human embryos at Carnegie stages (CS) 11-18 (approximately 31- 43 days post-fertilization) were obtained from the Human Developmental Biology Resource UK. We saw the first migratory neurons in the ventral thalamus, hypothalamus and telencephalon, decreasing in density along caudorostral and lateromedial axes. The network established by their long processes is the first component of neuropil of the thalamus, optic stock, basal telencephalon and ventrolateral cortex by CS14. Notably these cells were not CR or CB immunoreactive. To our surprise, we found TU-20-positive neurons scattering through the ectoderm and mesenchyma surrounding the forebrain before the formation of the olfactory placode. The CB and CR-positive neurons of placodal origin constitute a distinct migratory population at CS 13-14. Their processes form a network along the pial surface of the ventral telencephalon. At later stages, post-mitotic CB and CR-positive neurons begin to appear in the cortical wall, hypothalamus and central retina. However, we observed immunoreactivity for CB (but not CR) much earlier in certain neuroepithelial cells, before the onset of neurogenesis, specifically in the medio-dorsal part of the cortical wall, the dorso-caudal diencephalon and the periphery of the retina. Such CB expression was not seen in the neuroepithelium of lateral cortex, thalamus or central retina, where neurogenesis and differentiation advance more quickly. Calcium is known to play a variety of roles in stem cell proliferation, neural induction and differentiation, via influences on gene expression. CB in these particular groups of proliferative cells might modulate calcium in the nucleus and thus play a part in the patterning of the forebrain ventricular zone and retinal neuroepithelium. Supported by NIH, The Zvi and Ofra Meitar Family Fund, Knowledge Foundation and Arsenov Foundation

Disclosures: I. Bystron: None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.02/B37

Topic: A.08. Development of Neural Systems

Support: F31HL140823
5T32DK007058-48

Title: Mitochondrial leak metabolism patterns the neural ectoderm by establishing the Spemann-Mangold Organizer

Authors: *A. E. MACCOLL GARFINKEL[1], N. MNATSAKANYAN[2], K. ALAVIAN[3], A. WILLS[4], M. K. KHOKHA[5], E. A. JONAS[6];
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Abstract: Carbon metabolism is necessary to produce the energy for embryonic development. Although emerging studies have suggested a role for glycolysis in embryonic patterning, whether mitochondria play a similar role remains an open question. Here, we demonstrate that mitochondrial oxidative metabolism regulates dorsal and neural cell fate and patterning of the embryo via Hif-1α. Genetic disruption of mitochondrial function, or exposure to hypoxia, induces an expansion of the dorsal mesoderm, the Spemann-Mangold Organizer, that functions to specify the neuroectoderm in the embryo. Based on the hypoxia phenotype, we show that Hif-1α itself is sufficient to expand Organizer cell fate even when Wnt/β-catenin signaling is inhibited. However, oxygen consumption in the Organizer is 20% higher, rather than lower, than that of the ventral mesoderm, suggesting an increase in aerobic metabolism rather than regional hypoxia. We find that this increase in oxygen consumption is due to a mitochondrial inner membrane leak that decouples respiration from ATP production, and we identify the source of this leak is the “free” c-subunit ring of the F_1Fo ATP synthase which is enriched dorsally. Indeed, overexpression of the c-subunit is sufficient to induce Organizer cell fates, and an ectopic body axis, via Hif-1α activation. Taken together, our findings indicate that mitochondrial leak in the dorsal mesoderm, driven by the ATP synthase c-subunit ring, activates Hif-1α which establishes the Organizer, a tissue essential for the initiation of embryonic neural patterning. It is possible that mitochondrial leak metabolism has similar functions in many biological contexts, including diseases, in which Wnt signaling and Hif-1α play a role.

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Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.03/B38

Topic: A.08. Development of Neural Systems

Title: Spatial whole transcriptome profiling of mouse neurodevelopment at mid-gestation

Authors: C. WILLIAMS[1], ***S. ZIMMERMAN**[1], A. LASLEY[3], J. REEVES[3], M. HOANG[3], J. BEECHEM[2];
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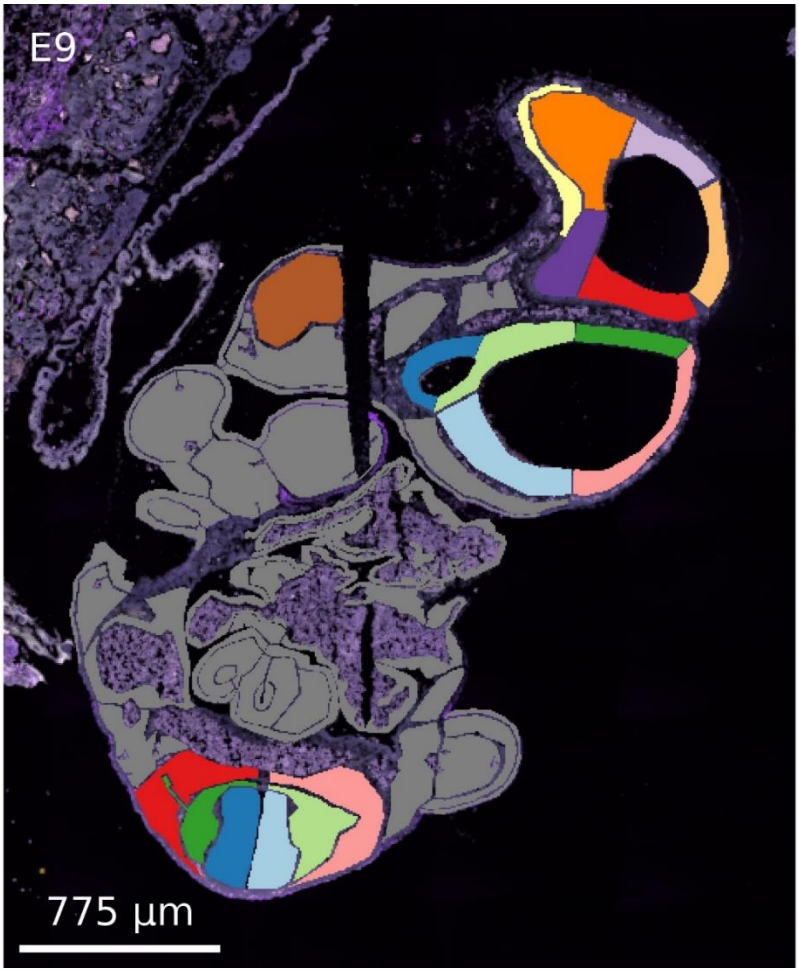
Abstract: The development of the mammalian nervous system is a complex process governed by gene expression that is precisely regulated in space and time. Insight into the transcriptional programs driving neurodevelopment has traditionally been acquired from low-plex *in situ* methods or single-cell transcriptomics of dissociated embryos, but new technologies are enabling spatial whole transcriptome profiling of defined regions of interest (ROIs). In this study, we used the GeoMx® Digital Spatial Profiler to characterize specific anatomical substructures of the mouse central nervous system (CNS) and enteric nervous system (ENS) at four timepoints from E9-E15 in healthy C57BL/6 embryos. Across the selected neuroepithelial and neuronal substructures (see examples in figure), an average of about 450 nuclei were detected in each ROI and the number of uniquely expressed genes ranged between 9,000 and 12,000. Within the CNS, we captured 25 ROIs in the forebrain across three sagittal sections and identified 75 genes that are differentially expressed between the developing telencephalonic and mesencephalonic regions at E9. Prominent among these genes are morphogens that are critical to the specification of neuronal fate. Compared to the CNS, the ENS is relatively understudied, so we additionally selected over 20 ROIs in the neuronal layer of the gut over eight sagittal sections for further analysis. The selections span across the rostrocaudal axis at E13 and E15, allowing observation of neural crest cells soon after migration into the gut and as they begin maturing into functional neurons. We identified gradients of gene expression in the enteric plexus ROIs from foregut to hindgut. Using a published single-cell RNA-seq dataset from the embryonic mouse, we deconvoluted the cell-type composition in these regions. These results provide a rich spatial and temporal atlas of gene expression in key structures of the developing central and enteric nervous systems, and can serve as a reference for future studies of developmental disorders.



Disclosures: **C. Williams:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **S. Zimmerman:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Lasley:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Reeves:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Hoang:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.04/B39

Topic: A.08. Development of Neural Systems

Title: Profiling the spatial, temporal and imprinting expression patterns of Mir125b-1 in the brain

Authors: *K.-C. HOU[1], M.-H. TSAI[2], H.-S. HUANG[2];
[1]Col. of Med., Natl. Taiwan Univ., Taipei, Taiwan; [2]GIBMS, Col. of Medicine, NTU, Taipei, Taiwan

Abstract: Genomic imprinting is highly prevalent in the brain and placenta. MicroRNAs (miRNAs) are abundant in neurons where they play key roles in development. However, few imprinted miRNAs have been discovered in the brain, and their functional roles remain unclear. We previously showed that *MIR125B2* was paternally expressed in human but not mouse brain. Knockout of *Mir125b-2* in mice led to deficits of hippocampus-related behaviors and increased neuronal excitability and increased excitatory synaptic transmission in hippocampal granule cells. *MIR125B1* is a homolog of *MIR125B2.* In this study, we aim to determine the imprinting status and functional role of *MIR125B1* in neurodevelopment. We performed Sanger sequencing on blood genomic DNA and brain cDNA from human family trios and quartets to show that Mir125b-1 was not imprinted in the human brain. We used real-time PCR to profile the expression patterns of mouse miR-125b-1 in different organs, brain regions, and brain developmental stages. We found a higher expression ($p<0.05$) of miR-125b-1 in the brain and eye compared with other organs (C57BL/6J, P28, n=3). Specifically, miR-125b-1 was enriched in olfactory bulb, thalamus, and hypothalamus (P28, n=12); such spatial distribution was different from miR-125b-2. Similar to miR-125b-2, miR-125b-1 expression reached a maximum at P0 and significantly decreased during brain development (P0-P49, n=6 for each age). Our findings would provide evidence regarding the potential roles of miR-125b-1 in the brain.

Disclosures: K. Hou: None. **M. Tsai:** None. **H. Huang:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR316.05/B40

Topic: A.08. Development of Neural Systems

Support: NLM Grant T15LM007442

Title: Identification of pediatric brain cancer neurodevelopmental origins using spatiotemporal landscapes

Authors: *A. RAJENDRAN[1,2], S. S. PATTWELL[2];
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Abstract: Normal brain development includes a synchrony of differentiation, specification, and maturation events that have yet to be entirely understood from a cellular or multi-omic perspective. Pediatric brain cancers may derive from dysregulation of normal brain development. The focus of this project is to characterize normal human brain development at the single cell transcriptomic level and identify the developmental and genetic patterns that may contribute to downstream aberrations such as pediatric brain cancers. This will result with a 'neurodevelopmental landscape', which includes the integration of existing single cell RNA sequencing data from human fetal brain during various embryonic ages and novel machine learning based application to integrate with similarly constructed cancer landscapes. Features included in the integration are derived from single cell RNA sequencing statistical analyses and novel applications of natural language processing (NLP) methods to more sensitively highlight cell type and differentiation state. Using this NLP pipeline, we expand possible weighted genetic markers for several cell types and molecular functions across developmental time and explore how they may be associated with cancer or neuropathology. These methods highlight molecular features within the neurodevelopmental landscape that forecasts the initiation and dynamics of the cancer landscapes---unearthing predictions of developmental genetic origins of pediatric brain cancers, while also advancing our knowledge of normal neurodevelopmental cell populations in embryonic development.

Disclosures: A. Rajendran: None. **S.S. Pattwell:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR316.06/B41

Topic: A.08. Development of Neural Systems

Support: Grants-in-Aid for Scientific Research 20K21584
AMED Grant 17ek0109145h003

Title: Clinical and genetic distribution of Joubert syndrome-related disorders, a neurodevelopmental disease group of ciliopathy

Authors: *Y. KITAMI[1], M. ITOH[2];
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Abstract: Joubert syndrome-related disorders (JSRD) are a clinical entity of neurodevelopmental diseases, including Joubert syndrome, Arima syndrome, Senior-Løken syndrome and Oral-facial-digital syndrome type VI among other things and commonly appear developmental delay with molar tooth sign (MTS; agenesis of cerebellar vermis and hypoplasia of brainstem) on imaging, renal dysplasia and retinal abnormalities. To date, more than 40 JSRD genes have been identified, however, there still remains variants of uncertain significance in JSRD patients. We investigated genetic distribution of 52 patients of 45 families with JSRD. The genetic analyses of 52 JSRD patients and their families were performed the genetic analysis with WES, Sanger sequencing and array CGH. The distribution was 19% (10/52) of *C5ORF42*(*CPLANE1*), 17 % (9/52) of *CEP290* gene mutations, 15 % (8/52) of *TMEM67*, 12 % (6/52) of *AHI1*, 4 % (2/52) of *TCTN2*, 2 % (1/52) of *TCTN1*, 2 % (1/52) of *KIAA0556* and 3 novel pathogenic genes; *PLEKHB1*, *RP1L1* and *SPTBN5*. To clarify the pathogenicity of variants of these 3 novel genes, we investigated the localization of these gene products and the intracellular function of the gene. We found that PLEKHB1 and RP1L1 localized near the basal bodies of primary cilia in the cytoplasm of RPE-1 cells. Knockdown of PLEKHB1, RP1L1 and SPTBN5 in xenopus recapitulated JSRD phenotypes, including renal cysts. Here, we demonstrate the evidence of pathogenesis and discuss the reverse translational study of unidentified gene variants.

Disclosures: **Y. Kitami:** None. **M. Itoh:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.07/B42

Topic: A.08. Development of Neural Systems

Support: RF1AG064822

Title: Loss of Pih1d3 Causes Prenatal Hydrocephalus with Abnormal Motile Cilia Development in Rats

Authors: T. ZHANG[1], S. CUI[2], X. XIONG[1], Z. YUAN[1], *H. ZHOU[2], X. XIA[1];
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Abstract: Primary ciliary dyskinesia (PCD) is a rare genetic disorder characterized by abnormalities in ciliary structure and function due to gene mutations. Pih1d3, located on the X chromosome in humans, has been associated with PCD. However, the precise role of Pih1d3 in PCD pathogenesis remains poorly understood. This study aimed to investigate the effects of Pih1d3 loss on hydrocephalus development and motile cilia development in a rat model. In this study, we generated a Pih1d3-knockout (KO) rat using the transcription activator-like effector nucleases (TALENs) strategy. The Pih1d3-KO rats experienced severe hydrocephalus and had a significantly reduced lifespan, typically dying within a few weeks. Western blot analysis

demonstrated abundant expression of the PIH1D3 protein in the brain. Immunostaining of WT and Pih1d3-KO rat brains at E19 and P5 revealed strong and specific staining in the ependymal epithelium lining the ventricular surfaces and the ciliated choroid plexus epithelium within the ventricular lumen. Loss of Pih1d3 in rats resulted in hydrocephalus during late perinatal stages, with severe disruption of brain structure by postnatal day 5 (P5). The Pih1d3-KO rats exhibited impaired cerebrospinal fluid (CSF) flow with a patent aqueduct of Sylvius. Immunofluorescent staining indicated that the polarity and permeability of the choroid plexus epithelium were not affected in Pih1d3-KO rats, as evidenced by normal distribution of cell junction markers N-cadherin and Na+/K+ ATPase α1 subunit. Investigation of cilia morphology in the choroid plexus and ependymal cells at prenatal and postnatal stages revealed a lack of essential molecular components for the inner and outer dynein arms of motile cilia in the Pih1d3-KO rats. Our study provides evidence for the essential role of Pih1d3 in preventing hydrocephalus and ensuring normal motile cilia development in rats. Overall, the Pih1d3-KO rat model offers valuable insights into the pathogenesis of PCD-related hydrocephalus and may facilitate future studies investigating potential therapeutic interventions for this condition.

Disclosures: **T. zhang:** None. **S. Cui:** None. **X. Xiong:** None. **Z. Yuan:** None. **H. Zhou:** None. **X. Xia:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.08/Web Only

Topic: A.08. Development of Neural Systems

Support: PD-FC2023

Title: Perinatal nicotine exposure impairs daily rhythm of PER1 and BMAL1 protein expression in the CA1 and the dentate gyrus of the hippocampus

Authors: D. LOMBARDI-MARTÍNEZ[1], ***M. FUENTES-CANO**[2], D. J. BUSTAMANTE-VALDEZ[1], P. DURAN[1];
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Abstract: The development of the central nervous system in mammals consists of several cellular and molecular processes that occur regularly under physiological conditions. There are windows of time in which the brain circuits underlying a certain function are especially receptive to the acquisition of certain types of information or stimuli to continue with their normal development If stimuli are displayed aberrantly or omitted entirely, the development of neural circuits can be permanently and negatively affected. Another process governed by temporality within organisms is the circadian rhythm, which depends, in part, on intracellular or molecular mechanisms (also called molecular clock) that form a self-regulating negative feedback

transcriptional circuit with a duration of approximately 24 hours. Chronic nicotine exposure during pregnancy is associated with changes in the CNS because it interferes with the regular modulation of acetylcholine in the developing brain, acting as an agonist at different types of nicotinic acetylcholine receptors. Similarly, nicotine administration appears to affect the expression of circadian expression genes in tissues. In the hippocampus, exposure to nicotine in the perinatal phases produces morphological, functional, and behavioral changes associated with it. The aim of the present study was to identify changes caused by perinatal exposure to nicotine in the standard rhythmicity of the molecular clock proteins BMAL1 and PER1, as well as changes in the morphology of CA1 and DG in the hippocampus. Maternal nicotine administration in the perinatal periods alters the temporal distribution and expression levels of the PER1 and BMAL1 proteins in the CA1 and GD areas of the hippocampus. On the other hand, perinatal administration of nicotine increases the number of cells in the CA1 and GD areas of the hippocampus. However, further studies are needed to determine a) whether this increase in cell density is directly related to impairments in hippocampal-dependent tasks observed in previous studies where nicotine doses were applied during development, and b) to better understand the intracellular mechanisms by which nicotine affects protein expression in the molecular clock.

Disclosures: D. Lombardi-Martínez: None. **M. Fuentes-Cano:** None. **D.J. Bustamante-Valdez:** None. **P. Duran:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: A.08. Development of Neural Systems

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JSPS Grant JP22K19245
Nagoya University Interdisciplinary Frontier Fellowship JPMJFS2120

Title: Neonatal letrozole treatment blocks defeminization of AVPV kisspeptin neurons and LH surge-generating system in male rats

Authors: *K. YAMADA, T. MANO, N. INOUE, Y. UENOYAMA, H. TSUKAMURA; Nagoya Univ., Nagoya/Aichi, Japan

Abstract: The mechanism regulating gonadotropin-releasing hormone (GnRH) and consequent luteinizing hormone (LH) surge is sexually differentiated in rodents. Kisspeptin neurons in the anteroventral periventricular nucleus (AVPV) are considered to be responsible for estrogen-

induced GnRH/LH surge in female rodents. Preovulatory level of estradiol (E2) upregulates AVPV *Kiss1* (kisspeptin gene) expression and induces LH surge in ovariectomized female rodents, whereas the E2 treatment fails to induce the AVPV *Kiss1* expression and LH surge in castrated males in adulthood. This suggests that AVPV *Kiss1* expression would be irreversibly suppressed in male rodents. Our previous study showed neonatal castration within 2 hours after birth rescued an estrogen-induced increase in AVPV kisspeptin expression and LH surge in male rats in adulthood (Homma et al., 2009). This suggests neonatal testicular testosterone defeminizes AVPV *Kiss1* expression and the GnRH/LH surge-generating system. The present study aimed to examine if E2 converted from circulating testosterone by aromatase is mainly responsible for the defeminization of AVPV kisspeptin neurons and the GnRH/LH surge-generating system in male rats. Male rats treated with letrozole (aromatase inhibitor) within 2 hours after birth showed higher numbers of AVPV *Kiss1*-expressing cells than vehicle-treated males and some letrozole-treated males showed LH surge in the presence of the preovulatory level of E2 in adulthood (over 8 weeks of age). Furthermore, female rats treated with testosterone propionate on the day of birth showed fewer AVPV kisspeptin neurons than vehicle-treated females and the animals failed to show E2-induced LH surge in adulthood. These results suggest that E2 converted from testis-derived testosterone secreted immediately after birth by neural aromatase is mainly responsible for irreversible suppression of AVPV *Kiss1* expression and defeminization of LH surge-generating system in male rats. Moreover, the present results indicate the neural aromatase functions in neonatal female rats.

Disclosures: K. Yamada: None. **T. Mano:** None. **N. Inoue:** None. **Y. Uenoyama:** None. **H. Tsukamura:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.10/B44

Topic: A.08. Development of Neural Systems

Support: Jane and Aatos Erkko Foundation
Magnus Ehrnrooth's F
Finska Läkaresällskapet
Finska Kulturfonden

Title: Developmental and behavioral characterization of zebrafish lacking histamine H1 receptor (hrh1)

Authors: Y. YAO[1], Y.-C. CHEN[2], D. BARONIO[3], C. JIN[3], ***P. PANULA**[3];
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Abstract: Three histamine receptors are expressed in vertebrate brain neurons and participate in behavioral control. They are also involved in different brain disorders. Histamine receptor 1 (HRH1) is involved in development of neurotransmitter systems and neurogenesis in addition to being the main postsynaptic histamine receptor in the brain. Abnormal HRH1 binding or expression is evident in patients with schizophrenia, depression, and autism. We assessed the role of hrh1 in zebrafish neurotransmitter system regulation and behavior by generating a zebrafish *hrh1*$^{-/-}$ line with the CRISPR/Cas9 system. The *hrh1*$^{-/-}$ zebrafish lacks a 11 bp fragment of the coding region. Quantitative PCR, *in situ* hybridization, and immunocytochemistry were used for the neurotransmitter systems and genes involved in brain development. Quantitative behavioral methods including locomotion, thigmotaxis, dark flash and startle response, novel tank diving and shoaling test were used for larval and adult fish. *Hrh1*$^{-/-}$ larvae and their *hrh1*$^{+/+}$ siblings displayed normal behavior. Transient abnormal expression of several neurodevelopmental markers was evident in *hrh1*$^{-/-}$ larvae, and reduction in the number of tyrosine hydroxylase 1 (Th1)-positive cells in the posterior recess and *hypocretin* (*hcrt*)-positive cells in hypothalamus. *th1* mRNA expression was also reduced in *hrh1*$^{-/-}$ larvae. These abnormalities were no longer found in adulthood. Abnormal shoaling behavior and novel tank diving behavior, low expression of *choline O-acetyltransferase a* and *LIM homeodomain transcription factor Islet1*, were found in adult *hrh1*$^{-/-}$ fish. The results show deficits in the dopaminergic and hypocretin systems during early development, and behavioral abnormalities in adult *hrh1*$^{-/-}$ zebrafish.

Disclosures: Y. Yao: None. **Y. Chen:** None. **D. Baronio:** None. **C. Jin:** None. **P. Panula:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.11/B45

Topic: A.08. Development of Neural Systems

Support: 5R01MH111918

Title: Phenotype-specific differences in neuronal activation across multiple brain regions following chronic social defeat stress in male and female mice

Authors: *G. ATWOOD[1,2], K. ANDERSON[2], S. JAGNARINE[2], A. LIPSHUTZ[2], P. ROGU[2], A. MANGANARO[2], D. DUMITRIU[2];
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Abstract: Stress is both ubiquitous and variable. Divergent responses to a particular stressor have been well established in rodent literature, with two behavioral phenotypes (susceptibility & resilience) reliably observed. The present research seeks to elucidate differential patterns of

neuronal activation between stress-susceptible and stress-resilient animals across two time points. A secondary goal is to explore sex differences in neuronal activation across both phenotype and time. We use chronic social defeat stress (CSDS), a well-validated model of divergent stress responses within a homogenous (in-bred) population of male and female mice. In this paradigm, aggressor male CD1 urine is applied to both male and female mice. The pair is then exposed to an aggressor for 10 minutes followed by co-housing with an aggressor. This process repeats for 10 days total and is followed by a social interaction (SI) test to determine susceptible (avoidant) and resilient (social preference) phenotypes. To capture and analyze activation patterns at two distinct timepoints, we used the TRAP2 mouse model to tag activated neurons within a specific temporal window alongside immediate early gene staining. This combination of techniques allows us to examine two connectomes in the same individual. The result is an ability to compare stress-induced activation patterns following an initial stressor and after 10 days of chronic stress. Preliminary data from 6 mice (1 male + 1 female from each behavioral group - control, susceptible, resilient) and 2 brain regions with known involvement in stress memory (amygdala and dentate gyrus (DG)) display region- and time-specific differences in neuronal activation. While preliminary analysis found phenotype-specific differences in neuronal activation patterns, this subset was not big enough to consider sex differences. To date, we expand this preliminary analysis using an unbiased brain-wide approach to continue to elucidate sex- and phenotype-specific differences in neuronal activation across time points in response to social stress.

Disclosures: G. Atwood: None. **K. Anderson:** None. **S. Jagnarine:** None. **A. Lipshutz:** None. **P. Rogu:** None. **A. Manganaro:** None. **D. Dumitriu:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.12/B46

Topic: A.08. Development of Neural Systems

Title: Gestational and Post Gestational Malnutrition alters the Redox Balance in the Heart of Adult Male Rats

Authors: B. BARRALES FUENTES, Jr[1], S. DAVILA SANTACRUZ, Jr[1], A. SANCHEZ RIVAS, Jr[1], L. NICOLAS TOLEDO[2], M. MARTINEZ GOMEZ[1], F. CASTELAN[1], *J. RODRIGUEZ[3];
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Abstract: Introduction: Developmental programming is a process determined by several factors including maternal diet generating both physiological and biochemical changes in the offspring during adult life, according to the origins of health and disease hypothesis or Barker's theory.

One of the main organs affected by nutrient deprivation during gestation is the heart due to its rapid development. The main objective of this study was to determine the structural and biochemical alterations of the heart in a model of gestational and post-gestational malnutrition. Hypothesis:The sugar consumption during gestation, post gestation and growth induce changes in cardiac morphology and biochemistry Methods: In a malnutrition model, 5% sucrose water was administered during gestation and post-gestation in the offspring of adult male rats. Wistar female rats were used to obtain male offspring, for this purpose they were divided into two experimental groups during gestation and lactation, one group that consumed plain water (CM) and another group that was administered 5% sucrose water (SM), once the lactation period was over, weaning took place and two groups were made from each group of mothers: from the mothers who consumed plain water, one group continued to consume plain water until the end of treatment (CM-CO) and another group consumed 5% sucrose water until the end of treatment (CM-SO), from the group of mothers who consumed sucrose, two groups were formed, one group consumed plain water at weaning (SM-CO) and another group continued to consume 5% sucrose water until the end of treatment (SM-SO), the treatment lasted 14 weeks. For the determination of cardiac histological characteristics, hematoxylin and eosin staining was used to determine change in morphology and biochemical tests for oxidative stress such as super oxide dismutase (SOD), catalase, thiobarbituric acid reactive substances (TBARS). Results: No significant changes were found in cardiac morphology and morphometry; however, in the oxidative stress tests, SOD was found to be decreased in the SM-CO condition. Conclusions: Factors that make up the nutritional status of the mother such as maternal diet can cause physiological or biochemical changes in the offspring during adult life. Exposure to sugar during pregnancy, lactation and growth decreases the protection against oxidative stress of super oxide dismutase in adult male rats and could originated pathologies like cardiomyopathies.

Disclosures: **B. Barrales fuentes:** None. **S. Davila santacruz:** None. **A. Sanchez rivas:** None. **L. Nicolas toledo:** None. **M. Martinez gomez:** None. **F. Castelan:** None. **J. Rodriguez:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.13/B47

Topic: A.08. Development of Neural Systems

Support: 15H05724
22H02941

Title: Developmental changes and gonadal steroid hormones regulation of the expression of two types of estrogen receptors in mice

Authors: *L. CAMPISTA LANA, **M. NAKATA**, S. OGAWA;
Comprehensive Human Sci., Univ. of Tsukuba, Tsukuba city, Japan

Abstract: Estrogen receptors (ERα and ERβ) are crucial for the development of sexually dimorphic neural networks. However, how their distribution patterns change in the developing brain, and how gonadal hormones influence their expression is largely unclear. We focused on distribution patterns of ERα, ERβ and their colocalization (ERα/β) throughout development and under different hormonal conditions during neonatal and prepubertal periods. Clarifying the changes of ERs distribution patterns during development reveals how gonadal hormones organize the developing brain. Using transgenic ERβ-RFP^{tg} mice, we analyzed sexually dimorphic brain areas that regulate socio-sexual behaviors, such as ventromedial hypothalamus (VMH), anteroventral periventricular nucleus (AVPV), and bed nucleus of stria terminalis (BNST). We mapped the distribution of ERα, ERβ, and ERα/β during neonatal (postnatal days [PD] 0, 7), prepubertal (PD14, 21) pubertal (PD28, 35, 42), and young adult periods (PD56). Since it's not clear whether gonadal hormones could also be up- or down-regulating the expression of ERs, we first analyzed the effects of neonatal (PD2 to PD6) estradiol-benzoate (EB, 0.02ml) treatment on ERs expression at PD14 in both sexes. We also examined the effect of prepubertal (PD25) gonadectomy (GDX) on ERs expression at PD42 and PD56 in both sexes. Our data showed that, in the VMH, ERβ and ERα/β expression was highest, with clear sex differences, during the neonatal period but decreased drastically after PD14 in both sexes. In the AVPV, ERα expression decreased from PD0 to PD14 but increased again with age in females only. In the BNSTp, females showed an increase of ERα whereas males showed an increase of ERβ with age from PD14 to PD56. ERα expression tended to increase in the VMH by neonatal EB treatment only in females. Prepubertal GDX had small effects on of ERα expression. However, ERβ expression in the VMH at PD14 was increased by neonatal EB treatment in both sexes. Prepubertal GDX also affected the number of ERβ expressing neurons in all three brain areas in both sexes, particularly at PD42, abolishing sex differences observed in intact mice. These data showed that ERs expression changed in sex- and region-specific manners in the developing brain, and also suggest that neurons expressing one type of ER might start or stop expressing the other during development. Moreover, neonatal and pubertal manipulation of gonadal hormones revealed possible underlying mechanisms of sexually dimorphic expression of ERs. Our data established the basis for future studies using brain-site manipulations of ERs to fully comprehend their specific functions.
Support: 15H05724 and 22H02941 to SO.

Disclosures: **L. Campista Lana:** None. **M. Nakata:** None. **S. Ogawa:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.14/B48

Topic: A.01. Neurogenesis and Gliogenesis

Support: Reed College RCSRF

Title: Identifying retinoic acid regulated neurogenic genes with computational and molecular approaches

Authors: S. XU[1], Y. ZHUANG[1], R. SACKSTEDER[1], *K. CERVENY[2];
[1]Reed Col., Portland, OR; [2]Reed Col., PORTLAND, OR

Abstract: The complex process of neuron generation during development or repair is driven by a combination of intrinsic and extrinsic signals. One such extrinsic signal, Retinoic acid (RA), can stimulate neuron formation by regulating the transcription of target genes upon binding with its intracellular receptors - the retinoic acid receptors (RARs). These receptors, together with their retinoid X receptors (RXR) co-receptors, engage with specific DNA sequences known as retinoic acid response elements (RAREs). RAREs are relatively conserved across numerous vertebrate species, comprising two direct 6-mer repeats separated by a 5-mer gap, and are frequently located in the upstream and noncoding regions of genes. Despite this, our understanding of RAREs and the full range of RA-regulated genes is still incomplete. Therefore, the identification of RAREs throughout the genome may reveal novel RA target genes and enrich our understanding of the RA regulatory network. In this study, we used an enhanced version of our recently developed motif-finding tool, RAREdar, to identify potential genes regulated by RA within the D. rerio genome. RAREdar is coded in Python and leverages a consensus motif of RARE and accounts for multiple flexible locations. Applying RAREdar to a sample set of genes expressed in the developing zebrafish eye between 1-5 dpf, successfully recognized known RA-target genes and a small collection of genes never before reported as RA-regulated. Based on multiple RARE occurrences, we selected a few genes as our primary interests. To validate these as RA-regulated, we are planning a series of gene expression assays to identify their status as immediate or secondary targets of RA. Our preliminary findings offer novel insights into the potential role of RA in retinal neurogenesis and set a solid groundwork for future exploration of an RA-regulated gene regulatory network.

Disclosures: S. Xu: None. **Y. Zhuang:** None. **R. Sacksteder:** None. **K. Cerveny:** None.

Poster

PSTR316. Development of Neural Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR316.15/B49

Topic: A.08. Development of Neural Systems

Title: Bidirectional Cognitive Interplay between Earth's Geo-Magnetic Field, Neurobiological Evolution and Behavioral Neurology

Authors: *D. ROSENFIELD;
Houston Methodist Hosp., Houston, TX

Abstract: Bidirectional Cognitive Interplay between Earth’s Geo-Magnetic Field, Neurobiological Evolution and Behavioral Neurology

David B. Rosenfield, M.D.
Robust data suggest that after our Solar System formed from an initially hot protoplanetary disk, the disk cooled and refractory (high condensation) temperature metals and metal oxides condensed into solid dust grains, followed by Fe metal and Mg silicates (K. Lodders, Astrophys, J., 591, 1220-1247, 2003).Subsequent activity formed the Earth's molten core with salient electrical conductions, producing the Geo-Magnetic Field (GMF), which shields all life forms from harmful radiation, especially solar wind and cosmic rays (Gauss, 1871).
The GMF affects evolution of all life forms, ranging from neuronal axonal flow to cellular and multi-organ systems, influencing the ability of plants to perceive/respond quickly by altering gene expression and phenotype, and magnetotactic bacteria's ability to orient/migrate along GMF lines. Further, there are substantial avian magnetic effects upon behavior as well as magnetic alignment that affects insects, amphibians, fish and mammals. Small crystals of human biogenic magnetite also affect the organism.
Hominin brains can detect geomagnetic fields, but it is not definite that this affects function (Maffei 2014; Erdman et al 2021; Wang et al, 2019; Yan et al 2012; Ritz et al 2009; Begall et al 2013; Kobayashi and Kirschvink, 1995).
What is clear is that the GMF is a major factor impacting the creation of life as well as the development and sustainability of all life forms. Since electrical currents bidirectionally relate to magnetism, possibly extending to the bidirectionality of DNA to output and back to DNA, this strongly overlaps and coalesces with all neurobehavioral activity. One can posit that hominins appeared as an image from this force and, given the bidirectionality, the power of human cerebration (e.g., thought processes, prayer) may also be geomagnetically relevant.

Disclosures: D. Rosenfield: None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.01/B50

Topic: A.10. Development and Evolution

Title: Causal relationships between Brain areas across Primate Evolution

Authors: *J. OGAWA[1], H. YE[3], S. SRINIVASAN[4], C. F. STEVENS[4], S. GEORGE[5], G. M. PAO[2];
[2]Biol. Nonlinear Dynamics Data Sci. Unit, [1]Okinawa Inst. of Sci. and Technol., Okinawa, Japan; [3]Univ. of Florida, Hlth. Sci. Ctr. Libraries, Gainesville, FL; [4]UCSD and KIBM, UCSD, La Jolla, CA; [5]Scripps Inst. of Oceanography, La Jolla, CA

Abstract: Across evolutionary time scales, different brain regions undergo species-specific adaptations that lead to selective enlargement and shrinkage depending on the ecological adaptive responses that suit the organism's niche. The relative enlargement and shrinkage of anatomically distinct brain region sizes could reveal relationships that reveal fundamental

architectural principles of building brains. Results using a variety of brain measurements across evolutionary time series ordered by diverging branching order across evolutionary time series show a high degree of co-variation of certain brain structures that are known to be functionally connected such as the piriform cortex and the olfactory bulb. Scaling of brain structures across evolutionary time series are not limited to linear relationships between structures as analysis of areas such as the hippocampus appear to show highly non-random patterns. Applying methods of Non-linear analysis of their variation across evolutionary time series using various embedding techniques reveal patterns of variation that are highly non-linear and appear that brain structures are in many cases dissipative systems in which a small number of factors determine the size of the particular structure. These non-linear signatures could be considered evidence for the existence of feed forward and feedback relationships across various brain structures in mammalian brain evolution.

Disclosures: **J. Ogawa:** None. **H. Ye:** None. **S. Srinivasan:** None. **C.F. Stevens:** None. **S. George:** None. **G.M. Pao:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.02/B51

Topic: A.10. Development and Evolution

Support: ERC Grant 818521

Title: Evolution of white matter lateralization in primates

Authors: *T. ORSET[1], J. ROYO[2], P. POUGET[2], M. THIEBAUT DE SCHOTTEN[3];
[1]ICM, Paris, France; [2]Inst. du Cerveau et de la Moelle Epinière (ICM), Paris, France; [3]BCBlab, Frontlab, Inst. Du Cerveau Et La Moelle Épinièr, Paris, France

Abstract: Understanding the organization of the brain through its origins is essential and comparative neuroscience offers a fundamental approach to achieve this. By studying conserved properties and species-specific phenotypes, we can learn about the evolutionary process behind them. Brain lateralization, which determines hemispheric functional dominance, is a crucial aspect of brain anatomy. To better comprehend functions and pathologies, exploring the origins of asymmetries throughout the animal kingdom is useful. Magnetic resonance imaging (MRI) is a non-invasive technique that allows for multi-species comparison of various brain characteristics. We utilized diffusion MRI data and fractional anisotropy (FA) maps to evaluate the properties of fibers in the white matter. By performing Tract-Based Spatial Statistics (TBSS) on symmetrized FA, we explored white matter asymmetries in several species, including humans, chimpanzees, macaques, and squirrel monkeys. Our findings show that while some conserved asymmetries date back more than 40 million years, other lateralization emerged later in primate evolution. These results are discussed with regard to the emergence of language and

emotions across different species. Additionally, we took a closer look at fronto-parietal connections through the three branches of the superior longitudinal fasciculus. These results provide new insights into the emergence and evolution of white matter lateralization.

Disclosures: **T. Orset:** None. **J. Royo:** None. **P. Pouget:** None. **M. Thiebaut De Schotten:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.03/B52

Topic: A.10. Development and Evolution

Title: Insights into the evolution of beat perception through comparative diffusion tractography

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Abstract: Humans show exceptional abilities among primates for auditory beat perception and synchronization (Patel et al., 2014). For example, when synchronizing taps with a metronome humans spontaneously tap predictively with high precision while macaques spontaneously tap reactively and require reward-based training to tap predictively (Gamez et al., 2018). Human beat synchronization abilities may have an evolutionary foundation in the auditory-motor circuits supporting advanced vocal learning, a critical ability for human spoken language development, which relies on precise audiomotor integration (Patel, 2021). While few studies have been carried out on beat synchronization in our closest primate relatives (chimpanzees), existing research shows chimpanzees can synchronize to a metronome predictively but with much less precision than humans and without tempo flexibility (Hattori et al., 2013). Neuroimaging work with humans indicates that a dorsal auditory stream pathway linking auditory and premotor cortical regions via the inferior parietal lobe is involved in beat processing, with a node in the vicinity of angular gyrus (AG). The white matter networks responsible for vocal learning in humans also include dorsal stream pathways that course through the inferior parietal lobe (Gierhan, 2013). Here, we used diffusion tractography in humans and chimpanzees to compare the organization of these white matter pathways in relation to the angular gyrus (AG). Based on previous work (Sierpowska, Bryant, et al., 2022), we hypothesized that the posterior segment of the arcuate fasciculus would be expanded to create a more interconnected network between AG and posterior temporal lobe as compared to chimpanzees. Further, we hypothesized that modifications to tracts reaching prefrontal areas from AG would also be observable. We ran tractography for three subsegments of the arcuate and the superior longitudinal fasciculus II on a sample of 32 human and 66 chimpanzee DWI scans using probtrackx (FSL 6.0.1; Jenkinson et al., 2012) and analyzed them based on the relative proportion of contribution to connectivity to

AG over all tractogram volumes for both hemispheres. Our preliminary results indicate that, compared with chimpanzees, humans show increases in the proportion of the fronto-parietal , fronto-temporal, and temporo-parietal segments of the arcuate to AG. Overall, these data provide more insight into the organization of AG connectivity relative to the beat processing network in an evolutionary context.

Disclosures: K.L. Bryant: None. **J. Sierpowska:** None. **A.D. Patel:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.04/B53

Topic: A.10. Development and Evolution

Support: NIH R01AG067419
NIH 3U42OD011197-19S1
NSF BCS-1846201

Title: Comparing neuron and glia densities in the cerebral cortex and hippocampus of humans and chimpanzees

Authors: S. R. DUNCAN[1], E. L. MUNGER[1], W. HOPKINS[3], P. R. HOF[4], C. SHERWOOD[5], *M. RAGHANTI[2], M. K. EDLER[1];
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Abstract: Comparative primate studies are critical for understanding the neuroanatomical correlates of human-specific cognition, aging, and neurodegenerative disease processes. In the present study, we aimed to gain insight into how two fundamental cell types, neurons and glia, respond to aging in humans and one of their closest living relatives, chimpanzees. The dorsolateral prefrontal cortex (DLPFC), middle temporal gyrus (MTG), entorhinal cortex (EC), and hippocampus are regions vulnerable to age-related changes and are involved in higher-order cognitive processes that include task switching and configuration, inhibition, language, sensory integration, and memory formation and retrieval. We used unbiased stereology to quantify neuron density (Nv) in the DLPFC (layer III), MTG (layer III), EC (layer II), and CA1 and CA3 (pyramidal layer) in humans (n = 14, 2-88 y) and chimpanzees (n = 46, 12-62 y) across the lifespan. In addition, glia densities (Gv) and glia to neuron (G:N) ratios were collected in the DLPFC, MTG, and EC. Chimpanzees possessed higher Nv in the DLPFC and MTG, while humans exhibited higher Nv in the CA1 and CA3 (p values ≤ 0.01). EC Gv was greater in chimpanzees compared to humans ($p \leq 0.01$). Regional variation in Nv was noted with chimpanzees exhibiting lower DLPFC and MTG Nv compared to the EC and hippocampus (p values ≤ 0.03). In humans, Nv was significantly higher in the CA3 than the EC and CA1 ($p \leq$

0.01). No sex differences were detected in either species. These data differ from an earlier study with a smaller cohort of chimpanzees (n = 28, 12-62 y), in which we identified mild age-related Nv decreases in the chimpanzee hippocampus. This discrepancy is likely due to the inclusion of several younger animals in the current report. Conversely, these data support a previous analysis that found the human hippocampus had higher Nv compared to chimpanzees and aligns with aging studies in humans in which no changes with age were identified in the CA1 or CA3. These preliminary findings may hint at potential underlying evolutionary mechanisms responsible for differences in memory and sensory processing in humans and chimpanzees. Greater hippocampal Nv could be partially responsible for increased plasticity in humans that allows for enhanced cognitive processing capabilities, yet, in turn, may contribute to a unique species-specific susceptibility to cognitive aging and neurodegenerative processes.

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Poster

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Program #/Poster #: PSTR317.05/B54

Topic: A.10. Development and Evolution

Support: NSF Grant IOS-2038528

Title: Artificial selection on wheel-running behavior alters sizes of brain regions

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Abstract: How might selection on a behavior alter brain structure? We used a unique and ongoing artificial selection model in which mice are bred for high voluntary wheel-running behavior, yielding four replicate lines of High Runner (HR) mice that run ~3-fold more revolutions per day than four replicate non-selected Control (C) lines. Previous studies reported that, with body mass as a covariate, HR mice had heavier whole brains, non-cerebellar brains, and larger midbrains than C mice. We sampled 100 female mice from generation 66 and used high-resolution microscopy to test the hypothesis that HR mice have greater volumes and/or cell densities in nine key regions from either the midbrain or limbic system. In addition, half of the mice were given 10 weeks of wheel access from weaning, and we predicted that chronic exercise would increase the volumes of the examined brain regions via phenotypic plasticity. We replicated findings that both selective breeding and wheel access increased total brain mass, with no significant interaction between the two factors. In HR compared to C mice, adjusting for body mass, both the red nucleus (RN) of the midbrain and the hippocampus (HPC) were significantly larger, and the whole midbrain (WM) tended to be larger, with no effect of wheel access nor any

interactions. Linetype and wheel access had an interactive effect on the volume of the periaqueductal gray (PAG), such that wheel access increased PAG volume in C mice but decreased volume in HR mice. Neither linetype nor wheel access affected volumes of the substantia nigra (SN), ventral tegmental area (VTA), nucleus accumbens (NAc), ventral pallidum (VP) or basolateral amygdala (BLA). We found no main effect of either linetype or wheel access on neuronal densities (numbers of cells per unit area) for any of the regions examined. Taken together, our results suggest that the increased exercise phenotype of HR mice is related to increased red nucleus and hippocampal volumes, but that chronic exercise alone does not produce such phenotypes.

Disclosures: Z. Thompson: None. **M. Schmill:** None. **T. Garland:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

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Program #/Poster #: PSTR317.06/B55

Topic: A.10. Development and Evolution

Support: FRQS
NSERC Discovery grant RGPIN-2018-05203

Title: A comparative neuroanatomical investigation of astrocytes and Purkinje cells in the cerebellum

Authors: *C. HERCHER[1,2], K. ELLERBECK[2], L. TOUTÉE[2], R. MPAI[1,2], X. YE[2], M. DAVOLI[2], N. MECHAWAR[1,2,3];
[1]McGill, Montreal, QC, Canada; [2]McGill Group for Suicide Studies, Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada; [3]Dept. of Psychiatry, McGill Univ., Montreal, QC, Canada

Abstract: Little is known about the morphological diversity and distribution of cerebellar astrocytes in humans, and how these features may differ from those of cerebellar astrocytes in species used to model human illnesses. In this study, we performed a comparative postmortem examination of cerebellar astrocytes and Purkinje cells (PCs) in healthy humans, macaques, and mice. Our sample included 2 male and 2 female human cerebella, obtained from the Douglas-Bell Canada Brain Bank, 2 male transgenic mice (ALDH1L1-Cre/ERT2; Rosa26-TdTomato), and 2 cynomolgus macaque cerebella. We used immunofluorescence to label canonical astrocyte markers glial fibrillary acidic protein (GFAP) and aldehyde Dehydrogenase-1 Family member L1 (ALDH1L1). At 3 anatomical positions, lateral, dentate nucleus, and vermis, the percent area coverages for GFAP immunoreactive (-IR) and ALDH1L1-IR astrocytes were assessed in the molecular layer (ML), Purkinje cell layer (PCL), granule cell layer (GCL), and white matter in lobules I-X. Stereological counts were performed in 3 functionally distinct regions, lobule III (motor), crus I (cognitive), and vermis VIIA folium (emotion). PCs were also quantified due to their intimate relationship with the specialized radial astrocyte, Bergmann glia. Overall, we

observed increased % area coverages of ALDH1L1-IR and GFAP-IR astrocytes with species progression. ALDH1L1-IR astrocytes were highly expressed in the PCL. GFAP-IR processes in the ML displayed increased complexity with species progression and presented varicosity-like protrusions in humans. In mice, GFAP-IR astrocytes were scarce in deep cerebellar nuclei. Stereological estimates revealed that in humans, the vermis lobule had the lowest astrocyte and PC densities. Species comparisons revealed modest increases in ALDH1L1-IR astrocyte densities in the PCL and GCL in humans. We observed an opposing trend for ALDH1L1-IR and GFAP-IR astrocytes where ALDH1L1-IR astrocytes increased with species evolution while GFAP-IR astrocytes decreased. However, GFAP-IR astrocyte defined territories increased with species progression. PC analyses revealed that while humans had the lowest PC densities, their cell body sizes were the largest with more ALDH1L1-IR astrocytes surrounding. Notably, crus I displayed the highest ratio of Bergmann glia to PC. This original study comprehensively characterized cerebellar astrocytes and PC across species, highlighting features unique to humans. These results indicate cerebellar astrocyte and PC divergence within and across species, possibly indicative of a role for these cells in cognitive and affective cerebellar processing.

Disclosures: C. hercher: None. **K. Ellerbeck:** None. **L. Toutée:** None. **R. Mpai:** None. **X. Ye:** None. **M. Davoli:** None. **N. Mechawar:** None.

Poster

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Topic: A.10. Development and Evolution

Support: Chair in Neuroscience UAM-Fundación Tatiana

Title: Noradrenaline innervation in the human, macaque and rat thalamus: Similarities and differences

Authors: *I. PÉREZ-SANTOS[1], L. DALL'ACQUA[2], E. RUBINO[2], M. OLIVA-MARTÍN[1], C. CAVADA[1];
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Abstract: The primate and rodent thalamus hold important differences: GABAergic interneurons are considerably more extensive in primates than in rodents, and neuromodulatory afferents, like dopamine, have quite distinct innervation patterns in the primate and rodent thalamus. In this work, we aimed at identifying similarities and differences between the noradrenaline (NA) innervation of the primate thalamus (human and macaque monkey) and rat thalamus. We used immunohistochemistry against the NA synthetizing enzyme dopamine-β-hydroxylase (DβH), and against the noradrenaline transporter (NET) to reveal NA axons. Maps of the thalamic distributions of NA axons were obtained using Neurolucida and Ilastik software.
NA axons were heterogeneously distributed in both the primate and rat thalamus, with some

nuclei showing dense innervation in the three studied species: the midline nuclei (e.g., paraventricular) and some intralaminar nuclei (parafascicular and paracentral).
Relevant interspecies differences were found in the lateral geniculate and in the reticular nuclei: dense innervation was present in rats, while sparser innervation was observed in macaques and humans. Also, NA innervation in some anterior nuclei and ventral motor nuclei was denser in rats than in primates. Conversely, the higher order mediodorsal nucleus displayed in macaques a medio-lateral gradient of NA innervation, with denser innervation medially, that was relatively preserved in the human thalamus but not obvious in the rat thalamus.
The pulvinar nuclei, fully developed in primates, were heterogeneously innervated in macaques and humans. Heterogeneity was also present in the posterior and lateral posterior nuclei of the rat, which are rodent higher order thalamic nuclei with functions and connections comparable to some of the primate pulvinar complex. Nonetheless, comparisons between the primate pulvinar and the rodent posterior and lateral posterior nuclei are difficult because of interspecies differences in thalamic organization.
In summary, the thalamus of primates and rats receives considerable NA innervation. Both similarities and differences are present. Differences are present mostly in the lateral geniculate, reticular, anterior, ventral anterior and mediodorsal nuclei. These differences point to a different role of thalamic NA in specific functions, including visual transmission, intrathalamic modulation, as well as limbic, motor, and cognitive mechanisms.
Supported by the Chair in Neuroscience UAM-Fundación Tatiana.

Disclosures: I. Pérez-Santos: None. **L. Dall'Acqua:** None. **E. Rubino:** None. **M. Oliva-Martín:** None. **C. Cavada:** None.

Poster

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Topic: A.10. Development and Evolution

Support: JSPS KAKENHI Grant Number 20K15597, 23K14022

Title: Specialization of compartments of the Dl, a visual telencephalic part, in gobies during evolution and the visual feedback system though the higher order visual center and cerebellum in teleost.

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Abstract: Most vertebrates possess two ascending visual pathways to the telencephalon (cerebral cortex). In mammals, one of the pathways is called the geniculate system, in which retinal information is sent to the striate cortex by the lateral geniculate nucleus in the

diencephalon. The other is called the extrageniculate system, in which retinal inputs reach the extrastriate cortex via the superior colliculus in the mesencephalon and then the lateral posterior nucleus-pulvinar complex in the diencephalon. In actinopterygians, cypriniform fishes (such as the goldfish and the carp) possess two visual pathways, while holocentriform fishes (such as the squirrelfish) and the yellowfin goby (Gobiiform is a taxon that emerged later than holocentriform) have only an extrageniculate-like pathway. We found that the retinal inputs reach mainly the lateral part of the dorsal telencephalon (Dl) via the optic tectum and then the nucleus prethalamicus (PTh) in the diencephalon, and four compartments of Dl are highly specialized in the goby. Therefore, we investigated the phylogenetic distribution of compartments of Dl in acanthopterygian fishes including holocentriform fishes and gobies, which probably possess only one visual pathway. We found that the Dl shows considerable species differences in the number of compartments and cytoarchitecture. It should be noted that four compartments of Dl occur only in gobies, while there are fewer specialized compartments in some other percomorph fishes. We revealed that the compartments of Dl in gobies are specialized than those in other acanthopterygian fishes. We also found descending pathways from forebrain visual centers to the cerebellum and other lower brain centers by tract-tracing methods. Our data shows that the Dl that receives inputs from the PTh projects to the central part of dorsal telencephalon (Dc), a higher order visual center, and visual information processed in the Dc reaches the PTh via optic tectum. We observed that the Dc also projects to the nucleus paracommissuralis (NPC). Furthermore, the cerebellum receives NPC inputs and projects to the PTh. Thus, these pathways starting from the PTh may eventually come back to the nucleus through feedback loops: PTh-Dl-Dc-tectum-PTh and PTh-Dl-Dc-NPC-cerebellum-PTh pathways. These loops may serve to coordinate oculomotor control and visual sensory processing systems.

Disclosures: H. Hagio: None. **N. Yamamoto:** None.

Poster

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Topic: A.10. Development and Evolution

Support: NSF BCS 1846201

Title: Dopaminergic innervation of the amygdala in human and other primates as it relates to affiliative behaviors

Authors: *H. G. MAYCON[1], D. N. JONES[1], H. N. SMITH[1], C. C. SHERWOOD[2], P. R. HOF[3], M. RAGHANTI[1];
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Abstract: Dopaminergic projections to the amygdala (AMG) contribute to the regulation of social behaviors. Previous studies in nonhuman primates have shown that a greater amount of DA in the AMG may be associated with increased affiliative behaviors. The current quantitative comparative analysis of dopaminergic innervation measured tyrosine hydroxylase-immunoreactive (TH-ir) axons in the AMG of human and other primates to assess species differences. Humans were predicted to have the greatest TH-ir axon density to support their highly social and cooperative behaviors. We further expected that more affiliative and/or monogamous nonhuman primate species would have greater TH-ir axon density relative to their less affiliative and/or non-monogamous relatives. Species included humans (2 F, 3 M; 33 - 53 years old), chimpanzees (3 F, 3 M; 24 - 36 years old), bonobos (1 F, 2 M; 5 - 52 years old), baboons (3 F, 3 M; 5 - 13 years old), Japanese macaques (3 F, 3 M; 9 - 19 years old), pigtailed macaques (3 F, 3M; 2 - 15 years old), rhesus macaques (3 F, 3 M; 8 - 14 years old), tufted capuchins (3 F, 3 M; 3 - 17 years old), common marmosets (3 F, 3 M; 4 - 6 years old), cotton-top tamarins (1 F, 1 M; 9 - 16 years old), and owl monkeys (2 F, 1 M; 3 - 18 years old). We used stereologic methods to quantify total neuron densities (Nv; Nissl) and TH-ir axon length density (ALv) in the basal, central, lateral, and accessory basal AMG (BA; CA; LA; AB). The variable used for species comparisons was ALv/Nv to account for species differences in brain size. We found no sex differences within species. ALv/Nv was analyzed using a mixed model ANOVA; AMG nucleus was the repeated measure and species was the between-subjects measure. Results showed a significant interaction ($F(14.062,61.871) = 3.542$, $p < 0.05$) and significant main effect of AMG nucleus ($F(1.406,61.871) = 33.262$, $p < 0.05$) and species ($F(10,44) = 4.086$, $p < 0.05$). Bonferroni post hoc testing revealed humans had the greatest amount of TH-ir axon density in the BA relative to all species (all p values < 0.05) except pigtailed macaque, tamarin, and owl monkey, and in the CA (all p values < 0.05). The BA regulates social learning, and the CA regulates behavioral, emotional, and physiological responses to social situations. These data support the hypothesis that increased DA within specific regions of the AMG are associated with human-specific behaviors, including increased sociality and cooperation. The increased expression in the (semi-) monogamous nonhuman primates (owl monkey and tamarin) is intriguing, but the increase in the pigtailed macaque and lack of increase in the (semi-) monogamous marmoset suggest more nuanced roles for DA within the CA that require further exploration.

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Poster

PSTR317. Comparative Anatomy and Physiology

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Program #/Poster #: PSTR317.10/B59

Topic: A.10. Development and Evolution

Support: FONDECYT 1210069

Title: Early development and late maintenance of the avian tecto-visual forebrain: retinal input is not necessary

Authors: M.-J. ROJAS[1], R. REYES-PINTO[1,2], E. SENTIS[1], *J. MPODOZIS[1];
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Abstract: The tectofugal pathway, a highly conserved retino-midbrain-fugal visual system, is the main visual pathway in birds and many mammals. In birds the tectofugal projection arises from the tectal ganglion cells (TGCs), which receive synaptic contacts from retinal fibers and from axon terminals of the nucleus isthmi parvocellularis (Ipc). The Ipc exerts a strong control on the propagation of visual activity from the TGCs to higher visual areas. TGCs project to the thalamic nucleus rotundus (Rt), which in turn project to the visual DVR, (vDVR), a three-layered, columnarly organized visual region of the lateral pallium. Previous works from our laboratory have shown that the establishment of the Rt-vDVR connectivity as well as the establishment of the intrinsic vDVR columnar circuitry are both independent of early retinal inputs. However, it is unclear whether the retinal inputs are relevant for maintaining the organization of this system at late embryonic and post-hatching stages. In this work we assess such question by performing "*in ovo*" mono and bienucleations in chick embryos of 14 days of incubation, to subsequently evaluate its effects on the neuroarchitecture and neurochemical features of the main structures of tectofugal pathway in perinatal and post hatching stages. We found that enucleations had a dramatic effect in the optic tectum, where the retinorecipient layers lose precise stratification and thickness. These changes were accompanied by an acute reduction in the characteristic expression pattern of calcium-binding protein (calbindin) in tectal layers 5b and 9-10, and by a disrupted expression pattern of GAD67 in all layers. In addition, our neurotracing experiments showed that enucleation strongly affected the distinctive "paintbrush" morphology of the Ipc axons but did not affect the characteristic ChAT expression of Ipc somata. Despite these strong effects in the optic tectum, the expression pattern of calbindin in the Rt was not affected nor it was the topographical organization of the Rt projection to the vDVR. However, an overall reduction of Rt volume was readily appreciable in bilateral enucleations. Furthermore, our *ex-vivo* experiments and neurofilament protein immunoassay showed that the reciprocal, homotopic, columnar axonal connections between the vDVR layers was not affected. Combined with previous findings, these results indicate that the organization of the higher stages of the tectofugal pathway is not influenced, at early or late stages, by the retinal activity. In particular, retinal inputs seem to be not necessary neither for the initial establishment nor for the posterior maintenance of the overall organization of the vDVR.

Disclosures: M. Rojas: None. **R. Reyes-Pinto:** None. **E. Sentis:** None. **J. Mpodozis:** None.

Poster

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Topic: A.10. Development and Evolution

Support: NIEHS ES 100221
NIH MH062044
NIH MH122622
NIH AR160055

Title: Identification of Hippocampal area CA2 in Hamster and Vole Brain

Authors: *P. N. SIEGLER[1,2], E. K. SHAUGNESSY[3], B. HORMAN[4], H. B. PATISAUL[4], K. L. HUHMAN[3], G. M. ALEXANDER[1], S. M. DUDEK[1];
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Abstract: Prairie voles (*Microtus ochrogaster*) and Syrian, or golden, hamsters (*Mesocricetus auratus*) are closely related to mice (*Mus musculus*) and rats (*Rattus norvegicus*, for example) and are commonly used in studies of social behavior, including social interaction, social memory, and aggression. The CA2 region of the hippocampus is known to play a key role in social memory and aggression in mice and responds to social stimuli in rats, likely owing to its high expression of oxytocin and vasopressin 1b receptors. However, CA2 has yet to be identified and characterized in hamsters or voles. In this study, we sought to determine whether CA2 could be identified molecularly in vole and hamster. To do this, we used immunofluorescence with primary antibodies raised against known molecular markers of CA2 in mice and rats to stain hippocampal sections from voles and hamsters in parallel with those from mice. Here, we report that, like in mouse and rat, immunofluorescent staining for many CA2 proteins in vole and hamster hippocampus reveals a population of neurons that express Regulator of G Protein Signaling 14 (RGS14), Purkinjie Cell Protein 4 (PCP4) and Striatal-Enriched Protein Tyrosine (STEP), which delineate the borders with CA3 and CA1. These cells were located at the distal end of the mossy fiber projections, marked by the presence of Zinc Transporter 3 (ZnT-3) and calbindin in all three species. In addition to staining the mossy fibers, calbindin also labeled a layer of CA1 pyramidal cells in mouse and hamster but not in vole. However, Wolfram Syndrome 1 ER Transmembrane Glycoprotein (WFS1) immunofluorescence, which marks all CA1 neurons, was present in all three species and abutted the distal end of CA2, marked by RGS14 immunofluorescence. Interestingly, although perineuronal nets (PNNs) are known to surround CA2 cells in mouse and rat, we found that staining for PNNs differed across species, with mouse showing CA2 expression, voles showing CA2 and CA3 expression, and hamsters showing CA3, but only some proximal CA2, expression. These results demonstrate that, like in mouse, CA2 in voles and hamsters can be molecularly distinguished from neighboring CA1 and CA3 areas, but PNN expression is less useful for identifying CA2 in the latter two species. These findings reveal commonalities across species in molecular profile of CA2, which will facilitate future studies of CA2 in these species. Yet to be determined is how differences in PNN expression might relate to differences in social behavior across species.

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Poster

PSTR317. Comparative Anatomy and Physiology

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Program #/Poster #: PSTR317.12/B61

Topic: A.10. Development and Evolution

Support: NSF IOS 1457291

Title: Brain changes during dog domestication: A gray matter morphometry comparison of pre-modern/primitive and modern breeds

Authors: *S. BARTON, M. ABDULLA, E. HECHT;
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Abstract: Domestication is often misconstrued as a single event, rather than an ongoing evolutionary process. Domestic dogs are a prime example of this phenomenon. The first domestic dogs were likely self-domesticated, free-ranging scavengers of human waste. Importantly, the vast majority of dogs in the world today still inhabit this initial stage of domestication. Some lineages of dogs later split into loosely defined types, but still retained many wolf-like traits, such as monoestrous. However, within the past couple of hundred years, Western dogs have been subject to intense artificial selection, transforming them into isolated breeds with few ancestral traits. Extant “pre-modern” or “primitive” dogs in earlier stages of domestication are genetically, cognitively, and behaviorally distinct from “modern” breeds in later stages of domestication. Yet we know extremely little about how dog brains may have changed between early and late stages of domestication. To assess this question, we examined T1-weighted MRI images of 12 primitive breed/type dogs and 36 modern breed dogs. We used a whole brain, data driven voxel-based morphometry approach to investigate differences in regional brain volume between the two groups. Our findings are relevant for our understanding of neural changes during domestication, as well as to the more specific, open question of human self-domestication.

Disclosures: S. Barton: None. **M. Abdulla:** None. **E. Hecht:** None.

Poster

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Program #/Poster #: PSTR317.13/B62

Topic: A.10. Development and Evolution

Support: Z01ES090089

Title: Mesoscopic analysis of cholinergic neuron populations in the developing and mature mouse brain

Authors: *R. O. GORAL[1], S. ROY[4], R. N. WINE[2], P. W. LAMB[2], J. L. YAKEL[3];
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Abstract: Acetylcholine (ACh) neurons in the central nervous system (CNS) are required for the coordination of neural network activity during higher brain functions and locomotion. Disturbed ACh signaling has been described in many diseases of the developing brain. Furthermore, ACh neuron subpopulations which co-transmit GABA have been linked to essential brain functions and disease. Despite more than 80 years of research, cholinergic contributions to brain development, either through ACh release or other co-transmitted molecules, remain unclear. Thus, understanding the timeline of how the cholinergic system develops in the healthy brain is crucial to understanding brain development. To do so, we used several transgenic mouse models that label ACh neurons with fluorescent markers. We generated whole-brain reconstructions from serial sections at different developmental time points and found two crucial time windows during which most of the ACh neuron populations emerged in the developing brain. In a second set of experiments, we developed a workflow using tissue clearing and light-sheet fluorescence microscopy to image entire brain hemispheres of different animal models. Using intersectional genetics, we quantified ACh neurons as well as ACh neuron subpopulations. Our results suggest that many ACh neurons express or previously expressed GABAergic markers. Future studies will investigate whether these neuron populations are changed in disease or environmental toxicant models.

Disclosures: R.O. Goral: None. **S. Roy:** None. **R.N. Wine:** None. **P.W. Lamb:** None. **J.L. Yakel:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.14/B63

Topic: A.10. Development and Evolution

Support: NIH P20GM103446
NIH R21HD101964
NIH 5P20GM103653
NIH P30AG013280

Title: Translating Time across species: cats as a useful model system for aging

Authors: *T. M. LEE[1], R. MOHAMEDELHASSAN[1], B. A. RIGBY DAMES[2], M. BRYANT[1], J. WHITE[3,4], K. OFORI[5], D. R. MARTIN[1], A. A. DE SOUSA[6], C. J. CHARVET[1];
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Abstract: Alzheimer's disease (AD) is an age-related neurodegenerative disease characterized by brain plaques, tangles as well as dementia. Progress in characterizing AD and other age-related disorders is hindered by a dearth of animal systems that spontaneously develop AD-related pathologies found in humans. Cats are of particular interest because they possess brain plaques and tangles at late-stages of life. We harnessed our Translating Time dataset, which relies on corresponding time points to find equivalent ages across species. We used temporal variation in transcription, behavioral milestones, and disease progression (e.g., age of plaque onset, tangle onset) to find corresponding time points across cats, humans, and established model systems (e.g., mice). Our Translating Time dataset now consists of 922 time points collected across 25 mammals (9 primate species, 6 rodent species, and 2 carnivore species). One major finding from this work is that it is difficult to find corresponding ages for humans at late stages of life (60s-70s) compared to great apes. Interestingly, a quadratic regression across time points expressed as the log-transformed age in days after conception equates corresponding ages across the lifespans of cats and humans. Accordingly, cats in their early teens equate to humans in their 60s, and cats in their mid-teens equate to humans in their 70s (R2=0.97, df=47). The identification of corresponding ages across the lifespan in cats and humans demonstrates that cats are a well suited model system to study aging.

Disclosures: **T.M. Lee:** None. **R. Mohamedelhassan:** None. **B.A. Rigby Dames:** None. **M. Bryant:** None. **J. White:** None. **K. Ofori:** None. **D.R. Martin:** None. **A.A. de Sousa:** None. **C.J. Charvet:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.15/C1

Topic: A.10. Development and Evolution

Support: Scott Fund, Auburn University College of Veterinary Medicine
Animal Health and Disease Research, College of Veterinary Medicine, Auburn University

Title: Translating time within species: predicting cat age from blood-based biomarkers

Authors: R. GIBSON[1], J. SANOSSIAN[1], E. C. GRAFF[2], R. GRAHAM[3], J. SMITH[4], M. IAZBIK[4], ***C. CHARVET**[1];
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Abstract: Cats develop plaques and tangles at late stages of their lives, and may be a useful model system to understand the neurobiology of human aging and aging-related diseases. Client-owned cats live long lives and share similar environments with humans. Therefore, cats may be especially useful models to study aging, but their age is often unknown and this lacuna is an

obstacle to their use as a model system of aging. Here, we used chemistry profiles to predict cat age. Chemistry profiles were gathered retrospectively from male and female cats from a colony (n=106) and blood donor cats from Ohio State University (n=10). We tested different machine learning models (e.g., support vector regression, extreme gradient boosting, random forest, gaussian process regression) to assess which models most accurately predict chronological age in cats. We found that several models were well suited to predict chronological age in cats given we observed strong correlations (r>0.9) between predicted and actual ages of cats. Our findings and bioinformatics pipelines provide novel tools to learn about the history and biology of companion animals and other model systems.

Disclosures: R. Gibson: None. **J. Sanossian:** None. **E.C. Graff:** None. **R. Graham:** None. **J. Smith:** None. **M. Iazbik:** None. **C. Charvet:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

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Topic: A.10. Development and Evolution

Support: NIH P20GM103446
NIH R21HD101964
NIH 5P20GM103653
NIH P30AG013280

Title: Translating time across species: machine learning approaches to align ages

Authors: *R. MOHAMEDELHASSAN[1], J. WHITE[3], K. OFORI[4], T. LEE[2], M. BRYANT[1], D. R. MARTIN[5], C. J. CHARVET[2];
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Abstract: We need better tools to map findings from model systems to humans. As part of this effort, we develop novel methods to find corresponding ages across different species. We used machine learning models applied to normalized gene expression to generate cross-species age alignments. We compared corresponding time points from these machine learning models to those from our Translating Time dataset. Our Translating Time dataset consists of 922 time points collected across 25 mammalian species. We collected RNA sequencing data from the frontal cortex of cats (n=8) and mice (n=16), and we used publicly available cerebral organoids from humans (n=20) and gorillas (n=20). We trained five machine learning models (e.g., R packages: glmnet, SVMlinear2, gaussprPoly) to establish age correspondence across species. We considered the root mean square error (RMSE) to assess which models are best suited to predict age. When predicting age in mice, we found that gaussprPoly had the lowest RMSE (0.651047)

of all tested models. We selected the model with the lowest RMSE to predict the ages of cats in mice days after conception (DAC) and between gorillas to human DAC. Age alignments generated from RNA sequencing data are similar to those collected from other methods in our Translating Time dataset. Our results demonstrate that the machine learning models can predict corresponding ages across species. Our work aids in the selection of suitable animal models and provides age equivalence across organoids and animals.

Disclosures: R. Mohamedelhassan: None. **J. White:** None. **K. Ofori:** None. **T. Lee:** None. **M. Bryant:** None. **D.R. Martin:** None. **C.J. Charvet:** None.

Poster

PSTR317. Comparative Anatomy and Physiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR317.17/C3

Topic: A.10. Development and Evolution

Title: First glimpse! A novel method for postmortem dissection and macroscopic viewing of the newly discovered meningeal layer in human brain

Authors: *A. KUMAR[1], R. KUMAR[2], C. KUMARI[4], A. ASGHAR[2], R. K. JHA[2], A. K. RASTOGI[3], P. KUMAR[5], R. K. NARAYAN[6];
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Abstract: Introduction: A recent study demonstrated the presence of an additional leptomeningeal layer in mice and human brains using advanced microscopic methods. This new layer is described as a thin but impermeable membrane between the arachnoid and pia mater, thus dividing subarachnoid space (SAS) containing cerebrospinal fluid (CSF) in two functional compartments. Despite having substantial clinical importance, the neuroanatomical approaches and macroscopic viewing of this meningeal layer, especially in the human brain, are still a lacuna in the literature. **Materials and Methods:** The fresh postmortem (n=7) and 10% formalin-fixed cadaveric (n=5) human brain specimens were retrieved using standard operative procedures and dissected *in situ*. The step-by-step dissection procedure was video recorded. An *in situ* dye test was used to examine the permeability of the new meningeal layer in fixed dissected brain specimens. Further tissue samples were retrieved from the fresh postmortem brains for histological analysis and scanning electron microscopic (SEM) viewing. In addition, postmortem/cadaveric specimens of the human spinal cord were also examined. **Results:** A standard dissection protocol was devised to approach the new meningeal layer in postmortem human brains. The presence of an intermediate leptomeningeal layer, between the arachnoid and pia mater was confirmed in all dissected specimens. It appeared as a thin translucent glistening membrane. The dye test and histological analysis validated the nonporous and predominantly cellular structure of the new meningeal layer. An intermediate leptomeningeal layer was also

noted in the spinal cord. The findings were further confirmed in the SEM examination of the tissue samples. **Conclusions:** The study enables neuroanatomical approaches and macroscopic viewing of the newly discovered meningeal layer in the human brain. The findings may help develop new neurosurgical diagnostic/operative procedures and train medical graduates.

Disclosures: A. Kumar: None. **R. Kumar:** None. **C. Kumari:** None. **A. Asghar:** None. **R.K. Jha:** None. **A.K. Rastogi:** None. **P. Kumar:** None. **R.K. Narayan:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.01/C4

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant AA026598

Title: Effects of acute and protracted alcohol withdrawal on cholinergic neuron activation and nicotinic acetylcholine receptor expression

Authors: S. ENGEL, S. MULLOY, R. DOBBELMANN, M. SCALF, *A. LEE;
Univ. of Minnesota, Minneapolis, MN

Abstract: The risk of developing alcohol use disorder (AUD) is impacted by the severity and frequency of alcohol withdrawal. Investigating the neurobiological changes produced by alcohol withdrawal provides insight into the mechanisms that contribute to AUD and identifies targets for pharmacological treatments for alcohol withdrawal. Based on our prior work, we hypothesize that cholinergic signaling and the nicotinic acetylcholine receptors (nAChRs) are activated by alcohol withdrawal mechanisms. Adult male wild-type mice (n=10/group) were given daily injections of alcohol (2.5 g/kg ip) or saline, both paired with alcohol dehydrogenase inhibitor 4-methylpyrazole (9 mg/kg ip) for nine days. Behavioral markers of anxiety and alcohol withdrawal were measured at 24h and 10 days after the last injection. Ethanol-treated mice showed increased withdrawal and anxiety-like phenotypes at 24hr withdrawal compared with saline-treated mice. Total somatic withdrawal signs and chewing behaviors were increased in ethanol-treated animals compared with controls (both $p<0.05$). In ethanol-treated mice, there was a 300% increase in the number of buried marbles by the ethanol-treated mice ($p=0.017$) in a marble burying test, a measure of compulsive-like behavior, and a 20% decrease ($p=0.048$) in open area time in an elevated-zero maze, indicating increased anxiety-like behavior. Mouse brains were then extracted, sectioned and probed for c-Fos protein expression using immunohistochemistry or nAChR subunit transcripts using multiplex RNAscope in situ hybridization. We examined the ventral tegmental area (VTA) and the mesopontine tegmentum (MPT), a cholinergic brainstem region consisting of the pedunculopontine tegmental nucleus (PPN) and the laterodorsal tegmental nucleus (LDT) that innervates midbrain regions. There was an increase in c-Fos expression and alpha-6 nAChR subunit expression in the VTA and an

increase in beta-2 nAChR subunit expression in the MPT at 24h of withdrawal. Our data suggests that alcohol withdrawal activates brainstem cholinergic neurons and its projection targets. Understanding cholinergic signaling in alcohol withdrawal will provide needed insight into the mechanisms mediating alcohol withdrawal and identify potential pharmacotherapies in mitigating these symptoms.

Disclosures: S. Engel: None. **S. Mulloy:** None. **R. Dobbelmann:** None. **M. Scalf:** None. **A. Lee:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.02/C5

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Ontario Graduate Scholarship

Title: Membrane trafficking of ionotropic acetylcholine receptors mediates extended desensitization of neuroendocrine cell responsiveness

Authors: *K. H. LEE[1], N. S. MAGOSKI[2];
[1]Biomed. and Mol. Sci., Queen's Univ., Kingston, ON, Canada; [2]Biomed. & Mol. Sci., Queens Univ., Kingston, ON, Canada

Abstract: Long-lasting neural activity in response to brief cholinergic input is a well-documented phenomenon, and underlies behaviors, such as executive attention, learning, and neuroendocrine control. However, the regulation of the cholinergic signal, especially that of the ionotropic receptor is poorly understood in this context. In the sea snail, *Aplysia* (hermaphrodite, adult; 200-600 g), activation of ionotropic acetylcholine receptors on neuroendocrine bag cell neurons causes a lengthy afterdischarge and egg-laying hormone secretion to trigger reproductive behavior. The afterdischarge is followed by an ~18-hr refractory period, during which only single action potentials can be evoked by a cholinergic synaptic input. Accordingly, in individual whole-cell voltage-clamped cultured bag cell neurons, inward currents elicited by consecutive, 2-sec pressure applications of 1 mM acetylcholine at 10-, 30-, 60-, 90-, or 120-min intervals, consistently yielded an outcome where the second response was ~60% of the first (n=5-9). The sustained desensitization was unaffected by 500 uM of the metabotropic acetylcholine receptor blocker, phenyl-trimethyl-ammonium (n=5). Nevertheless, at an interval of 6 or 24 hrs, the current nearly or completely recovered (~90% and ~110%, respectively; n=5 and 14) The response was also agonist-specific, as 10 mM of the nicotinic agonist, trimethyl-ammonium showed less desensitization with successive applications and weaker affinity compared to acetylcholine (n=6). Actual gating of the receptor is likely unrelated since 50-300 uM of the nicotinic receptor pore-blocker, hexamethoinum, did not rescue the desensitization at all concentrations tested (n=3-4). Furthermore, increasing intracellular cAMP using 100 uM of

the phosphodiesterase inhibitor, 3-isobutyl-1-methylxathine, decreased the second current by ~50% (n=14), which was significantly greater than control (65%; n=18), and sensitive to 10 uM of the PKA inhibitor, KT5720 (~65%; n=11), suggesting that cAMP is not directly involved in the desensitization. On the other hand, 12- and 24-hr pre-treatment with 20 uM of the proteasome inhibitor, lactacystin, which prevents forward trafficking of membrane proteins, resulted in a second current of ~80% (n=10 and 8, respectively). Hence, greater retrieval of ionotropic acetylcholine receptors from the membrane upon the initial cholinergic input may explain prolonged desensitization, and by extension, the maintenance of the refractory period and specific timing of reproduction.

Disclosures: K.H. Lee: None. **N.S. Magoski:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.03/C6

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: The human acetylcholinesterase C-terminal T30 peptide activates neuronal growth through alpha 7 nicotinic acetylcholine receptors and the mTOR pathway

Authors: *A. GRAUR[1], P. L. SINCLAIR[2], A. SCHNEEWEIS[3], D. T. PAK[4], N. KABBANI[5]; [1]George Mason Univ., Fairfax, VA; [2]Interdisciplinary Neurosci., George Mason Univ., Annandale, VA; [3]Pharmacol. and Physiol., Georgetown Univ., Washington, DC; [4]Georgetown Univ. Med. Ctr., Washington DC, DC; [5]Mol. Neurosci, Krasnow Inst., FAIRFAX, VA

Abstract: Acetylcholinesterase (AChE) is a highly conserved enzyme responsible for the regulation of acetylcholine signaling within the brain and periphery. AChE has also been shown to participate in non-enzymatic activity and contribute to cellular development and aging. In particular, enzymatic cleavage of the carboxy terminal region of the synaptic AChE isoform, AChE-T, is shown to generate a bioactive T30 peptide that binds to the α7 nicotinic acetylcholine receptor (nAChR) at synapses. Here, we explore intracellular mechanisms of T30 signaling within the human cholinergic neural cell line SH-SY5Y using high performance liquid chromatography (HPLC) coupled to electrospray ionization mass spectrometry (ESI-MS/MS). Proteomic analysis of cells exposed to (100nM) T30 for 3-days reveals significant changes within proteins important for cell growth. Specifically, bioinformatic analysis identifies proteins that converge onto the mammalian target of rapamycin (mTOR) pathway signaling. Functional experiments confirm that T30 regulates neural cell growth via mTOR signaling and α7 nAChR activation. T30 was found promote mTORC1 pro-growth signaling through an increase in phosphorylated elF4E and S6K1, and a decrease in the autophagy LC3B-II protein. These findings are corroborated in hippocampal neurons and show that T30 promotes dendritic arborization. Taken together, our findings define mTOR as a novel pathway activated by the T30

cleavage peptide of AChE and suggest a role for this pathway in cholinergic aspects of human disease.

Disclosures: A. Graur: None. **P.L. Sinclair:** None. **A. Schneeweis:** None. **D.T. Pak:** None. **N. Kabbani:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.04/C7

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant T32GM146604

Title: The balance of NACHO and Ly6H in the nucleus accumbens: Implications for nicotine addiction

Authors: *L.-B. SHINN, Y. SHERAFAT;
Psychology, California State University, San Marcos, San Marcos, CA

Abstract: Title: The balance of NACHO and Ly6H in the nucleus accumbens: Implications for nicotine addictionAuthors: Levi-Briana Shinn and Yasmine Sherafat Ph.D.
Nicotine addiction is recognized as the leading cause of premature death in the United States and current therapeutics have been demonstrated to yield moderate effects. Nicotine exerts its effects on neuronal nicotinic acetylcholine receptors (nAChRs) in the brain. Neuronal nAChRs in the Nucleus Accumbens (NAcc) have been demonstrated to modulate the rewarding effects of addictive drugs, including nicotine. Although the examination of nAChRs allows for an understanding of the actions of nicotine, the mechanisms by which nicotine acts on nAChRs are not well understood. Therefore, there is a critical need to further our understanding of nicotine's effects on the brain and behavior in order to study prospective endogenous proteins that may mediate nAChR activity. TMEM35a (NACHO), an endogenous protein has been demonstrated to modulate nAChRs. NACHO increases nAChR activity and has been shown to work antagonistically with the negative allosteric modulator Ly6H, to maintain optimal nAChR activity. Like Ly6H, Lynx2 is another negative allosteric modulator that has been demonstrated to inhibit nAChR function. Due to Lynx2 and Ly6H's familial and functional association, we sought to examine if global removal of Lynx2 alters NACHO expression in the NAcc in Lynx2 knockout and wild-type mice. We predicted that removing Lynx2 will result in increased NACHO expression in the NAcc. Using immunohistochemistry, we found no significant differences in NACHO expression in the NAcc in Lynx2 knockout mice when compared to their wild-type littermates. However, these findings are important and further establish that NACHO is indeed expressed in the NAcc. Thus, these findings reveal important insight regarding the relationship between Lynx2 and NACHO within brain regions critical to nicotine reward, learning, and memory.

Disclosures: **L. Shinn:** None. **Y. Sherafat:** 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.; Start up funds from CSUSM to Y.S..

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.05/C8

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant 5T34GM136481

Title: Investigating the balance between nicotinic receptor modulators in the hippocampus

Authors: ***N. JAMALIAN**, K. MARQUEZ, Y. SHERAFAT;
California State Univ. San Marcos, San Marcos, CA

Abstract: Investigating the balance between nicotinic receptor modulators in the hippocampus Authors: Nissa Jamalian*, Karina Marquez*, and Yasmine Sherafat*denotes co-first authors**Abstract**Recent studies have demonstrated that nicotine can enhance cognition, learning, andmemory. The hippocampus, a brain region vital for learning and memory contains nicotinicreceptors, which nicotine binds to. Novel Acetylcholine receptor Chaperone (NACHO)is achaperone protein that enhances nicotinic receptor function, while Lynx2 and Ly6H are negativeallosteric modulators that suppress receptor activity. NACHO and Ly6H have shown to rival foraccess to nicotinic receptors to maintain homeostasis. Thus, we were interested to see whetherremoval of Lynx2 would result in any changes of NACHO expression. Due to their reciprocalrelationship, we hypothesized that Lynx2 knockout (KO)mice would have increased NACHOexpression. Using immunohistochemistry and fluorescent microscopy, we were able to compareKO and control mice and our results found that there were no differences in expression ofNACHO in the hippocampus. These findings are important as we continue to understand moreabout the balance of allosteric modulators and how they maintain nicotinic receptor activity inthe hippocampus, which will be important for future nicotinic based cognitive therapeutics

Disclosures: **N. Jamalian:** None. **K. Marquez:** None. **Y. Sherafat:** 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.; Startup funds from CSUSM to Y.S..

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.06/C9

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant NS031744

Title: Assembly of Salt Bridges and Palmitoylation Sites Govern Ionic Current Through Single Nicotinic Receptor Channels

Authors: *L. ALHALHOOLY, S. M. SINE;
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Abstract: Ion channels are the fundamental units that confer cell membrane conductance and mediate cellular signaling. However, relationships between ion channel structure and function remain incompletely understood. Here we combine mutagenesis and single channel electrophysiology to determine how conserved pore-peripheral salt bridges of the muscle nicotinic receptor set the unitary current amplitude and maintain stability of the open channel current. Disrupting the salt bridges in all subunits reduces the current amplitude by 85 % relative to the wild-type receptor. Despite being equivalent, the salt bridges of individual subunits contribute unequally, and further, their contributions are interdependent. Namely, breaking the salt bridges in the α and β subunits reduces current amplitude, whereas breaking them in the ε and δ subunits increases amplitude. In addition, palmitoylation sites near the salt bridges in the ε and δ subunits affect the current amplitude like the salt bridges themselves. Mutating salt bridges in multiple subunits produces multiple stable open channel current levels whose relative contributions depend on the combinations of mutant subunits. The results reveal a system of coupled salt bridges and lipid anchors extending from the pore to the outermost boundary of the lipid membrane that controls the magnitude and stability of the ionic current.

Disclosures: L. Alhalhooly: None. **S.M. Sine:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

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Program #/Poster #: PSTR318.07/C10

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR PJT-153101 (E. Lambe)
CIHR; MOP89825 (E. Lambe)
Banting and Best Doctoral Canada Graduate Scholarship (S. Power)
Ontario Graduate Scholarships (S. Venkatesan, S. Power)

Title: Prefrontal cholinergic signalling: potentially-compensatory, functional nicotinic upregulation across different species and models of Alzheimer's disease

Authors: *E. K. LAMBE[1], S. VENKATESAN[1], S. QU[1], J. MCLAURIN[2], S. K. POWER[1]; [1]Physiol., Univ. of Toronto, Toronto, ON, Canada; [2]Biol. Sci., Sunnybrook Hlth. Sci. Ctr., Toronto, ON, Canada

Abstract: Deficits in attention and executive function occur relatively early in Alzheimer's disease (AD). To investigate the signalling pathway relevant for this aspect of cognition, we electrophysiologically interrogate cholinergic signalling in prefrontal cortex, *ex vivo*, in two models of AD. Examining a compound transgenic mouse that permits optogenetically-triggered release of endogenous acetylcholine in the presence of TgCRND8 pathology, we find a significant, unexpected upregulation of cholinergic responses in early AD that does not persist into late pathology. In the TgF344 AD rat model that closely recapitulates a human trajectory of AD, we also find a significant enhancement of responses to exogenous acetylcholine that does not persist into late disease. To identify the locus of the functional enhancement, we dissected pre- and post-synaptic components of cholinergic signaling pharmacologically. We identify that the cholinergic upregulation in AD is specific to increased postsynaptic nicotinic receptor signalling. Therefore, we probe how this cholinergic upregulation responds to broad versus nicotinic-selective pro-cognitive treatments. We find that acetylcholinesterase inhibition enhances cholinergic responses but greatly perturbs their kinetics. By contrast, nicotinic positive allosteric modulation enhances while retaining the kinetics of endogenous cholinergic signalling. Improving cognition in early AD is a potentially high-impact treatment goal, and these results suggest that pro-cognitive cholinergic signalling may be an efficient target.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.08/C11

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Intramural Research Program (Project 1ZIANS003137)

Title: Dynamic molecular organization of the central cholinergic synapse in developing and adult Drosophila brains

Authors: *J. ROSENTHAL[1], D. ZHANG[1], J. YIN[1], C. LONG[1], Y. LI[1], J. LI[2], Q. YUAN[1]; [1]Natl. Inst. of Neurolog. Disorders and Stroke, NIH, Bethesda, MD; [2]Janelia Res. Campus, Howard Hughes Med. Inst., Ashburn, VA

Abstract: At chemical synapses, presynaptic input is detected by the postsynaptic specialization of the receiving cell. This region contains a complex arrangement of proteins required for basic neurotransmission as well as synaptic plasticity. Historical models for studying the postsynaptic density (PSD) are the vertebrate cholinergic neuromuscular junction (NMJ) and the central glutamatergic synapse of the vertebrate brain. However, the nature of other synapse types is less well characterized. One such example is the cholinergic synapse of the central nervous system (CNS) which conducts synaptic transmission via nicotinic acetylcholine receptors (nAchRs). While postsynaptic nAchRs are sparse in the vertebrate brain, in many invertebrates they are the primary neurotransmitter receptor for conducting excitatory signaling in the CNS. Therefore, to better understand the molecular milieu in which postsynaptic nAchRs exist we investigated nAchR-interacting proteins in the *Drosophila* CNS by an in vivo spatially-resolved proteomics approach. Through a series of proximity-labeling experiments which utilize the modified Biotin Ligase "MiniTurbo", we endogenously labeled hundreds of proteins in the immediate vicinity of two nAchR subunits, Da1 and Da6. The resulting proteome included many synaptic proteins, such as PDZ domain-containing scaffolding proteins and actin-binding proteins, as well as additional classes of proteins like RNA-binding translational regulators and molecules responsible for protein modification and processing, indicative of protein flux at the postsynaptic region. Comparisons between these two proteomes also display a high degree of similarity between them, suggesting a close spatial overlap of Da1 and Da6, and potentially co-assembly into the same pentamer. In contrast, there is a clear divergence between different developmental stages. For both subunits there is a substantial increase in proteome size in adults, consistent with a more complex postsynaptic organization that emerges during development. Furthermore, many candidate genes with previously unidentified roles in synapse development and organization were discovered through the proteomics approach, providing new molecular targets for future functional characterizations and extended studies in vertebrate systems.

Disclosures: J. Rosenthal: None. **D. Zhang:** None. **J. Yin:** None. **C. Long:** None. **Y. Li:** None. **J. Li:** None. **Q. Yuan:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.09/C12

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: RIC3 and NACHO chaperone effects on alpha7 nicotinic and 5HT3 chimeric receptors

Authors: *R. H. LORING, Z. YAN;
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Abstract: How multi-subunit receptors fold and assemble is not well understood even though these proteins are the targets of many drugs and neurotransmitters. RIC3 (Resistance to inhibitors of cholinesterase3) and NACHO (Nicotinic Acetylcholine Receptor Regulator) are two

chaperones allowing α7 nicotinic receptor (α7 AChR) assembly in the endoplasmic reticulum of Human Embryonic Kidney (HEK) cells. However, serotonin 5HT3 receptors (5HT3R) do not require RIC3 or NACHO for assembly. Gee et al. (Br. J. Pharm. (2007) 152: 501) produced chimeras of α7 & 5HT3R (α7-5HT3R) showing that the four transmembrane domains (M1-M4) are critical for pentameric receptor assembly and that if M1-4 did not match, assembly of their constructs was not possible with RIC3. Kweon et al. (Cell Rep. 2020, 32: 108025) made an α7-5HT3R-T267 chimera with the splice site at threonine 267 in M2. α7-5HT3R-T267 expresses with NACHO, suggesting the incompatibility of mixed transmembrane domains is between M3 & M4. We have further investigated various α7-5HT3R chimeras with intact α7 AChR cytoplasmic loops to determine receptor expression measured by fluorescent α -bungarotoxin binding to transfected BOSC23 cells, an HEK variant. Surface receptor expression was evaluated in the presence of green fluorescent protein (negative control), NACHO, RIC3 or a 3:1 ratio of NACHO and RIC3. Results: As do others, we also find that compatibility between M3 and M4 is crucial for assembly and chaperone activity. Substituting the α7-5HT3R extracellular C-terminal with an α7 tail and/or the α7 ser-ala-pro (SAP) motif decreases surface expression and chaperone actions. Contrary to Kweon et al., substituting G265 with alanine is well tolerated in α7-5HT3R-T267 but L264 in M2 does appear crucial for NACHO activity and the synergistic effect of NACHO with RIC3. Further, amino acid C482 in M4 plays an important role in synergistic activity of NACHO and RIC3 on α7-5HT3R-T267. Molecular modeling suggests that C300 and C482 may form a disulfide bond in α7-5HT3R-T267- α7M4 that could inhibit assembly. However, preliminary data suggests that T300C mutation in α7 has little effect on expression, but that Y482C mutation in α7-5HT3R reduces expression by about half with or without chaperones. Further work is needed to determine if disulfide formation is involved in decreasing α7-5HT3R-Y482C expression. Therefore, many different regions of α7 AChRs are involved in the effects of RIC3 and NACHO, but the exact actions of these chaperones on α7 assembly remain obscure.

Disclosures: R.H. Loring: None. **Z. Yan:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.10/C13

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH grant R35 GM136430

Title: The role of alpha9-nAChRs in the immune cells in mediating alpha-conotoxin RgIA attenuation of oxaliplatin-induced neuropathic pain

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Abstract: Nicotinic acetylcholine receptors (nAChRs) in immune cells have been implicated in mediating pain and inflammation due to nerve injury. Oxaliplatin, a third generation diaminocyclohexane platinum drug and widely used chemotherapy agent, causes neuropathy and painful sensitivity to cold as a dose-limiting side effect. We have previously shown that specific block of α9α10 nAChRs with selective α-conotoxin RgIA analogs (RgIA) prevents oxaliplatin-induced cold allodynia in mice. Moreover, RgIA fails to prevent cold allodynia in α9 nAChR subunit knockout mice. Previous studies have not addressed the target tissue of RgIA. In the current study, we examined the role of α9-containing nAChRs in the mechanism of action of RgIA by using transgenic mice with conditional knockdown of the α9 subunit in immune cells. For this purpose, we used the Cre-loxP recombination system with transgenic Tie2Cre mice (to drive the expression of Cre recombinase in cells of hemopoietic origin) and transgenic LoxP mice (that have floxed α9 subunit genes). Tie2Cre_α9LoxP2 mice (which are hemizygous for Tie2Cre and homozygous for floxed α9) had an ~10-fold knockdown of α9 subunit in thymus compared to wildtype (WT) animals, without any change in α10 nAChR subunit levels, demonstrating selective knockdown of α9. The Tie2Cre_α9loxP2 mice developed cold allodynia to a similar degree as WT animals in response to acute (one time injection of 20 mg/kg) oxaliplatin treatment. RgIA prevented oxaliplatin-induced cold allodynia in WT animals, but failed to prevent this allodynia in the conditional α9 knockdown mice. These results further confirm the crucial role of α9 nAChRs in the mechanism of action of RgIA, and specifically demonstrate that α9-containing nAChRs in immune cells are necessary for mediating RgIA prevention of oxaliplatin-induced cold-allodynia.

Disclosures: L. Azam: None. **S. Christensen:** None. **Z. Riaz:** None. **A. Kendell:** None. **J. Cull:** None. **A. Hone:** None. **J.M. McIntosh:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

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Program #/Poster #: PSTR318.11/C14

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant URISE 5T34GM136481

Title: Nacho expression in the interpeduncular nucleus (ipn) in lynx2 knockout mice: implications for future withdrawal therapeutics

Authors: *A. ALMARAZ, Y. SHERAFAT;
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Abstract: Title: NACHO expression in the interpeduncular nucleus (IPN) in Lynx2 knockout mice: Implications for future withdrawal therapeuticsAuthors: Ariana Almaraz and Yasmine

Sherafat Ph.D. Nicotine dependency has grown rapidly in recent decades as vaping has gained popularity among all ages. Its easy accessibility has made it more difficult for consumers to quit as relapse rates have increased. It is important to examine how nicotine withdrawal syndrome affects long-term brain functioning using rodent models to find more efficacious treatment options.Exogenous nicotine directly acts on nicotinic acetylcholine receptors (nAChRs) throughout the brain. All nAChRs vary in their functioning across different brain regions. Within the interpeduncular nucleus (IPN), inhibitory neurons (Gamma-aminobutyric acid or GABA) are involved with nicotine dependance and fear responses. GABAergic neurons block signaling in the post-synapse desensitizing receptors until eventually greater concentrations of nicotine are achieved in nicotine dependent individuals. Specifically, the nAChR subunit, α7 is concentrated in the IPN and has been shown to play a role in nicotine aversion and in consumer's regulatory intake.Alpha7 is mediated by the recently discovered NACHO protein produced by the endoplasmic reticulum (ER) and is known to regulate the overall production of α7. Recent studies have confirmed that NACHO interacts with Ly6H, a negative allosteric modulator (NAM) that reduces the effectiveness of agonists when it binds to a nAChR by limiting the channel opening. Ly6H has shown to decrease α7 activity while NACHO is known to increase activity. Functionally and structurally similar to Ly6 is Lynx2 which has been reported to have a link to sensorimotor gating, learning, and memory.In the current study, we examined NACHO expression within the IPN from male Lynx2 knockout mice vs their wildtype littermates. The IPNs of coronally cut mouse brains were selected for immunohistochemistry and analysis of NACHO expression (N=5). Through manual quantification, there was a significant difference found between the two groups as Lynx2 knockout mice had significantly less NACHO expression than wildtype littermates, $p<0.05$. Future directions include validating the findings with quantification software, taking surface area into account, and using a larger sample size. However, from this finding, Lynx2 and NACHO have a linear relationship. As Lynx2 is absent, NACHO is also downregulated. Future withdrawal treatments may include downregulation of NAHCO expression as it is linked to α7 production and activity.

Disclosures: A. Almaraz: None. **Y. Sherafat:** 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.; Start up funds from CSUSM to Y.S..

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.12/C15

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant AG067029
NIH Grant DA042749

Title: Peptide ligands have selectivity for α7β2- over α7-only subtype nicotinic acetylcholine receptors

Authors: A. A. GEORGE[1], **S. J. JOHN**[1], L. M. LUCERO[2], B. EATON[2], E. JAISWAL[2], Y. CAO[3], W. IM[3], *P. WHITEAKER[1];
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Abstract: Background: Nicotinic acetylcholine receptors containing the α7 subunit (α7*-nAChRs) are widespread in both CNS and peripheral locations. Decades of research suggest that interactions between amyloid-β (Aβ) and α7*-nAChR play important roles in Alzheimer's disease (AD). Our work indicates that α7β2*-nAChR are prominently expressed on basal forebrain cholinergic neurons (BFCNs, that are lost early in AD progression), and that oligomeric $A\beta_{1\text{-}42}$ ($oA\beta_{42}$) directly activates α7β2*-nAChR, resulting in BFCN hyperexcitability. More detailed studies have been hampered by a lack of ligands to discriminate α7β2- from α7-only-nAChR subtypes (also likely expressed on BFCNs). Here we present a set of α-conotoxin (α-Ctx) ligands that selectively antagonize α7β2- over α7-only-nAChR. **Methods:** Two-electrode voltage clamp (TEVC) electrophysiology on *Xenopus* oocytes was used to functionally screen ≈500 novel α-Ctxs for selectivity towards α7β2*-nAChR. Molecular dynamics (MD) simulation was used to identify amino-acid residues of α7β2*-nAChR where selective α-Ctxs may bind. Site-directed mutagenesis was used to mutate those residues, probing whether selective peptides bind to a site at the α7/β2 subunit interface. **Results:** A small set of analogs of α-CtxPn1.2 was identified that selectively antagonize α7β2- over α7-only nAChR, with little activity at α3β4- or α4β2-nAChR. Kinetics analysis showed that association rates were similar across Pn1.2 analogs and between α7*-nAChR subtypes. Slower disassociation from α7β2- *vs.* α7-nAChR drove selectivity towards α7β2*-nAChR. The α-CtxPn1.2 [S4R] and [L10Y] analogs were the most selective towards α7β2-nAChR (18- and 57-fold vs. α7-only-nAChR, respectively). MD identified two sets of β2 subunit residues that were non-conserved between the known competitive α7/α7 subunit interface ligand binding site and a similar site at the α7/β2 subunit interface. Mutating either set of β2 subunit residues to their α7 subunit equivalents partially reduced α-CtxPn1.2 [S4R] selectivity towards α7β2-nAChR. **Conclusions:** We have identified the first α-Ctx antagonists with selectivity towards α7β2-nAChR. Site-directed mutagenesis confirms activity is mediated via non-ligand-binding α7/β2 interface sites. This implies a new, non-competitive, mode of action. Further development of these ligands will enhance α7β2-nAChR selectivity, significantly enhancing studies of α7*-nAChR/$oA\beta_{42}$ interactions. In turn, this will provide opportunities for innovative basic and translational scientific breakthroughs related to nAChR biology, AD, and cholinergic contributions to cognition more broadly.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

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Program #/Poster #: PSTR318.13/C16

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Intramural Research Program of the NIH, National Institute of Environmental Health Sciences/NIH/DHHS

Title: Cholinergic-sensitive theta oscillations in memory encoding

Authors: *Z. GU[1], J. L. YAKEL[2];
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Abstract: Cholinergic regulation of hippocampal theta oscillations has long been proposed as a potential mechanism underlying hippocampus-dependent memory encoding process. However, experimental evidence is largely lacking. Cholinergic transmission, especially the muscarinic acetylcholine receptors (mAChRs), has been traditionally associated with type II theta under urethane anesthesia. Cholinergic regulation of type I theta in freely moving animals is much less clear. In this study we examined the potential behavioral significance of cholinergic regulation of theta oscillations in the spatial memory encoding process in object location task. Cholinergic regulation of hippocampal theta oscillations and the behavioral outcomes was examined by intrahippocampal infusion of cholinergic receptor antagonists or knocking out cholinergic receptors in excitatory neurons or interneurons. We found that both mAChRs and α7 nicotinic acetylcholine receptors (α7 nAChRs) were involved in memory encoding (antagonists given before training trials) but not memory retrieval (antagonists given before testing trials) by engaging excitatory neurons and interneurons, respectively. There is a transient upregulated theta oscillation at the beginning of individual object exploration events that only occurred in the training trials but not in the testing trials. This transient upregulated theta is also the only theta component that significantly differed between training trials and testing trials and sensitive to mAChR and α7 nAChR antagonists. Thus, our study revealed a transient cholinergic-sensitive theta component that is specifically associated with memory encoding but not memory retrieval in object location task, providing direct experimental evidence revealing a potential mechanism underlying cholinergic regulated theta oscillations in hippocampus-dependent memory encoding process.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.14/C17

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Johns Hopkins University Catalyst Award
One Mind-Gifford Foundation Rising Star Award

Title: Imaging the α7 nicotinic acetylcholine receptor in affective and non-affective psychosis

Authors: *K. JENKINS[1], C. HARRINGTON[1], A. SOULE[1], W. G. LESNIAK[2], A. G. HORTI[2], M. G. POMPER[2], L. H. RUBIN[3], Y. DU[2], J. M. COUGHLIN[1];
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Abstract: Background: Low availability of the α7-nicotinic receptor (α7-nAChR) may be a relevant biomarker in the brains of individuals with non-affective psychosis (NP), as evidenced in post-mortem studies. An early pilot using positron emission tomography (PET) with [^{18}F]ASEM revealed evidence of low α7-nAChR in the brains of individuals with NP, compared to those with affective psychosis (AP) and to healthy controls (HCs). Here we aimed to increase the sample scanned with [^{18}F]ASEM PET to more rigorously test for group differences.
Methods: Data collected across HCs (N=24), individuals with AP (N=18), and individuals with NP (N=14) included a clinical research interview, magnetic resonance imaging, and one [^{18}F]ASEM PET scan with arterial line. Regional [^{18}F]ASEM binding (V_T) values were generated using Logan analysis with metabolite-corrected arterial input function. Group differences (healthy controls, AP, NP) in regional [^{18}F]ASEM V_T values were examined using a single linear mixed model with repeated measures. Primary predictor variables in the model included group, brain region, and the two-way interaction. Age was included as a covariate. Significance was set at $P < 0.05$.
Results: In the adjusted analysis, group differences in binding were found ($P<0.001$). The NP group had lower binding than both AP and HC groups, and there were no differences in binding between the AP and HC groups. There was a significant interaction between group and region ($P=0.01$), with the same overall pattern across all regions, except in cerebellum and frontal cortex where individuals with AP showed a trend of lower binding than HCs.
Conclusion: These [^{18}F]ASEM PET data are consistent with low α7-nAChR availability across the brains of individuals with NP relative to its availability in AP or HCs. Further investigation is necessary to assess the relationship between α7-nAChR availability and clinical signs within psychosis, including, but not limited to hallucinations, delusions, and cognitive deficits.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

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Program #/Poster #: PSTR318.15/C18

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant NS129676

Title: Assessing the potentiation of (α4)3(β2)2 nicotinic acetylcholine receptor by analogs of the allosteric agonist CMPI

Authors: *J. GAONA[1], **M. BERMUDEZ**[1], W. FELIX[2], A. ROLLING[1], G. THAKUR[2], A. K. HAMOUDA[1];
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Abstract: The (α4)3(β2)2 nicotinic acetylcholine receptor (nAChR) is the major heteromeric nAChR isoform expressed in the cortex and is believed to play a role in memory, cognition, and neuronal survival during aging. As such, selective potentiators of the (α4)3(β2)2 nAChR have potential therapeutic benefits in conditions associated with decline in the output of nAChR in the brain. In previous work, we have studied the pharmacology of the allosteric agonist CMPI (3-(2-chlorophenyl)-5-(5-methyl-1-(piperidin-4-yl)- 1H-pyrazol-4-yl) isoxazole) at the (α4)3(β2)2 nAChR and identified its binding site at the α4:α4 subunit extracellular interface. As part of our ongoing efforts to define structural features that confer CMPI binding selectivity at the α4:α4 interface, we are synthesizing and characterizing the *in vitro* pharmacology of a series of CMPI analogs using whole-cell current recording from *Xenopus laevis* oocytes expressing (α4)3(β2)2 nAChR. So far, we synthesized four analogs which have a thiazole (A12a) or imidazole (A12c, A12e, GAT2226) substitution instead of the pyrazole ring found in CMPI. These analogs maintained selectivity for (α4)3(β2)2 over (α4)2(β2)4 nAChR. They did not potentiate (α4)2(β2)3 nAChR when co-applied with either 1 μM or 10 μM ACh and potentiated current induced by 10 μM ACh at the (α4)3(β2)2 nAChR (>4 fold potentiation at 1 μM). The concentration response curve for these analogs is consistent with a rank of potency thiazole<pyrazole<imidazole. Furthermore, amino acid substitutions in the α4 subunit extracellular domain that decrease CMPI potentiation (e.g., G41M, E66I) also abolish/reduce A12a potentiation of (α4)3(β2)2 nAChR, indicating that the CMPI binding pocket can accommodate ligand structural changes, which allows the development of ligands that bind with higher affinity and selectivity at this positive allosteric modulator site. Ongoing experiments aim to characterize the pharmacology and determine the concentration dependent effects of additional CMPI analogs.

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Poster

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Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH NINDS NS077114

Title: Functional Role of Conserved Amino Acid Residues in the Intracellular Domain of α7 Nicotinic Acetylcholine Receptors

Authors: I. KIM CAVDAR[1], H. Q. DO[1], N. SARAYLI-BELIRGEN[1], L. A. PIERRE[1], *M. JANSEN[2];
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Abstract: Neuronal nicotinic acetylcholine receptors (nAChRs) represent drug targets for the treatment of neurological and neuropsychiatric diseases and disorders. The α7 nAChR in particular has been implicated in schizophrenia and Alzheimer's disease. In the central nervous system α7 nAChRs largely possess modulatory functions as compared to α2/4 and β2/3/4 containing nAChRs directly mediating neurotransmission. Resistance to Inhibitors of Choline esterase (RIC-3) is a chaperone protein that controls the number of functional nAChRs on neuronal plasma membranes. This number is altered in several diseases. For the development of potent or efficacious drugs with fewer side effects the intracellular domain (ICD) of α7 nAChR and in particular the protein-protein interaction with RIC-3 represents a promising target. This is largely based on the high diversity of the intracellular domain relative to the highly-conserved extracellular and transmembrane domains in this large receptor superfamily with more than 40 different subunits in humans. Here our goal was to identify and structurally characterize the RIC-3 α7 nAChR interface for future structure-based drug discovery efforts. To provide evidence for a direct interaction between the chaperone protein RIC-3 and the α7 nAChR ICD, we used synthetic peptides of α7 nAChR ICD in a pull down assay. We observed that a peptide of 17 amino acids consisting of the L1-MX-segment mediated interaction with RIC-3. When conserved residues within the MX-helix were substituted with Ala this interaction was disrupted. Two-electrode voltage-clamp (TEVC) recordings were performed to study the modulatory role of RIC-3 in functional α7 nAChR expression after injecting the corresponding cRNAs into *Xenopus laevis* oocytes. The electrophysiological approach with full-length receptors confirmed the results obtained with the reductionist peptide approach. For the first approach experiments were repeated with independently obtained peptide-coupled resins and also RIC-3 containing cell extracts. For the second in-vivo approach independent oocyte batches were used. All independent experiments were repeated 3 or more times. Our study identifies the L1-MX segment of α7 nAChR as sufficient to interact with RIC-3. This provides the basis for future detailed mechanistic and structural studies aimed at developing compounds to mimic or interfere with this protein-protein interaction that is crucial for determining the amount of functional α7 nAChR on the plasma membrane.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

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Program #/Poster #: PSTR318.17/C20

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: PAPIIT IN216319

Title: Mechanism of action underlying the antidepressant effects of mecamylamine

Authors: *A. MONDRAGON GARCIA[1], E. RAMÍREZ-SÁNCHEZ[3], J. GARDUÑO[4], S. HERNANDEZ-LOPEZ[5], *A. MONDRAGON GARCIA[2];
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Abstract: The dorsal raphe nucleus (DRN) serves as the primary source of serotonin (5-HT) to the forebrain. Previous studies conducted in our laboratory have demonstrated that nicotine increases the firing rate of 5-HT DRN neurons by facilitating glutamate release through the stimulation of presynaptic α4-β2 nicotinic acetylcholine receptors (nAChRs) (Garduño et al., 2012). Interestingly, other research suggests that mecamylamine (Mec), a non-specific and non-competitive nAChR blocker, also enhances the activity of 5-HT DRN neurons and triggers serotonin release (Mihailescu et al., 1997; Kenny et al., 2000; Reuben and Clark, 2000). Furthermore, behavioral studies have reported that Mec exhibits antidepressant effects in rats (Rebenstein et al., 2006). These conflicting findings suggest that Mec may have effects beyond nAChR blockade in the DRN. Hence, the objective of this study was to investigate the mechanisms by which Mec augments the excitability of 5-HT DRN neurons. We employed midbrain slices obtained from male Wistar rats aged 21-25 postnatal days and conducted calcium imaging experiments to record the activity of multiple 5-HT neurons simultaneously at single-cell resolution. Calcium imaging experiments revealed that Mec heightened the activity of the majority of DRN neurons. This effect was reversed by bath perfusion of serotonin or the selective 5-HT1A receptor agonist, 8-OH-DPAT (5μM), suggesting that the impact of Mec primarily targets serotonergic neurons. Our data also demonstrate that Mec enhances the activity of 5-HT neurons by stimulating α4-β2 nAChRs, which in turn promotes glutamate release (Garduño et al., 2012), and by reducing the inhibitory influence exerted by GABA release in the DRN. To record the electrical activity of 5-HT DRN neurons, whole-cell voltage and current clamp techniques were employed. We observed that Mec (3-6 μM) increased the firing rate (approximately 30%) and diminished the GABAergic sIPSCs recorded from 5-HT DRN neurons. Additionally, we conducted an animal model of depression like behaviour and behavioral tasks to assess the antidepressant effects of mecamylamine.

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Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

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Support: NIH Grant GM136430
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Title: Alpha-conotoxins containing amino acids of the sars-cov-2 furin cleavage site are antagonists of human alpha7 and alapha9alpha10 nicotinic acetylcholine receptors

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Abstract: SARS-CoV-2 is the pathogen responsible for the COVID-19 pandemic which has resulted in millions of deaths worldwide. A distinguishing feature of the SARS-CoV-2 virus is the presence of a unique sequence of amino-acid residues in the spike protein not found in closely related SARS-CoV-2-like viruses. This sequence, ^{681}PRRAR^{685}, forms a cleavage site for the enzyme furin and has been linked to increased viral pathogenicity. One hypothesis for the disease severity associated with COVID-19 proposes that the furin cleavage site interacts with and inhibits nicotinic acetylcholine receptors (nAChRs) expressed by immune cells resulting in increased systemic inflammation. However, many commercially available recombinant spike-proteins lack this critical furin sequence hindering testing of this nicotinic-receptor hypothesis. Here we used α-conotoxins from venomous marine snails as templates to engineer peptides that contain the SARS-CoV-2 furin cleavage site. We show that substitution of a portion of the native α-conotoxin sequence with the furin sequence, ^{680}SPRRARS^{686}, converts the inactive native peptide to one that potently inhibits human α7 nAChRs and subtypes containing α9 and/or α10 subunits. Remarkably, insertion of the delta variant sequence (^{680}SRRRARS^{686}) increased potency by >100-fold for inhibition of α7 and α9α10 nAChRs. Peptides with the alpha (^{680}SHRRARS^{686}) or omicron sequences (^{679}KSHRRAR^{685}) showed differential potencies for the two nAChRs subtypes. The observed potencies of the peptides containing the various furin cleavage-site sequences might be relevant to differences in pathogenicity of the SARS-CoV-2 variants. These studies suggest a plausible interaction between the SARS-CoV-2 spike protein and nAChRs.

Disclosures: A.J. Hone: None. **U. Santiago:** None. **C. Camacho:** None. **B. Tekarli:** None. **J. Gajewiak:** None. **P.J. Harvey:** None. **D.J. Craik:** None. **J. McIntosh:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.19/C22

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: R01DA043567

Title: Site of α3β4-nicotinic acetylcholine receptor current modulation by the prototoxin lynx1

Authors: *D. L. KNEISLEY[1], H. OH[1], Y. CAO[2], W. IM[2], J. MIWA[2], P. WHITEAKER[1]; [1]Pharmacol. & Toxicology, Virginia Commonwealth Univ., Richmond, VA; [2]Biol. Sci. & Bioengineering, Lehigh Univ., Bethlehem, PA

Abstract: Smoking, maintained by nicotine-seeking behaviors, is the leading cause of preventable death worldwide. Throughout the brain, nicotine acts on the nicotinic acetylcholine receptors (nAChR). The α3β4 nAChR subtype mediates aspects of nicotine withdrawal found onby modulating activity of GABAergic neurons of the interpeduncular nucleus, mediates aspects of nicotine withdrawal. Previous work shows that the prototoxin lynx1 is also highly expressed in these neurons, and that lynx1 allosterically diminishes α3β4 nAChR response differentially depending on receptor subunit ratio. Lynx1's allosteric modulation has advantages as a potential therapeutic target for anti-smoking drugs: Unlike competitive ligands which directly bind to the orthosteric site of the receptor to compete with acetylcholine (ACh), allosteric ligands allow the fine-tuning of receptor function without completely blocking the receptor or interfering with ACh binding. Their subtype specificity also reduces the potential side effects associated with binding at multiple nAChR subtypes. This study probes a subunit interface unique to the most sensitive α3β4 isoform to determine the molecular interactions through which lynx1 exerts its effects. Molecular dynamics simulations were used to identify residues of α3β4 where lynx1 may interact. These residues were mutated to alter potential side chain interactions with lynx1. Two-electrode voltage clamping electrophysiology on *Xenopus* oocytes was used to compare the difference in ACh-evoked currents between the wild type (WT) and mutant receptors coexpressed with increasing amounts of lynx1. 11 of 15 nAChR mutations tested showed altered sensitivity to the effects of lynx1 in comparison to WT α3β4. Mutating an aspartate (D157) or a glycine (G162) to alanine exhibited the largest decreases in sensitivity compared to WT, while changing a tyrosine (Y190) to alanine displayed the largest increase. Lynx1 also produces dose-dependent decreases in α3β4 function. Mutations at the putative interaction site show altered lynx1 sensitivity compared to WT nAChR, confirming the importance of this region for lynx1-nAChR interaction. Mutation effects appear to depend on location; those at residues corresponding to the conventional β4/α3 agonist-binding site produced increased sensitivity to lynx1, while mutations elsewhere in the receptor produced decreased lynx1 sensitivity. Though these studies are ongoing, the current results may suggest competition between ACh and lynx1 at the putative lynx1 binding site, which could indicate that the lynx1 binding site overlaps with a noncanonical, previously undiscovered α3/α3 agonist binding site.

Disclosures: D.L. Kneisley: None. **H. Oh:** None. **Y. Cao:** None. **W. Im:** None. **J. Miwa:** None. **P. Whiteaker:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.20/C23

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NS031744

Title: Functional and structural consequences of a mutation in the α4β2 nicotinic acetylcholine receptor associated with congenital epilepsy

Authors: *D. MSEKELA[1,2], S. M. SINE[2];
[1]Mayo Clin., Rochester, MN; [2]Receptor biology lab, Dept. of physiology and biomedical engineering, Mayo Clin. college of medicine, Rochester, MN

Abstract: Congenital mutations in genes encoding subunits forming α4β2 nicotinic receptor cause SHE(Sleep-related Hyperkinetic epilepsy). These mutations bring attention to crucial structures essential for the receptor's function, yet the precise mechanisms underlying their pathogenic effects have yet to be understood. In this study, we examine the structural and mechanistic underpinnings for SHE that arises from an insertion mutation in the α4 pore lining domain. We genetically reconstitute the SHE insertion and employ single-channel patch clamp electrophysiology to observe the functional consequences. Additionally, we use mutagenesis and structural modeling along with patch-clamp to explore the structural mechanism underlying the insertion mutation. This integrated approach allows us to reveal novel insights into structure - function relationship of the α4β2 receptor. Through our investigation of a gain-of-function mutation linked to SHE, we have discovered previously unknown intra-molecular interactions that regulate the stability of the open channel. The mechanistic consequences of this SHE lay the foundation for rational therapy, while the newly identified interactions contribute to our understanding of the mechanisms behind the transition of the receptor channel from the open to the closed state.

Disclosures: D. Msekela: None. **S.M. Sine:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.21/C24

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Creation and characterization of a stably transfected cell line expressing alpha3 beta2 neuronal nicotinic acetylcholine receptors

Authors: *S. N. SUDWEEKS[1], C. CALLISON[2];
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Abstract: The nicotinic acetylcholine receptor (nAChR) is a ligand-gated ion channel created by a pentameric combination of subunits. There is a family of genes that can create these subunits (alpha 1-10, beta 1-4, gamma, delta, and epsilon). Various subunit combinations create subtypes of this receptor with unique properties. The alpha1, beta1, gamma, delta, and epsilon subunits are used to make nAChRs at the neuromuscular junction in skeletal muscle. The alpha 2-10 and beta 2-4 subunits are used to create the neuronal nAChRs in both the peripheral nervous system (PNS - mainly alpha3 with beta4) and the central nervous system (CNS - many subunit combinations). We report here on the creation and characterization of a stably transfected HEK-293 (human embryonic kidney) cell line expressing the alpha3 and beta2 subunits, together with the nAChR chaperone protein NACHO to help increase subunit expression. We have identified the alpha3 + beta2 subunit combination of neuronal nAChRs using single-cell RT-PCR as being highly expressed in rat hippocampal GABA-ergic interneurons. This subunit combination does not appear to be widely expressed in other brain regions, or in other non-neuronal tissues. Electrophsyiological voltage-clamp recordings from the co-expression of alpha3 and beta2 subunits demonstrates that these subunits can make functional nAChRs of at least two subtypes, depending on the relative levels of expression of each subunit, presumably by changing the stoichiometry of the pentamers. This cell line provides a screening tool for identifying potential novel drug compounds that are subtype specific for targeting hippocampal interneurons and potentially influencing learning and memory.

Disclosures: S.N. Sudweeks: A. Employment/Salary (full or part-time):; Brigham Young University. **C. Callison:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.22/C25

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH/INBRE grant P20GM103395

Title: Design and optimization of nicotinic acetylcholine receptor subtype selective peptides

Authors: *B. C. V. OBRIEN, L. WEBER, M. WELTZIN;
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Abstract: Nicotinic acetylcholine receptors (nAChRs) are widespread throughout the brain, express as distinct subtypes, and play pivotal roles in neurologic diseases such as major depressive disorder (MDD) and Alzheimer's disease. Two of the most significant barriers to treatments targeting these receptors are the ability to cross the blood brain barrier (BBB) and nAChR subtype specificity. Previous work has demonstrated that small cell-penetrating peptides (CPPs) can be used to transport therapeutics across the BBB. CPPs developed from the rabies viral glycoprotein (RVG) are capable of interacting with nAChRs but in a non-subtype selective

manner. As neurological diseases are often caused by dysfunction of a specific subtype (i.e., α7 subunit-containing nAChRs in Alzheimer's disease and MDD), CPPs which lack nAChR subtype specificity are less desirable as potential therapeutics. We hypothesize that subtype selective chimeric CPPs are therapeutically beneficial and can be generated from regions of the RVG fused with proteins possessing endogenous nAChR subtype selectivity. We designed nine chimeric peptides generated from three unique proteins with different nAChR subtype targets. Two-electrode voltage clamp (TEVC) electrophysiology was used to determine functional changes in the acetylcholine-induced response of *Xenopus laevis* oocytes expressing distinct nAChR subtypes and isoforms via injection of subunit cRNA biased ratios. Ten nAChR subtypes were separately examined by pre-exposure to a high inhibiting concentration (100 μM) of each chimeric peptide, or the parent RVG peptide. One chimeric peptide displayed an increased selectivity for α7 nAChRs over the parent RVG peptide. Peptide concentration response profiles (0.01 – 300 μM) were performed on α7 nAChRs and the potency for the lead chimeric peptide was determined to be 20 μM, which is improved in comparison to the RVG parent peptide (35 μM). UCSF ChimeraX 1.4 and AlphaFold were used to identify residues important for peptide structure and possible nAChR interactions. Alanine mutagenesis of these residues identified several regions that were further refined to improve α7 nAChR potency and subtype selectivity. Our final generated chimeric peptide has an α7 nAChR potency 3.5-fold greater (10 μM) than that of the parent RVG, while minimally altering the activity of the other nAChRs, including the abundantly expressed α4β2 isoforms. This novel chimeric peptide has the potential to be beneficial in the delivery of research and therapeutic cargo to further the study and treatment of neurological diseases, including Alzheimer's disease and MDD.

Disclosures: B.C.V. OBrien: None. **L. Weber:** None. **M. Weltzin:** None.

Poster

PSTR318. Ligand-Gated Receptors and Ion Channels-Acetylcholine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR318.23/C26

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: 2P20GM103395

Title: Cytotoxicity and internalization properties of a novel alpha7 nicotinic receptor selective peptide

Authors: *L. WEBER, B. C. V. O'BRIEN;
Univ. of Alaska, Fairbanks, AK

Abstract: Cell-penetrating peptides (CPPs) contain less than 30 residues, have the unique ability to enter cells and can cross the blood-brain-barrier (BBB), making them attractive agents for Central Nervous System (CNS)-targeted drug delivery. Examples are CPPs derived from the rabies virus glycoprotein (RVG). RVG-derived CPPs make use of a nicotinic acetylcholine

receptor (nAChR)-mediated mechanism to enter the mouse CNS to deliver cargo, such as siRNA, macromolecules, nanoparticles, or small molecules. However, previously developed RVG-derived peptides lack selectivity for distinct nAChR subtypes, which are associated with different neurological diseases and are unevenly distributed in the CNS. As an example, dysregulation of the homomeric α7-nAChR is involved in Alzheimer's disease and major depressive disorder. An α7-nAChR-selective CPP may be useful in the treatment of above-mentioned CNS diseases, and the enhanced nAChR selectivity will reduce the risk for off-target effects. In an effort to develop a CPP that is subtype-selective for α7-nAChRs, we combined regions of the RVG-CPP with segments of a protein known to specifically interact with the α7-nAChR. However, insertion of protein segments into the RVG-CPP brings the possibility for altering its cytotoxic and cell internalization properties. To investigate these changes for our RVG-chimeric peptide, we cultured and transfected mammalian neuronal-like N2a cells with α7-nAChR subunits and the nAChR chaperone NACHO, and verified α7-nAChR plasma membrane expression using a pH-sensitive fluorescent tag. Cells were treated with the RVG-CPP or our chimeric peptide (0.3 nM to 100 mM) for 24h to obtain their cytotoxic profiles using a redox indicator for cell viability. Separately, α7-nAChR cells were treated with our chimeric peptide fluorescently-tagged with FITC to determine cell internalization properties. Cells were visualized using live cell confocal microscopy and fluorescence was quantified using ImageJ. We observed no cytotoxic effects of the original RVG-CPP or chimeric peptide. Additionally, our data suggests that our lead peptide can enter and transport cargo into neuronal-like cells using an α7-nAChR-mediated mechanism. Our novel α7-nAChR subtype-selective CPP may be useful in research applications requiring cargo delivery and as a vehicle for CNS-targeted drug delivery.

Disclosures: L. Weber: None. **B.C.V. O'Brien:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.01/C27

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Intramural Award to CJM

Title: Regulation of somatostatin interneurons by excitatory GluN1/3A glycine receptors influences hippocampal network rhythms

Authors: *J. KIM[1], A. VLACHOS[1], K. PELKEY[1], R. CHITTAJALLU[1], S. HUNT[1], D. LIU[2], Z. ZHU[2], H. YUAN[2], S. F. TRAYNELIS[3], S. L. SUMMER[4], D. C. LIOTTA[4], C. J. MCBAIN[1]; [1]Section on Cell. and Synaptic Physiol., Eunice Kennedy Shriver Natl. Inst. of Child Hlth. and Human Development, NIH, Bethesda, MD; [2]Emory Univ. Sch. of Med., Atlanta, GA; [3]Emory Univ. Sch. of Med., Sch. of Med., Atlanta, GA; [4]Dept. of Chemistry, Emory Univ., Atlanta, GA

Abstract: Spontaneously synchronized neuronal activity within the central nervous system is critical for activity-dependent neural plasticity, encompassing the maturation of nascent circuits, as well as learning and memory processes in mature circuits across species. Recent studies have highlighted crucial roles for GluN1/GluN3a dihetemeric receptors in influencing the membrane potential and excitability of neurons in the cortex, medial habenula, and amygdala. These unconventional NMDA receptors (NMDARs) differ from conventional GluN1/GluN2 NMDARs, as they are voltage-independent (ie. Mg2+ insensitive) and solely activated by glycine, a co-agonist of conventional NMDARs. Within the hippocampus, somatostatin-expressing interneurons (SOM-INs) prominently express GRIN3A throughout development. Our investigation reveals that the excitability of SOM-INs is profoundly influenced by these enigmatic excitatory glycine receptors (eGlyRs) from early postnatal stages to adulthood. We have observed that eGlyRs have the capacity to strongly modulate the pacemaking activity of SOM-INs in the hippocampal circuit, regulating the frequency of sharp wave ripples (SWRs), network oscillations closely associated with memory consolidation. Remarkably, our findings indicate that endogenous glycine tonically engages SOM-IN eGlyRs, rather than transiently through phasic afferent input, in line with previous observations of tonic occupation of conventional NMDAR co-agonist binding sites. Collectively, our data shed light on the physiological roles of the elusive GluN3A subunit. By tonically exciting SOM-INs, eGlyRs play a crucial role in regulating synchronized network rhythms, ultimately influencing circuit function and memory formation. This novel insight provides a deeper understanding of the interplay between excitation and inhibition in maintaining the delicate balance of neuronal activity.

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Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.02/C28

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Deutsche Forschungsgemeinschaft VI586

Title: The unaffected glycine receptor beta subunit in startle disease -beneficial or detrimental?

Authors: A.-L. ECKES[1], A.-S. HASENMÜLLER[1], I. FUHL[1], C. MILLE[2], O. CORTES CAMPO[1], N. REINHARD[1], *N. SCHAEFER[1], C. SPECHT[2], **C. VILLMANN**[1];
[1]Univ. Hosp. Wuerzburg, Wuerzburg, Germany; [2]French Inst. of Hlth. and Med. Res., Univ. Paris-Saclay, Le Kremlin-Bicêtre, France

Abstract: The rare neurological motor disorder startle disease is caused by disinhibition in the spinal cord and brainstem due to genetic variants in genes (*GLRA1*, *GLRB*) encoding inhibitory

glycine receptor (GlyR) subunits. The adult GlyR is a heteropentamer composed of alpha1 and beta subunits which replaces embryonically expressed GlyRalpha2 homomers. The GlyRbeta subunit is thereby important for synaptic localization of the complex via interaction with the scaffold protein gephyrin. Different human GlyR mutations of *GLRA1* and *GLRB* have been intensively studied *in vitro*. However, the role of unaffected GlyRbeta in the context of mutated GlyRalpha1 *in vivo* remains unclear. Here, we used knock-in mice expressing endogenous mEos4b-tagged GlyRbeta that were crossed with startle disease mutant mice harboring a mutation in the GlyRalpha1 subunit. We explored the role of unaffected GlyRbeta under lethal disease conditions in mice completely missing GlyRalpha1 (*oscillator*) or carrying a missense mutation in alpha1(*shaky*). We identified highest GlyRbeta expression in spinal cord and brainstem nuclei but also in higher brain regions e.g., the periaqueductal gray, thalamus, and hypothalamus. In most cases, colocalization of GlyRbeta and GlyRalpha1 was observed.Interestingly, in both mouse models either lacking GlyRalpha1 or expressing mutated GlyRalpha1 a decreased GlyRbeta expression was found. In *oscillator* animals lacking GlyRalpha1, significantly increased expression of alpha2 was demonstrated arguing for alpha2 becoming a partner of GlyRbeta in heteromeric receptor complexes. Even if increased GlyRalpha2 expression may represent an attempt for compensation, compensation is insufficient and thus unable to overcome lethality of homozygous *oscillator* animals. In *shaky* animals, increased expression of mutated GlyRalpha1 subunits has been identified shifting GlyR complexes to a larger portion of homomeric and thus not synaptically expressed receptors and/or an increase in homomeric presynaptic GlyRs. Considering synapse size, the synapse size was decreased in animals lacking GlyRalpha1 while mice suffering from startle disease but still expressing GlyRs composed of unaffected GlyR beta and mutated GlyRalpha1 did not reveal changes in synapse size. To conclude, the role of unaffected GlyRbeta subunits in startle disease can be interpreted in two directions. A beneficial effect of GlyRbeta in mutant mice is the preservation of the structural integrity of the synapses. In contrast, the presence of mutant GlyRbeta containing receptor complexes at synapses might be detrimental as it may prevent the efficacy of compensation by other GlyRalpha subunits.

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Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.03/C29

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH/NINDS Intramural Support

Title: Effect of chronic stress on inhibitory synaptic development and function

Authors: *S. PANDEY[1], W. HAN[2], W. LU[3];
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Abstract: Chronic stress is a major factor contributing to various neurodevelopmental and neuropsychiatric disorders, including posttraumatic stress disorder (PTSD), anxiety, and depression. A number of studies have suggested that acute stress triggers a cascade of signaling through neuroendocrine system including hypothalamus-pituitary-adrenal glands (HPA axis) to cope up the incoming challenge at the metabolic, immunological and psychological levels. HPA axis also exerts its powerful effect at the synaptic level to modulate the activity of synapses in the brain to maintain a homeostatic environment and adaptivity. Although short term or acute stress has been considered as a beneficial adaptive mechanism, the chronic or recurrent stress has been reported to disrupt the HPA axis and cause a variety of neurological disorders by impairing normal synaptic transmission and their signaling. Hence, it is important to understand the effect of chronic stress on the synapses to develop potential therapeutic approaches. It is well-established that proper functioning of the brain is maintained by the coordinated excitatory and inhibitory synaptic activity, and chronic stress has been shown to affect both glutamatergic excitatory and GABAergic inhibitory synaptic transmission in the brain. The effects of chronic stress on excitatory synaptic transmission have been studied extensively over the last 2-3 decades. However, the involvement of inhibitory synapses and their roles in chronic stress remain largely unclear. We have used two chronic stress models to study the effect of chronic stress and its consequences on inhibitory synaptic development and transmission by utilizing biochemical, physiological, pharmacological, and behavioral approaches. We have observed that chronic stress altered expression of several GABAergic synaptic proteins and synaptic transmission in mouse hippocampal neurons. We have further discovered a stress sensitive signaling pathway and crucial molecules involved in the process. We have also employed pharmacological inhibition approaches targeting signaling molecules to rescue molecular and behavioral deficits in chronically stressed mice. Taken together, our data reveal key molecules and signaling pathways involved in stress-sensitive regulation of inhibitory synapses in the brain, which might provide insights into developing potential therapeutic targets for chronic stress and anxiety disorders.

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Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.04/C30

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NS123933

Title: Tonic GABAA receptor-mediated inhibition in cerebellar Purkinje cells

Authors: *S. MITRA[1], S. N. KHATRI[2], J. PUGH[3];
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Abstract: Cerebellar Purkinje cells show high spontaneous firing rates which are modulated by a dynamic balance of excitation and inhibition. Previous work was shown that phasic inhibition, arising primarily from molecular layer interneurons, is critical for sensory evoked pauses in Purkinje cell, synchrony of firing across Purkinje cells, and regulation of synaptic plasticity at excitatory parallel fiber synapses. However, there is little work examining the influence of tonic GABAA receptor currents on Purkinje cell behavior. We used a variety of electrophysiological, pharmacological and biochemical techniques to investigate tonic GABAA receptor currents in Purkinje cells. In whole cell recordings from juvenile (P14-30) and adult (P50-100) mouse Purkinje cells, we find the application of saturating concentrations of GABAA antagonists (20 µm bicuculline or 25 µm gabazine) results in a ~20 pA decrease in the holding current, indicating tonic GABAA receptor activity. Tonic currents are often contributed to by extrasynaptic GABAA receptors containing delta subunits. To examine if delta subunits are expressed by Purkinje cells, we bath applied delta subunit-specific positive allosteric modulators (20 µM DS2 or 500 nM THIP) and observed a ~20pA increase in the holding current, which was abolished by GABAA receptor antagonists. Additionally, tonic GABAA receptor currents were absent in Purkinje cells from a delta subunit KO animal. Finally, single cell qPCR found significant levels of delta subunit mRNA expression in Purkinje cells. These experiments establish that Purkinje cells express a tonic GABAA current primarily mediated by delta subunit-containing receptors. In order to investigate how tonic inhibition interact with phasic inhibition, we used the mdx mouse model, a model for Duchenne Muscular Dystrophy (DMD) which shows impaired clustering of synaptic GABAA receptors and reduced phasic inhibition. Using photolytic uncaging of RuBi-GABA with a 473nm laser, we recorded GABA currents elicited over a range of laser powers from WT and mdx Purkinje cells. Mdx cells displayed larger responses and a leftward shift in the power-response curve, consistent with increased expression of high-affinity delta-subunit-containing receptors. Likewise, we observed a trend toward larger tonic GABAA receptor currents in mdx Purkinje cells compared to WT, suggesting enhanced tonic currents may compensate for loss of phasic inhibition in Purkinje cells. These data suggest that Purkinje cells have significant tonic currents dependent on delta-subunit-containing GABAA receptors, which may play a critical role in maintaining Purkinje cell firing at an optimal level.

Disclosures: S. Mitra: None. **S.N. Khatri:** None. **J. Pugh:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.05/C31

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: DP1EY033975

Title: Tonic GABAergic activity facilitates dendritic calcium signaling and short-term synaptic plasticity

Authors: *C. Q. CHIU[1], T. M. MORSE[1], K. AIT OUARES[1], F. NANI[2], F. KNOFLACH[2], M.-C. HERNANDEZ[2], M. J. HIGLEY[1];
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Abstract: Brain activity is highly regulated by GABAergic synaptic activity, which acts via diverse GABA-A receptors to suppress somatic spike generation as well as dendritic synaptic integration and calcium signaling. GABA can also evoke a tonic membrane conductance thought to be mediated via distinct GABA-A receptors to inhibit neuronal excitability, though the consequences for dendritic calcium signaling are unclear. Here, we used 2-photon laser scanning microscopy to investigate the role of tonic GABAergic signaling in apical dendrites of layer 2/3 pyramidal neurons from the mouse prefrontal and visual cortex. Unexpectedly, pharmacological blockade of GABA-A receptors paradoxically suppressed dendritic calcium influx in response to somatic action potentials (APs). This effect was mimicked by the selective negative pharmacological modulation of α5 subunit-containing subtypes. Conversely, application of a novel α5-specific positive allosteric modulator enhanced dendritic calcium influx. We then imaged calcium signals in the apical dendrites of layer 2/3 PNs from awake, head-fixed mice and found that negative modulation of α5-containing GABA receptors suppressed dendritic calcium influx without an alteration in neuronal activity. This pharmacological profile is reminiscent of GABAergic synapses formed by somatostatin-expressing interneurons (SST-INs). Indeed, optogenetic stimulation of SST-INs evoked a tonic GABAergic conductance in postsynaptic PNs, suggesting that these cells may be an important contributor to tonic signaling. Computational modeling revealed that tonic GABAergic hyperpolarization of the dendritic membrane potential can de-inactivate low-threshold calcium channels and boost their subsequent activation by back-propagating APs. Confirming this hypothesis, experimental blockade of low-threshold T-type channels reduced the effects of the tonic GABAergic conductance on dendritic signaling. Finally, we found that bidirectional pharmacological manipulation of tonic GABAergic activity influenced the presynaptic short-term suppression of phasic inhibitory inputs to PN dendrites in a calcium-dependent manner. Overall, these results demonstrate that tonic GABAergic signaling can counterintuitively enhance dendritic calcium influx and short-term plasticity of GABAergic synapses. Our work highlights a novel physiological role for α5 subunit-containing GABA-A receptors in the cortex and suggests new avenues for the exploration of GABAergic control of neuronal activity in both health and disease.

Disclosures: **C.Q. Chiu:** None. **T.M. Morse:** None. **K. Ait Ouares:** None. **F. Nani:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **F. Knoflach:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **M. Hernandez:** A. Employment/Salary (full or part-time):; F. Hoffmann-La Roche. **M.J. Higley:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.06/C32

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Pharmacological activation of unfolded protein response promotes proteostasis of epilepsy-associated $GABA_A$ receptors

Authors: *X. CHEN[1,2], Y.-J. WANG[2], T.-W. MU[2];
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Abstract: Protein homeostasis between $GABA_A$ receptor folding, trafficking and degradation is essential to ensure normal physiological functions. Mutations in $GABA_A$ receptors lead to numerous neurological disorders, including genetic epilepsy, and many patients suffering from which are resistant to current drug treatments. Therefore, developing novel therapeutic strategies to target defective $GABA_A$ receptors is critical to effectively treat genetic epilepsy. In this study, we used HEK293T cells that exogenously express disease-associated variants in the β2 subunit of $GABA_A$ receptor (i.e., I246T) with wild type α1 and γ2 subunits. Cell surface biotinylation assay demonstrated that these β2 variants result in significantly reduced surface expression of the β2 protein compared to the wild type (n=4), mainly by decreasing folding and surface trafficking of the mutant receptors. Mechanistically, we further investigated how these mutations affect functional surface $GABA_A$ receptor, including but not limited to, cycloheximide-chase protein degradation assay, immunofluorescence confocal imaging; we studied whether terminally misfolded mutant proteins are preferably degraded through proteasome or lysosome pathways, and of particular interest, which factors are involved in these pathways. Additionally, we searched for small molecules that restore the functional surface expression of the pathogenic receptors. We found that $GABA_A$ receptors-specific pharmacological chaperones restored the surface expression of these β2 mutants (n=4). Western blot analysis further showed that pharmacologically activating the unfolded protein response enhanced the total and surface protein levels of β2 variants (n=4). Co-immunoprecipitation studies are performed to determine whether direct interactions with certain chaperones and factors are enhanced upon drug treatment and how they mediate $GABA_A$ variant receptor trafficking and clearance. Overall, our results demonstrated that these compounds, while having distinct mechanisms of action, hold great therapeutic potentials to treat genetic epilepsy by targeting the disease-associated $GABA_A$ receptor variants.

Disclosures: X. Chen: None. **Y. Wang:** None. **T. Mu:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.07/C33

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: JSPS KAKENHI; grant numbers 19H03409
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JST SPRING, Grant Number JPMJSP2132

Title: Detailed analysis of the mechanism underlying propofol-induced PKC translocation and activation.

Authors: *S. NOGUCHI[1,2], T. KAJIMOTO[5], T. KUMAMOTO[3], T. URABE[4], S. NARASAKI[2,4], K. HARADA[2], I. HIDE[2], S. TANAKA[2], N. SAKAI[2];
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Abstract: Propofol is an intravenous anesthetic agent that exerts its anesthetic effects by activating $GABA_A$ receptors. We have previously shown that propofol induces PKC translocation. In this study, we performed the following experiments to clarify the detailed feature of propofol-induced PKC translocation. (1) To elucidate the character of propofol-induced PKC translocation using various subtypes of PKCs and proteins other than PKC. (2) To elucidate whether PKC is activated at the site where PKC was translocated by propofol using C kinase activity reporter (CKAR). (3) To identify the structural motif involved in the propofol-induced translocation of protein using derivatives of propofol. We used HeLa cells transiently expressing various PKC-GFP, Targeting-CKAR, and 3xGFP proteins by electroporation. The propofol-induced changes in protein localization were observed by time-lapse imaging under a fluorescence microscope or confocal laser-scanning microscope. The PKCs used in the experiments were PKCα (conventional PKC), δPKC (novel PKC), and PKCζ (atypical PKC). Propofol above 100 μM markedly translocated PKCα and PKCδ from the cytoplasm to the plasma membrane, while PKCδ also migrated to the Golgi apparatus. On the other hand, PKCζ migrated from the cytoplasm to the nucleus with a uniform protein concentration in and out of the nucleus. Propofol also induced the translocation of proteins other than PKC into and out of the nucleus, similarly to that of PKCζ. This indicates that PKC translocation into the nucleus is not PKC-specific; Propofol triggers propofol-induced PKC translocation to the plasma membrane and Golgi apparatus in an active fashion, whereas PKC translocation into the nucleus seemed to occur in an passive fashion probably by altering nuclear membrane permeability. Measurement of PKC activity at subcellular sites using CKAR revealed that PKC is activated at the plasma membrane and Golgi apparatus in a propofol concentration-dependent manner. In experiments using isomers and derivatives of propofol, some of the derivatives translocated PKCα to the nucleus rather than to the plasma membrane. This suggests that the structural motif of propofol involved in the translocation of PKC to the plasma membrane differs from that of propofol involved in the translocation of PKC into and out of the nucleus. These results suggest that propofol-induced changes in the localization of PKC and other proteins may be involved in exerting propofol's effects, including adverse effects.

Disclosures: **S. Noguchi:** None. **T. kajimoto:** None. **T. Kumamoto:** None. **T. Urabe:** None. **S. Narasaki:** None. **K. Harada:** None. **I. Hide:** None. **S. Tanaka:** None. **N. Sakai:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.08/C34

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: University of Pennsylvania Department of Psychiatry

Title: Physiologically based pharmacokinetic and pharmacodynamic modeling of midazolam: free brain concentrations associated with clinical endpoints

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Abstract: Introduction: With benzodiazepine administration in human subjects, there are close relationships between brain concentrations, γ-aminobutyric acid type A receptor ($GABA_AR$) modulation, neurophysiological function, and clinical endpoints . Midazolam is a 1,4 benzodiazepine used for sedation, anxiolysis, anterograde amnesia, and anesthesia induction. Its active metabolite, 1-hydroxymidazolam, modulates $GABA_ARs$ at nanomolar levels and has amnestic effects in vivo. Computed midazolam brain concentration-time profiles derived from plasma concentrations can be associated with clinical endpoints utilizing PBPK-PD modeling. **Methods:** Data and initial parameter values were obtained from PubMed and DrugBank searches. Data from the published literature were extracted with WebPlotDigitizer 4.5, and analyzed with Microsoft Excel and Origin 2021b. PBPK models for midazolam and 1-hydroxymidazolam were developed with PK-Sim software. PBPK model parameters were optimized with a Monte Carlo algorithm while simultaneously fitting midazolam and 1-hydroxymidazolam plasma concentrations. Free brain concentrations were determined after PBPK model confirmation. PD models for sedation, cognitive impairment, amnesia incidence, and EEG drug effect were developed with the MonolixSuite 2021R platform. PD model parameters were optimized using the SAEM algorithm. **Results:** Midazolam peak plasma concentration is 85 ng/mL after a 1 mg IV administration. Following a 2.5 mg IV midazolam administration, 1-hydroxymidazolam peak plasma concentration is 4.7 ng/mL. Simulated midazolam and 1-hydroxymidazolam free brain concentrations increase linearly with midazolam dose. Peak potentiation of EC_5 responses from α1β2γ2L and α5β3γ2L receptors is 63% and 42% for a 7.5 mg midazolam administration. PD models demonstrate midazolam free brain concentration-time profiles correspond to changes in peak saccade velocity, digit symbol substitution test scores, amnesia incidence, and EEG effects. Midazolam brain concentrations associated with unresponsiveness, responsiveness, orientation, drowsiness, partial amnesia, and being awake in individuals from this analysis are 52 nM, 35 nM, 14 nM, 10 nM, 8 nM, and 6 nM, respectively. **Conclusions:** Midazolam free brain concentrations correspond to several clinical endpoints following single intravenous administrations. With midazolam administrations at doses used clinically, 1-hydroxymidazolam concentrations reach levels that modulate recombinant $GABA_ARs$ in vitro and likely contribute to endpoints in human subjects.

Disclosures: **P. Burkat:** None.

Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.09/C35

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant F31NS12410
NIH Grant R01MH119154
NIH Grant R01MH128199
NIH Grant 5T32NS099042

Title: Translational control of long-term inhibitory synaptic plasticity

Authors: *T. M. WELLE, D. RAJGOR, J. D. GARCIA, D. KAREEMO, S. M. ZYCH, S. E. GOOKIN, T. P. MARTINEZ, M. L. DELL'ACQUA, C. P. FORD, M. J. KENNEDY, K. R. SMITH;
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Abstract: mRNA translation is crucial for synaptic plasticity and sculpting persistent activity-dependent changes in synaptic strength. Despite its importance in many forms of excitatory plasticity, our understanding of how translation controls synaptic inhibition remains limited. The clustering of $GABA_A$ receptors ($GABA_ARs$) at the inhibitory postsynaptic domain is a key mechanism which rapidly increases synaptic inhibition during inhibitory long-term potentiation (iLTP). However, it is unclear how clustering of $GABA_ARs$ and the inhibitory scaffold gephyrin are maintained long-term during iLTP. We have recently shown that translation of $GABA_ARs$ and gephyrin is required for persistent iLTP and identified an essential excitation-transcription (E-T) coupling pathway which drives $GABA_AR$ translation following iLTP stimulation. This pathway signals transcriptional suppression of miR376c thus promoting translation of its targets, synaptic $GABA_AR$ subunit transcripts, increasing $GABA_AR$ clustering at synapses, and sustaining synaptic inhibition. In contrast, the mechanisms controlling gephyrin translation during iLTP are unknown. Here, we identify miR153 as a novel regulator of gephyrin mRNA translation and protein expression at synapses. We find that reduced miR153 expression is required for persistent iLTP and is driven by the same E-T coupling pathway that downregulates miR376c following stimulation. Our results support a model wherein neuronal activity leverages miRNAs to coordinate changes in expression for multiple target transcripts involved in inhibitory synaptic transmission. Overall, this work provides insight into the role of mRNA translation for sustaining long-term changes in synaptic inhibition and contributes to our broader understanding of molecular mechanisms underlying synaptic plasticity.

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Poster

PSTR319. Ligand-Gated Receptors and Ion Channels-GABA and Glycine

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR319.10/C36

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: T32GM007635
R01MH119154

Title: The nanoscale organization of inhibitory synapses throughout the somato-dendritic axis

Authors: *A. STEWART, S. GOOKIN, J. GARCIA, K. CROSBY, K. SMITH;
Univ. of Colorado, Aurora, CO

Abstract: GABAergic inhibitory synapses mediate synaptic inhibition and regulate neuronal excitability, cell firing, and synaptic plasticity. Inhibitory synapses are distributed across the soma and throughout the dendritic arbor where they are innervated by distinct interneurons which contribute to their diverse functions. Somatic inhibitory synapses synchronize circuits through sculpting neuronal output and spike timing, whereas synapses formed on the dendrites control excitatory activity and dendritic integration. Advances in super-resolution imaging techniques have allowed for increased appreciation of a common, highly organized nanoscale architecture at synapses. Neurotransmitter receptors, scaffolds, and adhesion molecules all cluster into subsynaptic domains (SSDs) aligned with presynaptic neurotransmitter release sites. This nanoscale organization is thought to contribute to efficient neurotransmission by placing low affinity receptors close to sites of neurotransmitter release. For inhibitory synapses this organization may contribute to their unique and striking functional diversity. Here we investigate inhibitory synapse diversity using super-resolution microscopy to interrogate the nanoscale organization of different inhibitory synapse subtypes. We show that throughout the somato-dendritic axis postsynaptic $GABA_A$ receptors and the scaffold gephyrin are differentially organized. Somatic synapses appear consistently larger than dendritic, containing larger postsynaptic domains and larger active zones. Additionally somatic synapses are structurally more complex compared to their dendritic counterparts, exhibiting a more intricate nanoscale organization of $GABA_A$ receptors and the scaffold, gephyrin. Together our data suggest that nanoscale organization of inhibitory synapses could be a key driver underlying the diversity of synaptic inhibition.

Disclosures: **A. Stewart:** None. **S. Gookin:** None. **J. Garcia:** None. **K. Crosby:** None. **K. Smith:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.01/C37

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Fondecyt 1221030
Fondecyt 1161375

Title: Structural Patterns in Mouse and Human TAAR1 Activation: Insights from Molecular Modeling

Authors: *A. I. ROBLES[1], G. TORRES[2], A. FIERRO[3];
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Abstract: Trace amine-associated receptor 1 (TAAR1) is a G protein-coupled receptor (GPCR) activated by phenethylamine, Tyramine, and other endogenous trace amines. TAAR1 has emerged as a novel target for the treatment of neuropsychiatric diseases, and animal models have proven valuable for drug discovery in this context. However, a recent study has demonstrated specie-dependent affinity of EPPTB, an antagonist of this receptor. To further investigate this phenomenon, we conducted a comprehensive structural analysis to explore the conformational changes induced by the interaction of mouse and human TAAR1s with endogenous substrates. Employing in-silico methodologies such as molecular docking (AutoDock 4.0.2), all-atom, and coarse-grained molecular dynamics simulations (Amber18), along with free energy calculations, we examined the structural similarities and differences between mTAAR1 and hTAAR1. Our findings elucidate the distinct role of intracellular loop 3 (ICL3) in the conformational changes of these receptors when interacting with agonists or antagonists. These results offer a potential avenue for understanding species-specific regulatory mechanisms.

Disclosures: A.I. Robles: None. **G. Torres:** None. **A. Fierro:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.02/C38

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant EY002520
Research to Prevent Blindness Physician-Scientist Award
Research to Prevent Blindness Unrestricted Award

Title: Htr1b is required for normal retinal ganglion cell function in mice

Authors: *S. GIBSON, X. TAO, J. MA, G. SHEN, M. POLO-PRIETO, P. PITALE, B. FRANKFORT;
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Abstract: Serotonin plays a critical role in retinal development, neural processing, and visual acuity. Clinical evidence suggests that increased serotonin signaling may be neuroprotective against retinal diseases including glaucoma, a common neurodegeneration of the optic nerve and retinal ganglion cells (RGCs). Here, we demonstrate that serotonin receptor 1B (*Htr1b*) is expressed in RGCs at high levels in both mice and humans. Deletion of *Htr1b* in all cells disrupts vision, retinal electrical activity, and RGC function, but despite these disturbances, retinal anatomy remains preserved in adult mice. Visual behavior tests revealed that $Htr1b^{-/-}$ mice exhibit reduced contrast sensitivity and visual acuity compared to wildtype controls. In vivo measurements of RGC activity using an electroretinogram in $Htr1b^{-/-}$ retinas showed significant temporal processing delays and reduced gain in response to increasing light stimuli. Recordings of individual RGCs using a multielectrode array revealed that $Htr1b^{-/-}$ retinas displayed abnormal RGC light responses in the presence of and after washout of exogenous serotonin. In summary, we found that HTR1B is a key receptor in retinal serotonergic circuitry and is essential for RGC function and vision.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.03/C39

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH Grant AG005214

Title: Acetylcholine mustard and acetylethylcholine mustard irreversibly activate all five muscarinic acetylcholine receptor subtypes

Authors: *J. ELLIS, G. ELMSLIE;
Psychiatry and Pharmacol., Penn State Univ., Hershey, PA

Abstract: Mustard analogs of many receptor ligands have been used to irreversibly label or inactivate receptors. In aqueous solution, mustard groups cyclize to form aziridinium ions that

closely mimic quaternary amines. Such a reactive intermediate may therefore bind in the same orientation within the same binding pocket of the receptor that accommodates the related reversible ligand. Once oriented, it is capable of alkylating any of a number of nearby amino acid side chains. In principle, a mustard analog of an agonist might yield irreversible activation of the receptor. However, acetylcholine mustard (AChM) has been widely reported to possess agonist activity at muscarinic receptors only until alkylation is achieved, at which point the receptor becomes inactivated. Contrary to these previous reports, we have found that agonist activity persists after irreversible binding of AChM and its analog, acetylethylcholine mustard (AEChM), at all five subtypes of muscarinic receptors. The Gq-linked subtypes (M_1, M_3, and M_5) were evaluated by measuring release of radiolabeled arachidonic acid (AA) from intact CHO cells expressing the relevant receptor. After cells were preincubated with cyclized agonist mustards and thoroughly washed, the basal AA release was found to be significantly elevated. This elevation could be prevented if atropine (ATR) was included in the preincubation phase, but was not inhibited by the presence of ATR in the assay phase. By contrast, inclusion of amiodarone in the assay phase dramatically increased the response of cells that were preincubated with the agonist mustards. We have previously shown amiodarone to be a positive allosteric modulator (PAM) at these receptors. Once again, ATR in the assay itself did not prevent the effect of amiodarone, but ATR in the preincubation phase prevented it completely. At the M_1 subtype, the well-known M_1-specific PAM BQCA enhanced the mustard-induced activity in a manner similar to that observed for amiodarone. For the G_i-linked subtypes (M_2 and M_4), enhancement of the binding of the GTP analog GTPγ^{35}S was assayed in membranes of CHO cells expressing the appropriate receptor. Again, pretreatment with the agonist mustards produced a significant response that survived thorough washing and was not inhibited by ATR. Furthermore, the M_2/M_4 PAM LY2119620 enhanced that response in a dose-dependent manner. These studies confirm that amiodarone, BQCA, LY2119620 and other ligands investigated act at an allosteric site and show that muscarinic receptors that have been alkylated by AChM and AEChM do adopt active conformations that can be further enhanced by appropriate allosteric ligands.

Disclosures: **J. Ellis:** None. **G. Elmslie:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.04/C40

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: USDA
NIFA

Title: High-fat diet alters hypothalamic 5-HT_{2C} receptor expression in chicken

Authors: B. ROY[1], S. NAHASHON[2], ***H. M. FENTRESS**[3];
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Abstract: Serotonin 2C (5-HT_{2C}) receptors are G protein-coupled receptors which are expressed on GABAergic, glutamatergic, and dopaminergic neurons. 5-HT_{2C} receptors have been identified as important regulators of obesity in humans and animals. It is well known that 5-HT_{2C} receptor expression levels are inversely proportional to the obesity of any individual. To date, no research has examined the expression levels of the 5-HT_{2C} receptor on birds with high fat content. The aim of the current study was to evaluate the expression levels of the 5-HT_{2C} receptor in broiler chickens when fed a low fat versus high fat diet. In order to perform the experiment, we raised a total of 200 birds (100 males and 100 females) with two main groups: birds fed a high-fat diet (HFD) (100 birds with 10 replications) and birds fed a low-fat diet (LFD) (100 birds with 10 replications). Birds in the HFD and LFD groups were fed high energy ratios containing 11.65% fat and low energy ratios containing 5.62% fat respectively. The hypothalamus of the birds was collected every two weeks until eight weeks of age (WOA). 5-HT_{2C} receptor mRNA expression levels in the hypothalamus of chicken brains were identified by the real-time qPCR reactions based on threshold cycle (Ct) values of the samples. The expression levels of hypothalamic 5-HT_{2C} receptor mRNA were higher in the LFD group of birds compared to the HFD group of birds with significant differences at 6 and 8 WOA ($P<0.05$). However, there were no significant differences in 5-HT_{2C} receptor mRNA expression levels between male and female birds in either of the diet groups. The diet and age both have a significant effect on the expression of hypothalamic 5-HT_{2C} receptor in birds. The LFD increases the expression levels of hypothalamic 5-HT_{2C} receptor after 4 weeks of continuous feeding. On the contrary, HFD gradually decreases the expression levels of hypothalamic 5-HT_{2C} receptor mRNA from 2-6 WOA.

Disclosures: B. Roy: None. **S. Nahashon:** None. **H.M. Fentress:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.05/C41

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NSERC DISCOVERY GRANT (E LAMBE)
CIHR CGS D (S POWER)

Title: Opto-5-HT: interrogating endogenous serotonergic regulation of medial prefrontal cortex with optophysiological approaches

Authors: *S. K. POWER[1], D. SARGIN[4], E. K. LAMBE[1,2,3];
[1]Physiol., [2]Obstetrics and Gynecology, [3]Psychiatry, Univ. of Toronto, Toronto, ON, Canada; [4]Univ. of Calgary, Calgary, AB, Canada

Abstract: The medial prefrontal cortex (mPFC) is essential for cognition, executive function, and emotional behaviour. Serotonergic afferents innervate the mPFC and pharmacological interventions targeting the serotonin system influence emotion and cognition. Exogenous serotonin and related agonists alter neuronal activity in the mPFC. However, these manipulations do not capture many important features of synaptically-released endogenous serotonin signaling (opto-5-HT). Since recent work shows that brief optogenetic stimulation of serotonergic inputs to the mPFC enhances cognitive flexibility in mice, we interrogate the impact of opto-5-HT release for the electrophysiological state and activity of this region's major output neurons. Using stimuli with behaviourally-relevant timing, we find that endogenous serotonin release inhibits deep-layer pyramidal neurons *ex vivo*. With pharmacological intervention, we probed the molecular mechanisms of this inhibition and find that, while it persists in the presence of synaptic blockers, it is significantly suppressed by antagonism of 5-HT_{1A} receptors. Since endogenous 5-HT signaling is richer and more complex than that achieved with exogenous agonism, we are probing its pre- and post-synaptic regulation. An area of particular interest is the impact of selective serotonin reuptake inhibitors (SSRIs). Optophysiological approaches provide a novel opportunity to examine this cognitively- and emotionally essential synapse.

Disclosures: S.K. Power: None. **D. Sargin:** None. **E.K. Lambe:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.06/C42

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Camden Health Research Initiative (CHRI), Rowan University

Title: Unlocking Antipsychotics Molecular Mechanisms in Brain: Insight into 5-$HT_{2A}$and D_2receptor heterodimers

Authors: *G. A. CARRASCO;
Biomed. Sci., Cooper Med. Sch. of Rowan Univ., Camden, NJ

Abstract: Dopamine 2 (D_2) and serotonin 2A (5-HT_{2A}) receptors are implicated in the pathogenesis of psychotic disorders due to their involvement in modulating neurotransmission and synaptic plasticity. Hyperactivity of dopaminergic transmission in the mesolimbic pathway, particularly at D_2 receptors, has been associated with positive symptoms of schizophrenia, such as hallucinations and delusions. On the other hand, 5-HT_{2A} receptors, widely expressed in cortical pyramidal neurons, have been linked to the negative symptoms and cognitive deficits of schizophrenia, with atypical antipsychotics targeting both D_2 and 5-HT_{2A} receptors to alleviate these symptoms. Importantly, interactions between $G_{q/11}$-coupled 5-HT_{2A} receptor and the $G_{i/o}$-coupled D_2 receptor would form heterodimers that would be specifically targeted by antipsychotics. These heterodimers, observed in prefrontal cortex and striatum, could explain

some of the therapeutics effects of antipsychotics.
Here, we tested a non-selective cannabinoid agonist (CP55940), a selective CB1 agonist (ACEA), and a selective CB2 agonist (GP1a) over a 72-hour period. Our findings showed that CP55940 treatment significantly increased D_2 receptor protein levels by 99 ± 7% compared to controls ($p<0.01$). Surprisingly, both GP1a and ACEA also increased D_2 receptor protein levels, with increases of 35 ± 7% and 39 ± 5%, respectively. Co-treatment with GP1a and ACEA resulted in a 76 ± 8% increase. Notably, while CP55940 and GP1a upregulated D_2 mRNA expression, ACEA did not, indicating distinct signaling mechanisms for CB1 and CB2 receptors in regulating D_2 protein expression. Furthermore, we discovered that both selective and non-selective cannabinoid agonists significantly enhanced the formation of a 5-HT_{2A}-D_2 receptor heterodimer in neuronal cells ($p<0.01$), assessed through co-immunoprecipitation of 5-HT_{2A} and D_{2L} receptors. Importantly, the cannabinoid-induced formation of this heterodimer was inhibited in cells expressing GRK5, but not GRK2, shRNA. Olanzapine, a typical antipsychotic, effectively suppressed the cannabinoid-enhanced formation of the 5-HT_{2A}-D_{2L} receptor heterodimer ($p<0.01$). This was associated with reduced membrane-associated protein levels of 5-HT_{2A} and D_{2L}. Indeed, our preliminary results suggest that the heterodimer may be influenced by GRK5 levels, which promotes its formation, while another GRK2 protein levels inhibits its formation.
A deeper understanding of their mechanism of antipsychotics action could pave the way for the development of safer and more effective treatments, improving the quality of life for millions of patients.

Disclosures: G.A. Carrasco: None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.07/C43

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NINDS Intramural Research Program

Title: Characterization of positive allosteric modulators that enhance dopamine's binding affinity and potency for signaling at the D_1 dopamine receptor

Authors: J. N. HANSON[1], A. N. NILSON[1], F. WANG[2], J. RAYADURGAM[2], K. D. LUDERMAN[1], B. FREE[1], K. J. FRANKOWSKI[2], *D. R. SIBLEY[1];
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Abstract: The D_1 dopamine receptor (D1R) is a G protein-coupled receptor that facilitates multiple functions in the CNS including motivation, movement, and reward following dopamine (DA) stimulation. Reduction in DA signaling at the D1R is linked to several neurological

disorders such as Parkinson's disease. Additionally, drugs that enhance DA signaling at the D1R may prove beneficial for the treatment of cognitive impairment. Unfortunately, D1R orthosteric agonists have largely failed in the clinic due to poor bioavailability, rapid metabolism, and tolerance. However, positive allosteric modulators (PAMs) may provide an alternative and more effective treatment approach. PAMs may exhibit higher target selectivity by binding to less conserved regions of the D1R and provide a larger therapeutic window. Previously our lab implemented a high throughput screen of the NIH Molecular Libraries Program 400,000+ small molecule library to identify potential PAMs of the D1R. Following the primary screen/hit selection, validation of the hit compounds, and optimization of the hits, we identified MLS6585 as a lead compound. MLS6585 potentiates D1R-mediated β-arrestin signaling, increasing the potency of DA by 5-fold, without showing any intrinsic agonist activity in the assay. Also, MLS6585 increases DA affinity by ~5-fold in radioligand binding assays. Analogs of MLS6585 were made to increase its efficacy for potentiating DA affinity and signaling at the D1R. Over 110 analogs of MLS6585 were tested in a β-arrestin recruitment assay and two analogs, UNC9815 and UNC10062, were identified as compounds that could potentiate DA signaling more effectively than MLS6585. At a single high dose of the PAM, UNC9815 increased DA potency (EC_{50}) by 11-fold, whereas UNC10062 only increased DA potency by 4-fold but increased the maximum response (Emax) by 220%. Also, UNC9815 showed a 6-fold increase in DA affinity, whereas UNC10062 showed no increase in DA affinity. We used both MLS6585 and UNC9815 to interrogate the D1R structure using mutagenesis studies to identify the PAM binding site. Preliminary studies show that the binding site for MLS6585 and its analogs likely involves the transmembrane 7 (TM7) region of the receptor. Using point mutations of certain amino acids within the TM7 region, phenylalanine at position 319 (F319) of the D1R was identified as an important residue needed for PAM activity. Identification of this binding site may prove beneficial for optimization of future D1R PAMs using structure-guided analog design and may aid in the discovery of therapeutically viable compounds in the future.

Disclosures: **J.N. Hanson:** None. **A.N. Nilson:** None. **F. Wang:** None. **J. Rayadurgam:** None. **K.D. Luderman:** None. **B. Free:** None. **K.J. Frankowski:** None. **D.R. Sibley:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.08/C44

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIDA-Intramural Research Program

Title: Biased agonism may explain the different striatal presynaptic/postsynaptic profile of preferential dopamine D3 receptor agonists

Authors: *A. HICKS[1], W. P. REA[1], E. MORENO[2], N.-S. CAI[1], V. CASADÓ[2], A. H. NEWMAN[1], S. FERRE[1];

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Abstract: Previous studies using selective dopamine D_3 receptor (D_3R) ligands in reserpinized mice have provided evidence for a significant role of striatal postsynaptic dopamine D_1 receptor (D_1R)-D_3R heteromers in the locomotor activity mediated by D_1R agonists. D_3R antagonists such as PG01037 show cross-antagonism in the heteromer and counteract the D_1R agonist-mediated locomotor activity, while preferential D_3R agonists such as pramipexole, quinpirole, and PD128907 synergistically potentiate D_1R agonist-mediated locomotor activity. The synergistic effect of D1R and D3R agonists is mediated by a G protein-independent and β-arrestin-dependent mechanism, likely involved in the pathogenesis of L-dopa-induced dyskinesia. When we evaluated the effect of the same preferential D_3R agonists in non-reserpinized mice, we observed the expected result of locomotor depression by a presynaptic mechanism not present in reserpinized mice. Interestingly, the minimal doses of pramipexole and quinpirole to promote locomotor depression were between 30 and 100 times lower than those promoting potentiation in reserpinized mice counterparts. However, we found no difference in the minimal doses of PD128907. We therefore questioned the mechanism behind the differential striatal presynaptic/postsynaptic profiles of pramipexole and quinpirole. Since the three compounds are not highly selective for D_3R, we first assumed that activation of other D_2-like receptor subtypes could be involved in their differential behavioral profile. For example, $D_{2S}R$ and D_4R are other D_2-like striatal presynaptic receptors that promote inhibition of dopamine release when directly or indirectly activated. Furthermore, we also considered the differential effect of the drugs on D_2-like receptor heteromers. Hence, we first analyzed the ability of the three compounds to activate Gi/o proteins (Go1) in bioluminescence resonance energy transfer (BRET) experiments, with the BRET donor and acceptor chromophores fused to the α and γ subunits, respectively, in HEK-293T cells transfected with D_3R, $D_{2S}R$, $D_{2L}R$, $D_{4.4}R$, $D_{4.7}R$ alone or co-transfected with D_3R and D_1R or $D_{2S}R$ and $D_{4.4}R$ or $D_{4.7}R$. The relative potencies (EC_{50} values) for the isolated and co-transfected receptors were overall very similar. Moreover, they all showed highest affinities for the D_3R alone or co-transfected with D_1R. We therefore suggest that the different striatal presynaptic/postsynaptic profiles of the three compounds may be related to a G protein-biased agonism of pramipexole and quinpirole. Experiments on β-arrestin recruitment are in progress to test this hypothesis.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.09/C45

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: CIHR PJT-173511
NSERC RGPIN/04394-2019

Title: Dopamine receptor expression in the mesencephalic locomotor region of mice

Authors: *S. DI VITO[1,2], C. R. BELWAY[3,2], S. SHARMA[2,3], P. J. WHELAN[3,4];
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Abstract: Dopaminergic (DA) control of motor function is understood to be indirect via substantia nigra pars compacta (SNc) projections to the striatum. DA interaction with dopamine D1or D2-receptor (D1R or D2R) expressing cells in the striatum leads to locomotion via disinhibition of Mesencephalic Locomotor region (MLR) neurons. The MLR is known to initiate locomotor movements when electrically stimulated in the absence of forebrain inputs. It consists of the cuneiform nucleus (CnF) and pedunculopontine nucleus (PPN). These regions are heterogeneous and show differential effects on locomotion following stimulation of either glutamatergic or GABAergic cells. The MLR receives predominantly inhibitory input from the basal ganglia. Recently, our lab discovered that a dopaminergic nucleus within the medial zona incerta (mZI); the A13, projects to the MLR. Direct dopaminergic projections that modulate locomotion, from the SNc to the MLR, have also been identified in lampreys and rats. This suggests at least two direct dopaminergic control pathways to the MLR that lie parallel to the canonical nigrostriatal DA pathway for motor control.It is not well understood how dopamine receptors are distributed across cellular subtypes in the MLR. We used adult C57BL/6 male and female mice to address this gap. RNAscope® was performed to determine the distribution of DA receptor mRNA on vGLUT2 and VGAT neurons within the PPN and CnF. Preliminary results suggest that D1R and D3R are sparsely expressed, while D5R shows modest expression in both PPN and CnF. On the other hand, D2R were robustly expressed in both the CnF and PPN showing coexpression in both vGAT and vGLUT2 neuronal populations. Our work suggests that DA modulation of MLR neurons may be predominantly D2R mediated and could play a role in opposing behaviours, due to expression on both glutamatergic and GABAergic cells. This work forms the foundation for future studies which will probe the role of dopamine in MLR function.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.10/C46

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NINDS Intramural Research Program
NCATS Intramural Research Program

Title: Developing a novel D_2 dopamine receptor antagonist from hit to lead candidates for the treatment of neuropsychiatric disorders

Authors: *A. N. NILSON[1], J. N. HANSON[1], A. E. DULCEY GARCIA[2], T. POUDEL[2], R. FREE[1], R. R. CALVO[2], J. J. MARUGAN[2], D. R. SIBLEY[1];
[1]Mol. Neuropharm. Section, Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [2]Div. of Pre-Clinical Innovation, Natl. Ctr. for Advancing Translational Sci., Rockville, MD

Abstract: The D_2 dopamine receptor (D2R) is a G protein-coupled receptor (GPCR) and a validated drug target for many neuropsychiatric disorders. All currently available antipsychotic medications are antagonists of the D2R; however, most antipsychotics also exhibit poly-pharmacology in that they have affinity for other GPCRs. As such, there are a plethora of off-target side effects associated with antipsychotics including weight gain, dysphoria, and sedation, among others. A highly selective D2R antagonist could overcome these pitfalls and increase patient compliance with antipsychotic medications. Thus, our group conducted a high throughput screen in search of a novel chemical scaffold that exhibits high selectivity for the D2R compared to other GPCRs. One hit compound, MLS6916, was screened for global selectivity in the Psychoactive Drug Screening Program and for activity at 168 GPCRs in the Eurofins gpcrMAX panel. Both screens used a single high concentration of MLS6916 and revealed extreme D2R selectivity with the only other target showing activity being the D4R. The MLS6916 scaffold was chemically optimized to remove a reactive hydrazide moiety and structure activity relationship analyses were conducted to improve metabolic stability. The criteria for chemical optimization included a D2R binding affinity between 50-100 nM, increased metabolic stability, and maintenance of D2R selectivity over the highly related D3R and D4R. Over 115 analogs were analyzed using D2R, D3R, and D4R radioligand binding assays as well as antagonism of dopamine-stimulated beta-arrestin recruitment. Additionally, compound stability in mouse, rat, and human liver microsomes was assessed. Combining these results led to the identification of NCGC1360. NCGC1360 has >100-fold selectivity for the D2R compared to the D3R and D4R in functional beta-arrestin recruitment assays and a Ki of 81 nM for the D2R. NCGC1360 was advanced into pharmacokinetic studies using a 30 mg/kg (i.p.) dose in mice. The half-life of NCGC1360 was 1.8 hours in plasma and 1 hour in brain with excellent brain penetration at ≥1:1 brain:plasma ratio indicating adequate pharmacokinetics for further *in vivo* studies. We are currently testing NCGC1360 in animal models that are predicative of antipsychotic efficacy and on-target side effects such as amphetamine-induced hyperlocomotion and catalepsy, respectively. Finally, we are testing NCGC1360 in preliminary toxicity studies including cytotoxicity, cytochrome P450 inhibition, and hERG channel inhibition. Taken together, these studies provide strong support for the development of NCGC1360 into a potential new antipsychotic medication with fewer side effects.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.11/C47

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Effects of dopamine D3 receptor activation on mouse medial prefrontal cortical layer 5 pyramidal cell resonance

Authors: N. MOHAMED, ***M. THOMAS**;
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Abstract: Dopamine plays an important role in cognition, including working memory, but little is known on the cellular level about dopamine's actions on its D3-type receptor (D3R). Theta rhythms localized to the medial prefrontal cortex (mPFC) are important components of working memory, and "Type I" layer 5 pyramidal cells, defined by subcortical projection patterns, are resonant at theta frequency. Theta resonance is mediated by expression of the HCN channel, readily identified in current clamp recordings as a prominent "sag" in the membrane potential in response to a strong hyperpolarizing pulse. We examined the effects of two selective D3R agonists on HCN current (as determined indirectly by effects on "sag" amplitude) and electrical resonance in mouse mPFC Type I layer 5 pyramidal cells.Tissue slices from mPFC were prepared from adult mice (C57 BL/6). Whole-cell patch recordings were made from layer 5 cells at 32 C using standard methods to acquire and analyze current clamp recordings. Hyperpolarizing current pulses (500 msec, 200 pA) were applied and the "sag" was calculated as (maximum hyperpolarization amplitude - amplitude at 400 msec) and expressed as percent max hyperpolarization. In cells determined to be type I cells (described below), a constant-amplitude (100 pA) sinusoidal current was injected with frequency swept from 0-10 Hz over 10 sec. The resonant frequency (Fr) was determined as the frequency where maximum P-P amplitude occurred during the sweep. Sag amplitude and Fr were determined for each Type I cell in control solution and following a 5 min application of a D3R agonist (PD128907 or SK609, both at bath concentrations of 10 uM). Resting membrane potential and cell input resistance were also measured for each cell in control and drug solutions, to account for any D3R effects not targeted directly to the HCN current.Neurons designated as type I displayed significantly larger "sags" (18.2 ± 4. 4%) than those designated as type II (8.30 ± 5. 6%). D3R activation significantly inhibited sag amplitude
in these cells [mean of 15.5% in control versus 6.5% in PD-128907 (P = 0.008, n=6), mean of 17.3% in control versus 9.8% in SK609 (P = 0.0004, n=5)]. Consistent with sag depression, both agonists significantly decreased Fr in type I cells [Overall: mean Fr was 2.1 Hz (n= 8); mean Fr 1.8 Hz in control versus 0.7 Hz in PD-128907 (P = 0.02, n=4), mean Fr 2.4 Hz in control versus 0.5 Hz in SK609 (P = 0.0002, n=4)]. Thus, D3R activation resulted in changes in mPFC layer 5 resonance properties, with potential implications for network connectivity during working memory tasks and other cognitive functions dependent on optimal theta coupling between mPFC and other CNS regions.

Disclosures: **N. Mohamed:** None. **M. Thomas:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.12/C48

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: DA R33041876
Department of Pharmacology and Toxicology
Stark Neurosciences Research Institute
IUSM Strategic Research Initiative

Title: Role of Spinophilin in dopamine 2 receptor neuroadaptations in a striatal dysfunction context

Authors: *B. HENS, A. BAUCUM, II;
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Abstract: The striatum is a major input to the basal ganglia. The striatum is involved with neuronal activity that is responsible for reward, movements, and connection between them. Dysfunction in the striatum is associated with the symptoms of myriad disorders, including substance use disorder, schizophrenia, obsessive compulsive disorder, Huntington's disease, and Parkinson’s disease. The dorsal striatum has two different neuronal cell types: medium spiny projection neurons (MSNs) and interneurons such as cholinergic interneurons (CINs). MSNs consist of direct pathway (dMSNs) and indirect pathway (iMSNs) neurons. dMSNs express dopamine 1 receptor (D1Rs) and function in promoting movement. In contrast, iMSNs express dopamine 2 receptors (D2Rs) and function in movement inhibition. Alterations in striatal dopamine, as is observed in substance use disorder and Parkinson’s disease, bidirectionally modulates dopamine release leading to an imbalance in reversible protein phosphorylation downstream of the dopamine receptors. Serine/threonine protein phosphorylation is mediated by kinases and highly promiscuous phosphatases. Spinophilin is the major postsynaptic density protein phosphatase 1 (PP1) targeting protein. Spinophilin is highly expressed in the striatum and we have found that spinophilin is regulated by, and modulates behavioral impacts of, dopamine on motor outputs. While spinophilin interacts with D2R, but not the D1R, how spinophilin impacts D2R function and subcellular localization in the context of striatal dysfunction is unclear. Given our previous studies, we hypothesize that spinophilin modulates dopamine-dependent motor output by actions on postsynaptic D2R function. Consistent with this hypothesis, we found that global loss of spinophilin led to a tolerance to the locomotive-suppressing effects of D2R agonist, quinpirole (3 mg/kg), a dose found to consistently impair locomotion. Potential mechanisms by which spinophilin mediates quinpirole-induced locomotor tolerance will be reveled through proteomics analysis. Together, understanding spinophilin’s role in modulating D2R function and subsequent downstream signaling could contribute to potential therapeutic drugs for diseases associated with striatal dysfunction.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.13/C49

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Lundbeck Fundation
Independent Research Fund Denmark 1030-00452B

Title: Emerging functional heterogeneity of D1-medium spiny neurons challenges the paradigm of low-affinity dopamine D1 receptors

Authors: *A. KONOMI PILKATI[1], T. ANDREASSEN[2], A. T. SØRENSEN[3], K. L. MADSEN[3], U. GETHER[4];
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Abstract: The basal ganglia are a collection of subcortical nuclei that have been linked to movement, emotion, motivation, and learning. The striatum, which serves as the gateway to the basal ganglia circuit, receives dopaminergic neurons from the midbrain as well as other forms of input from various brain regions. The striatum is structurally and functionally segregated; the dorsal striatum is involved in habit formation whereas the ventral striatum is in goal-directed actions. Its main cell type is the medium spiny neurons (MSN), further divided into dopamine D1-receptor (D1R-MSNs) and D2-receptor MSNs (D2R-MSNs). The D1R is considered to have less affinity to dopamine (DA) (responding at the µM range) as compared to the high-affinity D2R (nM range). It has accordingly been assumed that D1R-MSNs sense phasic release of DA while D2R-MSNs sense lower levels of tonic DA release. Here, we study D1R-MSNs sensitivity to DA in striatal primary cultures derived from D1-Cre mice pups or wild-type E19 rat embryos and in acute adult brain slices transduced and stereotactically injected with the genetically encoded protein kinase A (PKA) sensor ExRai-AKAR2 respectively. By using live imaging epifluorescence and 2-photon microscopy, we can track PKA activity in individual neurons with high temporal and spatial resolution. Strikingly, we observe that individual D1R-MSNs exhibit differential responsiveness to DA with a subpopulation of D1R-MSNs responding to nanomolar concentrations of DA while other D1R-MSNs require micromolar concentrations. The D1R-MSNs display a DA-response spectrum. This phenotype appears to be spatially segregated in the striatum and is seemingly characteristic of MSN when compared to other neuronal types. Furthermore, the heterogeneity among MSN not only relies on the DA concentration-response, but also on how this signal is translocated to the nucleus. In addition to revealing a so far unappreciated dynamic signaling heterogeneity of D1R-MSNs, the data challenge the classical assumption of low-affinity D1Rs and high-affinity D2Rs. Further ongoing studies are targeted toward dissecting the underlying cellular mechanism and the putative role in DA-linked memory processes.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: MCIU-AEI-FEDER-EU Grant PID2020-118511RB-I00

Title: Insights of schizophrenia-associated dopamine D_2 receptor variants: interplay with the adenosinergic system

Authors: M. VALLE LEON[1], C. LLINAS DEL TORRENT[2], P. ÁLVAREZ-MONTOYA[3], M. LÓPEZ-CANO[3], N. CASAJUANA-MARTIN[2], S. FERRÉ[1], L. PARDO[2], K. SAHLHOLM[4], ***F. CIRUELA**[3];
[1]NIH, NIDA, IRP, NIH, NIDA IRP, Baltimore, MD; [2]Autonomous Univ. of Barcelona, Bellaterra, Spain; [3]Pathology and Exptl. Therapeut., Univ. de Barcelona, L'HOSPITALET DE LLOBREGAT, Spain; [4]Karolinska Institutet, Karolinska Institutet, Stockholm, Sweden

Abstract: Schizophrenia has a prevalence rate of approximately 1 in 300 people worldwide. Researchers have proposed various hypotheses to better understand the complex nature of schizophrenia, with a primary focus on investigating the roles and interactions of different neurochemical systems (i.e., dopaminergic, serotonergic, and glutamatergic, among others) in its development and manifestation. Recently, we contributed towards the understanding of the involvement of the adenosine A_{2A} receptor ($A_{2A}R$) in the pathophysiology of schizophrenia. Interestingly, the adenosinergic control of the striatal dopaminergic system is highly dependent on the molecular and functional interactions between the A_2R and dopamine D_2 receptor (D_2R) localized in the GABAergic striatopallidal neurons. A hypoadenosinergic state, with a decrease in extracellular adenosine concentration or a relative downregulation of $A_{2A}R$ versus D_2R expression, should reduce the well-established tonic allosteric inhibition of D_2R function mediated by $A_{2A}R$ within the context of the $A_{2A}R$-D_2R heteromer. Therefore, it has been postulated that increasing this allosteric inhibition could represent a promising strategy for the treatment of schizophrenia. Here, we aimed at evaluating the impact of schizophrenia-associated D_2R variants, namely S311C, K327E, R360H, R150H, and R220H mutants, on the ability of the receptor to heteromerize with $A_{2A}R$. First, D_2R mutants were expressed in HEK-293 cells and the subcellular distribution was analyzed by confocal microscopy analysis. All D_2R mutants expressed well and co-distributed with $A_{2A}R$. Next, we analyzed cell surface expression by biotynilation experiments. While the S311C, K327E, R360H and R220H mutants reached the cell surface similarly to the wild-type receptor, the R150H mutant was significantly less expressed at the cell surface, remaining intracellularly. All D_2R variants showed comparable signaling properties as determined by ability to reduce cAMP accumulation and capacity to activate G protein-gated inwardly rectifying potassium channels (GIRKs). Finally, we determined the ability of D_2R mutants to heteromerize with $A_{2A}R$ in mammalian cells using a NanoBiT assay. The results revealed that all D_2R variants retained the ability to form heteromers with $A_{2A}R$. However, we observed variations in the effects of haloperidol and aripiprazole on the density of $A_{2A}R$-D_2R heteromers among some of the D_2R mutants, which could explain some

differences in the response to these medications mediated by differences in the $A_{2A}R$-mediated allosteric control of mutant D_2R.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR320.15/C51

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Italian Ministry for University Education and Research (Prin: 2017K2NEF4)

Title: Gpr158 activation enhances excitability of medium spiny neurons in the nucleus accumbens

Authors: G. ACETO[1,2], L. NARDELLA[1,2], C. COLUSSI[3,2], C. GRASSI[1,2], ***M. D'ASCENZO**[1,2];
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Abstract: It has been recently demonstrated that the amino acid glycine is the endogenous agonist of GPR158, a class C orphan G protein-coupled receptor (GPCR) (Laboute et al., 2023). The effect of glycine on cAMP signaling was potentiated when GPR158 was coexpressed with regulator of G protein signaling 7 and protein β5 (RGS7-Gβ5), indicating that GPR158 exerts its effects on cAMP via this protein complex. The levels of GPR158 expression are particularly high in the nucleus accumbens (NAc), a major input structure of the basal ganglia that integrates information from cortical and subcortical structures to mediate goal-directed behaviors. However, it is yet unknown how glycine, through activation of GPR158, could affect the intrinsic excitability of medium spiny neurons (MSNs) in the NAc. To fill this gap, we performed whole-cell patch-clamp recordings and found that postsynaptic GPR158 activation by glycine administration elevates the excitability of MSNs measured as number of action potentials in response to a given depolarization. The evoked firing increased after 1-2 minutes of bath glycine administration and remained elevated for minutes. Consistent with the negative modulation of low voltage-activated potassium current (M-current), GPR158 activation reduced the latency to fire and diminished action potential afterhyperpolarization. The increased excitability was protein kinase A-dependent (PKA) and associated with decreased M-currents measured in voltage-clamp configuration. Furthermore, selective pharmacological inhibition of the Kv7.2 channel, the main molecular determinant of M-currents in MSNs, mimicked the

increased excitability induced by GPR158 activation. Together, our findings indicate that glycinergic transmission in the NAc increases MSN intrinsic excitability through GPR158-dependent modulation of Kv7.2 channels.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.16/C52

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

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RI-MUHC Studentship Award 2020

Title: Heterogeneous transmission at cerebellar parallel fiber-Purkinje cell synapses

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Abstract: The cerebellum is known for its involvement in diverse motor and non-motor functions. However, the cerebellar cortex has a homogeneous cytoarchitecture that repeats across cerebellar lobules, and is thought to perform similar computations. How does such a homogeneous circuit architecture lead to a diversity of functions? Recent studies indicate that heterogeneity in spiking properties and synaptic plasticity could account for some of the heterogeneity in cerebellar function. In addition, molecular heterogeneity has been described, which largely covaries with the expression of aldolase C, also known as zebrin. Here, we demonstrate that synaptic transmission on longer timescales is also heterogeneous across the cerebellum. We focused on synaptic transmission at one of the key synapses of the cerebellar cortical circuit: the parallel fiber (PF)- Purkinje cell (PC) synapse, which carries the majority of the information to Purkinje cells, the sole output neurons of the cerebellar cortex. Using C57BL/6J mice (both sexes) of age P(postnatal day)23-P35, we performed patch-clamp electrophysiology on PCs from acute cerebellar slices. We measured slow synaptic currents via the excitatory glutamatergic, metabotropic glutamate receptor 1 (mGlu1) at the PF-PC synapses. These currents are sustained for a few hundred milliseconds and therefore work on timescales relevant to behaviors mediated by the cerebellum. Surprisingly, we discovered a marked heterogeneity in these currents across the three different cerebellar regions tested: vermis lobules

4/5, vermis lobule 10, and the flocculus. The slow currents were strikingly delayed in lobule 10 and flocculus compared to lobules 4/5. Owing to this heterogeneity in slow currents, we found that PC action potential firing in response to PF synaptic inputs was also markedly different. Moreover, we interrogated the relationship of the heterogeneity we observe with zebrin, since many molecules downstream of mGlu1 activation are correlated with zebrin expression. We found that although cells with longer delays were in a predominantly zebrin-positive zone and cells with shorter delays in a predominantly zebrin-negative zone, the zebrin identity of the cells alone could not predict the timing. Thus, our findings demonstrate a remarkable and previously unknown heterogeneity in excitatory slow synaptic transmission in the cerebellum. We show that such heterogeneity in synaptic currents can drive differences in the timing of PC spiking in response to seemingly similar PF synaptic inputs across functionally different regions.

Disclosures: R.E. Thomas: None. **F. Mudlaff:** None. **A. Suvrathan:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.17/C53

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: ANR French grant agency

Title: A pharmacological signature to selectively determine mGlu7 containing heterodimers

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Abstract: mGlu7 homodimers have an apparent very low affinity for glutamate much lower than that of the other mGluRs. We hypothese that mGlu7 is mainly present on the brain as heterodimer. It was already highlighted that mGlu7 subunit is able to heterodimerize, especially with the mGlu2 subunit. Here we determined the pharmacological in order properties of mGlu2-7 and compared it to those of mGlu7 and mGlu2 homodimers. Our final aim is to be able to identify the mGlu2-7 heterodimer throught its pharmacological features in cerebral tissue. We used second messenger signaling assays to study the pharmacological properties of mGlu2, mGlu7 and mGlu2-7 transiently expressed in HEK cells together with the chimeric G protein GqTop. For the mGlu2-7 we used the optimized quality control system based on the GABAB receptor to make sure that only the heterodimer reached the cell surface. Both orthosteric and allosteric ligands for mGlu2 and mGlu7 was tested on each homodimer, and then tested on mGlu2-7 heterodimers. We identified a specific combination of ligands that can be used as a pharmacological signature of this heterodimer relative to the homodimers, as already found for

the mGlu2-4 heterodimer. Functionnally, there is a symmetric activation of the heterodimer mGlu2-7 leading preferentialy to mGlu7 G protein coupling. The assays on mGlu2-7 allowed us to identify ligands that act differently on mGlu7 depending on it's involvement in an homo or heterodimeric entity. This pharmacological signature will be used to identify mGlu2-7 heterodimers in native tissues.

Disclosures: K. Belkacemi: None. **F. Bertaso:** None. **I. McCort:** None. **F. Acher:** None. **L. Prezeau:** None. **J. Pin:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.18/C54

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Intramural funds of NDIA

Title: Heteromers of μ-opioid receptor and corticotropin release factor receptor CRF_1 control glutamatergic transmission in the central amygdala

Authors: *W. N. SANCHEZ LUNA[1], E. MORENO[2], A. YAUCH[3], N. CASAJUANA[4], N.-S. CAI[1], V. VICENT CASADÓ[2], L. PARDO[4], L. D. PLANT[3], S. FERRE[1];
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Abstract: The Central amygdala (CeA) plays an important role in negative-like states such as anxiety, fear, stress, and in opioid withdrawal. μ-opioid receptor (MOR) and corticotropin release factor receptor CRF_1 (CRF_1R) are expressed within CeA neurons and locus coeruleus, where they mediate biochemical changes associated with opioid withdrawal. Both receptors are co-localized in the locus coeruleus, but their co-expression in the CeA needs to be still demonstrated. We investigated if when co-expressed in the same cells MOR and CRF_1R form heteromers, which could then provide new targets for opioid withdrawal syndrome. We first studied MOR-CRF_1R heteromerization in vitro in co-transfected HEK-293T using different complementary techniques, including bimolecular fluorescence complementation (BiFC), complemented donor-acceptor resonance energy transfer (CODA-RET), Fluorescence Resonance Energy Transfer (FRET) with donor decay photobleaching and total internal reflection fluorescence microscopy (TIRF). Synthetic peptides with the amino acid sequence of all transmembrane domains (TMs) of the two receptors were used in BiFC experiments to evaluate the possible TM interfaces for MOR-CRF_1R heteromerization and MOR-MOR and CRF_1R-CRF_1R homomerization. Subsequent computer modeling analysis provided a very

similar tetrameric structure to that recently reported for MOR-galanin Gal_1R heteromer, composed of Gs-coupled CRF_1R and Gi-coupled-MOR homodimers. CODA-RET experiments demonstrated significant reciprocal negative allosteric interactions between CRF_1R and MOR ligands. G Signaling experiments of cAMP accumulation demonstrated a canonical Gs-Gi antagonistic interaction at the adenylyl-cyclase level which was dependent on MOR-CRF_1R heteromerization, since it was disrupted with TM peptides that specifically aimed at the heteromeric interface. Finally, *in vivo* experiments in Sprague Dawley rats demonstrated that the MOR agonist methadone locally perfused in the CeA produces an increase in extracellular glutamate concentration. CRF did not produce any change when locally infused, although completely counteracted the effect of methadone. Experiments with the local infusion of heteromer-disrupting peptides are in progress to demonstrate that the interactions between methadone and CRF are mediated by MOR-CRF_1R heteromers. *In situ* hybridization experiments are also in progress to analyze the CeA neuronal elements expressing both receptors and possibly harboring functional MOR-CRF_1R heteromers. Work supported by the intramural funds of NIDA.

Disclosures: **W.N. Sanchez Luna:** None. **E. Moreno:** None. **A. Yauch:** None. **N. Casajuana:** None. **N. Cai:** None. **V. Vicent Casadó:** None. **L. Pardo:** None. **L.D. Plant:** None. **S. Ferre:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.19/C55

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Endocannabinoid regulation of tearing

Authors: *N. MURATAEVA, J. BILLINGSLEY, K. ANDREIS, E. RICHTER, A. THAYER, K. YUSTE, T. BOSQUEZ-BERGER, J. WAGER-MILLER, H. BRADSHAW, A. STRAIKER; Indiana Univ. Bloomington, Bloomington, IN

Abstract: Introduction: Cannabis users frequently report dry mouth and dry eye. We recently showed that cannabinoid CB1 receptors sex-dependently regulate tearing in mice: activation of CB1 receptors reduces tearing in males but increases tearing in females. CB1 receptors are expressed in the axons of cholinergic neurons innervating the lacrimal gland. Two endogenous cannabinoids, N-acyl ethanolamines (NAEs), including anandamide, or 2-arachidonoyl glycerol (2-AG) likely mediate these effects. NAEs are synthesized enzymatically by NAPE-PLD and metabolized by FAAH or NAAA. 2-AG is synthesized by diacylglycerol lipases and metabolized chiefly by monoacylglycerol lipase (MAGL).
Methods: To explore which endocannabinoid regulates tearing we tested tearing, examined protein and mRNA expression of key cannabinoid genes, and measured the effect of FAAH deletion on endocannabinoid levels using lipidomics.

Results: We find evidence that both anandamide- and 2-AG-degrading enzymes contribute to regulation of tearing. Deletion and block of FAAH increased tearing in females, but not males, partly mirroring the sex-dependence of CB1. Blocking NAAA had no effect on tearing. NAEs may therefore mediate the CB1-dependent increase in tearing via FAAH. Acutely blocking MAGL had no effect in either sex but deletion of MAGL impaired tearing in males, but not females. MAGL deletion appears to also contribute to degradation of the lacrimal gland. FAAH protein is seen within restricted to the acinar cells while NAPE-PLD is enriched in myoepithelial cells. We do not detect a difference in mRNA expression for FAAH, NAPE-PLD, or MAGL by sex. In lipidomic experiments comparing males vs. females, female mice have higher levels of several NAEs, though not anandamide. In contrast males have higher levels of several acylglycerols, including 2-AG. Female FAAH knockouts have higher levels of two NAEs including anandamide than males.
Conclusion: Our results suggest that some of the sex-dependence of effects on lacrimation that we have reported for activation of neuronal CB1 receptors in the lacrimal gland may extend to the endocannabinoid messengers. FAAH appears to mediate the increase in tearing seen in females while MAGL function appears to be critical for the viability of the lacrimal gland in males.

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Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.20/C56

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Title: Complement c5a receptors facilitate spontaneous excitatory currents in pyramidal neurons in a non-canonical calcium independent mechanism

Authors: *S. E. PARKER[1], M. C. BELLINGHAM[1], T. M. WOODRUFF[1,2];
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Abstract: The complement cascade, a vital component of the innate immune system, has been proposed to play a significant role in adult neurophysiology. Recent studies have suggested that various complement factors are employed by microglia to facilitate synaptic phagocytosis and encode physiological forgetting of memories. Furthermore, terminal complement activation factors, including C5a and its corresponding receptor C5aR1, have been found to be expressed in neurons within the CNS. Whilst C5aR1 is a G-protein coupled receptor known for its involvement in peripheral innate immune cell function, its role in the adult brain remains unclear. In this study, we aimed to investigate the role of neuronal C5aR1 by employing whole-cell

electrophysiology. We recorded spontaneous excitatory currents (sEPSCs) from layer 5 pyramidal neurons in the prefrontal cortex of adult wild-type and C5aR1-/- mice. Our results revealed that mice lacking C5aR1 exhibited a 40% increase in sEPSC interevent intervals compared to their wild-type counterparts, indicating that C5aR1 is essential for appropriate synaptic transmission in pyramidal neurons. However, as C5aR1 is necessary for appropriate CNS development, we next aimed to see whether acute pharmacological blockade of C5aR1 in WT adult animals could mirror the phenotype observed in the knockout animals. To target C5aR1, we used the selective antagonist PMX53, and recorded sEPSCs following acute C5aR1 blockade. Notably, pharmacological inhibition of C5aR1 in wild-type pyramidal neurons led to a 50% reduction in sEPSC frequency, supporting the hypothesis that C5aR1 signaling facilitates synaptic transmission in mature pyramidal neurons. To investigate the underlying mechanisms, we explored the role of calcium in mediating this reduction in synaptic transmission. We observed that C5aR1 antagonism in wild-type pyramidal neurons resulted in a 40% decrease in the frequency of quantal miniature EPSCs, which serve as a measure of calcium-independent neurotransmitter release. Additionally, paired-pulse analysis was conducted to explore calcium-dependence, but no significant alterations in paired-pulse ratios were observed upon C5aR1 antagonism. These findings suggest that C5aR1 supports synaptic transmission through a calcium-independent mechanism. In conclusion, our study provides novel evidence for the functional role of neuronally-expressed C5aR1. Pyramidal neurons lacking C5aR1 or treated with a C5aR1 antagonist exhibited reduced excitatory synaptic transmission. These results support recent findings highlighting the fundamental contribution of the complement system to healthy brain neurophysiology.

Disclosures: S.E. Parker: None. **M.C. Bellingham:** None. **T.M. Woodruff:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.21/C57

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Supported by: Department of Defense, Multiple Sclerosis Research Program GRANT13212139

Title: C1ql1 is expressed by oligodendrocyte progenitor cells and promotes recovery after demyelination.

Authors: *Z. M. ALTUNAY[1], J. BISWAS[1], R. S. PIJEWSKI[1], L. C. KRESIC[1], A. TANG[1], A. D. SCHOUW[1], A. NISHIYAMA[2,3], S. J. CROCKER[5,4], D. C. MARTINELLI[6,4];
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Abstract: The central nervous system's (CNS) axon myelination is necessary for the CNS to operate properly. Oligodendrocytes are responsible for the myelination of the CNS. Oligodendrocytes originate from oligodendrocyte progenitor cells (OPCs), a possible source of newly generated oligodendrocytes for replacing those damaged by inflammation or injury in diseases like multiple sclerosis (MS). C1QLs bind to adhesion GPCR B3 (ADGRB3; a.k.a. BAI3), which is expressed by neurons and some types of glia. Additionally, *ADGRB3* and *C1QL1* have been linked to MS in several studies. However, the oligodendrocyte lineage has not yet been investigated in the context of C1QL1 or ADGRB3, which inspired us to investigate whether C1QL1 has a function in the regulation of OPC maturation. We found that *C1ql1* was detected in the oligodendrocyte lineage, in both the gray matter and the white matter. We show that the gene *C1ql1* is expressed by OPCs, and not neurons, in most regions of the brain. To study the function of *C1ql1* in OPCs, we created conditional knockout mice (cKO). To induce demyelination, 8-week-old mice were fed a diet of ground standard mouse chow mixed with cuprizone powder, for 5 weeks. Following this period, mice were perfused or allowed to recover on a normal diet for an additional 2 weeks prior to perfusion. We evaluated the histology of control and cKO mice to examine if C1QL1 would have a function in regulating the propensity of an OPC to differentiate into a mature oligodendrocyte. We found that the cKO mice had a significant decrease in the density of oligodendrocytes in both the white and gray matter after the cuprizone recovery period. In this research, we demonstrate clearly that *C1ql1* is expressed by OPCs. Additionally, our findings revealed that OPCs are the only cell type that expresses *C1ql1* in most brain areas. With the use of cKO mice, we demonstrate that *C1ql1* KO mice have a developmental delay in oligodendrocyte cell density and a reduced or slowed remyelination after a demyelination insult. Our results suggest that the C1QL1-ADGRB3 signaling pathway may have therapeutic potential for treating demyelinating diseases such as MS.

Disclosures: Z.M. Altunay: None. **J. Biswas:** None. **R.S. Pijewski:** None. **L.C. Kresic:** None. **A. Tang:** None. **A.D. Schouw:** None. **A. Nishiyama:** None. **S.J. Crocker:** None. **D.C. Martinelli:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.22/C58

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NIH U18 DA052416

Title: The development of diphenyleneiodonium analogs as GPR3 agonists

Authors: B. BLOUGH[1], D. HARRIS[1], J. WILSON[1], S. CHEEKS[2], C. D. FOWLER[3], ***E. GAY**[1];
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Abstract: G protein coupled receptor 3 (GPR3) is an orphan receptor potentially involved in many important physiological processes such as drug abuse, neuropathic pain, and anxiety and depression related disorders. Pharmacological studies of GPR3 have been limited due to the restricted number of known agonists and inverse agonists for this receptor. In this study, we report the discovery of GPR3 agonists based off the diphenyleneiodonium (DPI) scaffold. The most potent full agonist was the 3-trifluoromethoxy analog with an EC_{50} of 260 nM and 90% efficacy compared to DPI. Development of a homology model of GPR3 indicated a binding site rich in potential π-π and π-cation interactions stabilizing DPI-scaffold agonists. MMGBSA free energy analysis showed a good correlation with trends in observed EC_{50}s. DPI analogs retained high receptor selectivity for GPR3 over GPR6 and GPR12. We tested the lead analog in C57BL6/J mice and found that administration of a GPR3 agonist, attenuated nicotine intake in the intravenous nicotine self-administration procedure.

Disclosures: B. Blough: None. **D. Harris:** None. **J. Wilson:** None. **S. Cheeks:** None. **C.D. Fowler:** None. **E. Gay:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.23/C59

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: R00 DA048970
AP-0323-04

Title: Allosteric modulator biases neurotensin receptor 1 toward both β-arrestin and noncanonical G protein coupling

Authors: *A. R. ALWIN[1], M. N. MOORE[1,2], C. L. KRUSEMARK[1], L. M. SLOSKY[1];
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Abstract: NTSR1 is a G protein-coupled receptor (GPCR) that modulates brain dopamine signaling and is a promising anti-addiction therapeutic target. NTSR1 signals through G protein- and β-arrestin-mediated pathways. Clinical development of balanced NTSR1 agonists that activate both pathways is precluded by their on-target side effects. The biased allosteric modulator SBI-553 confers β-arrestin bias to neurotensin (NT), the endogenous ligand, by selectivity antagonizing its Gαq signaling. Biased allosteric modulators may be the path forward for NTSR1 drug development efforts, as SBI-553 lacks the thermal and hemodynamic side

effects characteristic of balanced NTSR1 agonism. Neuronal GPCRs can signal through the activation of one or more of 16 Gα G proteins (e.g., Gαq, Gαi, Gαo, Gαs) as well as through β-arrestins. While NTSR1's calcium-dependent physiological effects implicate Gαq/11 as preferred signaling mediators, NTSR1 is promiscuous in *in vitro* assays, activating at least 12 different G proteins. SBI-553 does not activate Gαq and blocks NT-induced Gαq activation, but its effect on other G proteins required investigation. Here, we completed the most comprehensive assessment of SBI-553's ability to modulate NTSR1 signaling to date. We assessed SBI-553 efficacy using an effector panel that included 14 G proteins, β-arrestin1 and β-arrestin2. NT and the competitive antagonist SR142948A served as positive and negative controls, respectively. Using BRET2-based G protein activation assays and BRET1-based β-arrestin recruitment assays in HEK293T cells, we found that NTSR1 ligands have distinct G protein selectivity profiles. Critically, SBI-553 exhibited previously unappreciated G protein activity, acting as a weak partial agonist at the Gαo and Gα12/13 G proteins. In combination with NT, SBI-553 exhibited complex allosteric interactions that were highly G protein dependent. SBI-553 simultaneously antagonized NT-NTSR1 Gαq/11 coupling and promoted NT-NTSR1 Gαi/o/12/13 activation and β-arrestin1/2 recruitment. These findings suggest that SBI-553 biases NTSR1 not only toward β-arrestin recruitment, but also toward noncanonical G protein signaling. A potential role for noncanonical NTSR1 G protein signaling in SBI-553's behavioral effects is an exciting area of investigation. These findings reveal uniquely complex allosteric interactions and suggest the ability to generate small molecule compounds with exquisite effector selectivity, laying the foundation for targeted, pathway-selective drug discovery efforts.

Disclosures: **A.R. Alwin:** None. **M.N. Moore:** None. **C.L. Krusemark:** None. **L.M. Slosky:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.24/C60

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: K99/R00 DA048970
F32 DA043931

Title: G protein independent GRK2 and β-arrestin2 recruitment by the neurotensin receptor 1

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Abstract: The neurotensin receptor 1 (NTSR1) is a G protein-coupled receptor (GPCR) with diverse central and peripheral activities, including the regulation of brain dopamine signaling, body temperature, and blood pressure. NTSR1 predominantly couples to the Gq family of Gα

proteins. Agonist binding stimulates dissociation of the Gα subunit from the Gβγ subunits. Balanced agonism of the NSTR1 results in Gq signaling as well as the recruitment of β-arrestin, which mediates receptor internalization and a spatially and temporally distinct pattern of kinase activation. We recently developed β-arrestin-biased allosteric modulators of the NTSR1 typified by the compound SBI-553. These modulators stimulate NTSR1-β-arrestin recruitment in the absence of Gq signaling. Canonically, ligand-induced recruitment of β-arrestin is preceded by G protein activation, the binding of dissociated Gβγ to GPCR kinase 2 (GRK2), and GRK2 mediated receptor phosphorylation. Herein, we present evidence to suggest that the NTSR1 recruits GRK2 and β-arrestin2 via an alternative G protein independent mechanism. In HEK293T cells, the endogenous ligand neurotensin (NT) and SBI-553 both stimulated NTSR1-GRK2 recruitment in a concentration-dependent manner. Moreover, SBI-553-induced β-arrestin2 recruitment to the NTSR1 was enhanced by the overexpression of GRK2 and reduced by pharmacological and genetic inhibition of GRK2. Critically, and consistent with a G protein-independent mechanism, SBI-553-induced recruitment of GRK2 to NTSR1 was unaffected by truncation of GRK2's Gβγ association domain. We further investigated the G protein dependency of NTSR1 GRK2 and β-arrestin2 recruitment using G protein knockout (KO) HEK293 cells. NT-induced GRK2 and β-arrestin2 recruitment to the NTSR1 was reduced in Gq/11 KO, G/i1/i2/i3/o/z KO, and Gq/11/s/olf/12/13 KO cell lines while SBI-553-induced recruitment remained intact. This body of evidence indicates that the β-arrestin-biased ligand SBI-553 induces GRK2 and β-arrestin2 recruitment to the NTSR1 via a noncanonical, G protein-independent mechanism. The finding that the NTSR1 may directly engage GRK2 independent of activated G protein expands our understanding of the mechanisms of GPCR-β-arrestin engagement. Additionally, this work identifies GRK2 as a target for potentiating or inhibiting the effects of β-arrestin-biased NTSR1 ligands.

Disclosures: **M.N. Moore:** None. **C.L. Krusemark:** None. **A.R. Alwin:** None. **N. Atluri:** None. **L.S. Barak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); US Patents 9,868,707 and 10,118,902 relating to the chemistry of SBI-553 and its derivatives have been issued to the Sanford Burnham Prebys Medical Research Institute and Duke University (L.S.B.).. **L. Slosky:** None.

Poster

PSTR320. G-Protein Coupled Receptors: Signaling Pathways and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR320.25/C61

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: Tier 1 Internal Grant, Northeastern University

Title: Golf-biased pharmacological actions in vitro and ex vivo

Authors: *H. YANO, A. NGUYEN, A. SEMEANO;
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Abstract: The dopamine D1 receptor (D1R) plays a pivotal role in locomotion, reward, cognition, and memory and learning, therefore, is an important drug target in various neurological and neuropsychiatric disorders. Besides the affinity and selectivity towards D1R, little attention has been given to signaling differences between Gαs (Gs) and Gαolf (Golf) - two D1R-coupling Gα subunits highly homologous yet very discrete in their expression patterns (i.e., Gs - cortex and Golf - striatum). Since typical drug screening does not discriminate Gs and Golf coupling, we have developed novel drug screening methods with bioluminescence resonance energy transfer (BRET) that can differentiate coupling between Gs and Golf. Using the assay, we characterized various D1R ligands including several non-catechol agonists as they represent a recently developed novel scaffold. We uncovered biased D1R ligands in Gs / Golf pathways. In particular, two ligands behave as full agonists for Golf but partial agonists for Gs signaling. We further studied domain analysis on the interface region of the D1R intracellular domain and that of Gs or Golf. The analysis suggests a couple of intriguing conformational differences between the two G proteins while comparison between the Gs and Golf biosensor readouts suggests largely the similar movement in Gs and Golf containing heterotrimers. The biased behavior of pharmacological findings in the BRET assay were studied in depth with various cellular signaling, electrophysiological and psychomotor activation experiments exploiting selective Golf expression pattern in the striatum. Specifically, we have developed two methods (i.e., cAMP imaging and pathway-specific optogenetics) to study D1R-mediated neuronal functions with cell-type specificity and observed differences in Gs- and Golf-expressing neurons. The combination of full efficacy at Golf and partial efficacy at Gs may constitute a new therapeutic approach for neurological conditions with reduced side effects due to lower Gs signaling in the periphery. Our study demonstrates that D1R biased signaling at the closely related Gs and Golf subtypes can translate to the neuronal activities in the brain, and brain region selective targeting of a specific receptor can be achieved via biased agonism.

Disclosures: H. Yano: None. **A. Nguyen:** None. **A. Semeano:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.01/C62

Topic: B.04. Synaptic Transmission

Support: Hungarian Academy of Sciences, Momentum Grant
ERC Fronthal

Title: Specialized cortico-thalamic connections between the layer 5 of the frontal cortex and the thalamus

Authors: *N. HADINGER[1], H. BOKOR[4], J. K. MAKARA[2], B. TÓTH[3], N. YAMAWAKI[5], G. M. SHEPHERD[6], L. ACSADY[7];
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Abstract: Top-down cortico-thalamic communication from layer 5 pyramidal cells has profound impact on the activity of layer 5 recipient thalamic nuclei. Giant corticothalamic terminals arising from the collaterals of layer 5 pyramidal cells are critical components of these pathways. The properties of layer 5 inputs, however, have mainly been investigated in sensory cortical regions so far. In this project we asked whether cortico-thalamic L5 terminals arising from frontal cortical regions have similar properties. We found that L5 afferents arising from M1, M2, cingulate and prelimbic cortices and innervating the ventromedial (VM), intralaminar, parafascicular, reuninens and parts of the mediodorsal nuclei do not form giant terminals. Upon optogenetic stimulation small L5 terminals could still reliably elicit action potentials in VM neurons. Small L5 boutons displayed less short-term depression in VM than their large counterparts in the S1/L5 pathway innervating the nucleus posterior, both in in vitro and in vivo preparations. At the ultrastuctural levels the small L5 terminals also differed in size, targets and number of mitochondria from the L6 corticothalamic terminals. Surprisingly, the main targets of small frontal L5 to VM axons were dendritic spines of various size and form. L5 recipient thalamic spines contained spine apparatus and multivesicular bodies similar to cortical spines. Two-photon glutamate uncaging revealed that large thalamic spines in VM could function as calcium compartments. The degree of compartmentalization in the thalamus was similar to the that measured in the spines of CA1 pyramidal cells. We conclude that the presence of functional spines on thalamic dendrites with a specialized cortical input can potentially endow thalamocortiocal cells with the ability of scaling and plastic regulation of incoming cortical inputs.

Disclosures: **N. Hadinger:** None. **H. Bokor:** None. **J.K. Makara:** None. **B. Tóth:** None. **N. Yamawaki:** None. **G.M. Shepherd:** None. **L. Acsady:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.02/C63

Topic: B.04. Synaptic Transmission

Support: NIH Grant DK092651 to JHP

Title: Spontaneous and evoked neurotransmission use a shared pool of synaptic vesicles and receptors in the nucleus of the solitary tract

Authors: *R. A. ARNOLD, J. H. PETERS;
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Abstract: Synaptic transmission is a fundamental point of information processing in the nervous system that relies on vesicle fusion and release via functionally distinct pathways. Previous work in the central nervous system supports the model that different release pathways use separate vesicle pools; however, the extent to which this is true in other neuronal populations has not been determined. Here we investigated the synaptic vesicle pool organization of primary viscerosensory neurons of the vagus nerve. Vagal afferent neurons form excitatory glutamatergic synapses onto second-order neurons in the brainstem nucleus of the solitary tract (NTS) and exhibit three distinct release pathways. These include evoked release (both synchronous and asynchronous) which require presynaptic action potentials, and spontaneous quantal release, which occurs stochastically and independent from action potentials. Experimentally, we targeted vesicle release presynaptically by blocking vesicle refilling with bafilomycin and concanamycin, or postsynaptically via use-dependent blockade of N-methyl D-aspartate receptors (NMDARs) with MK-801. Using patch-clamp electrophysiology on acute brainstem slices from Sprague-Dawley rats we measured evoked excitatory postsynaptic currents (eEPSCs) from local electrical stimulation of vagal terminals before and after treating the slice with bafilomycin (0.5 µM) or concanamycin (2 µM) at rest, to prevent spontaneously released vesicles from refilling with glutamate. We observed a proportionate reduction in both spontaneous EPSC (sEPSC) frequency and eEPSC amplitude, indicating that selective targeting of spontaneous release impacted subsequent evoked release pathways. Similarly, the use-dependent blockade of postsynaptic NMDARs with MK-801 (10 µM) at rest (to block NMDARs used during sEPSCs) also inhibited the NMDA component of subsequent eEPSCs. We also recorded sEPSCs before and after treatment with MK-801 during stimulation of vagal terminals to block NMDARs used during evoked release. Again, we saw a reduction in both sEPSC frequency and eEPSC amplitude. We conclude that, in contrast with other neuronal populations, neurotransmission from vagal afferent neurons to the NTS uses a common pool of synaptic vesicles and postsynaptic receptors for both spontaneous and evoked neurotransmission. Thus far, these shared vesicle pools are unique to vagal afferent neurons. A common pool of presynaptic vesicles and postsynaptic receptors for spontaneous and evoked neurotransmission may allow for shared resources during high frequency activity and compensate for spatial limitations at the active zone of the synapse.

Disclosures: R.A. Arnold: None. **J.H. Peters:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.03/D1

Topic: B.04. Synaptic Transmission

Support: NIH Grant 20GM109098

Title: Nanoscale Organization of thalamocortical synapses onto individual dendritic spines of L5 pyramidal neurons revealed by staining for endogenous proteins using single-domain camelid nanobodies.

Authors: *Y. AKTER, G. JONES, V. SHIFFLETT, M. HRUSKA;
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Abstract: Synapses in the central nervous system are small, diffraction-limited cell-cell junctions designed to rapidly transfer and process information on a millisecond timescale. One of the characteristic features of central synapses is their remarkable structural and functional variation. Indeed, a single pyramidal neuron receives thousands of synapses that differ in their strength, release probability, and signaling properties that form the basis for cortical computation and information storage in the brain. The discovery of unitary pre- and post-synaptic proteins that scale in number with increasing spine size suggests the logic of diverse synaptic function. Whether modular nano-organization is a universal feature of all synapses is unknown. Defining the organizational principles of diverse set of synapses in the brain will require applying high-throughput super-resolution approaches in their native environment. Here, we have characterized the use of directly conjugated single-domain camelid nanobodies for imaging synapses in thin brain cryosections using Stimulated Emission Depletion (STED) super-resolution microscopy. Our results demonstrate that nanobodies are significantly more efficient in labeling synapses in brain sections than conventional antibodies and they outperform conventional antibodies in obtaining the sub-diffraction resolution needed to quantify synaptic organization at the nanoscale level. Using nanobodies, we investigated the differences in the nano-organization of thalamo-cortical (TC) and cortico-cortical (CC) synapses in apical (Layer 1) and basal (Layer 5) dendrites of layer 5 pyramidal neurons in the mouse primary somatosensory cortex. Both, TC and CC synapses are glutamatergic that can be distinguished by the presence of the Vesicular Glutamate Transporter, VgluT1 (CC) and VGluT2 (TC). TC synapses differ from CC synapses by their low abundance in the cortex, but much higher reliability. Yet, there is still a debate about how the TC synapse organization enables their function in the brain. Our results indicate that a significantly larger fraction of TC synapses in L1 contain multiple synaptic nanodomains compared to CC synapses, despite their similar spine sizes. In contrast, L5 TC and CC synapses do not exhibit differences in nanomodule organization. Overall, by applying novel tools to label synaptic nano-architecture in situ, our results shed light on key differences between the nanoorganization of different excitatory synaptic subtypes across cortical layers.

Disclosures: **Y. Akter:** None. **G. Jones:** None. **V. Shifflett:** None. **M. Hruska:** None.

Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR321.04/D2

Topic: B.04. Synaptic Transmission

Support: NIH R01 MH119347

Title: Distribution of the Synapse Differentiation Induced Gene 1 (SynDIG1) protein in mouse brain throughout development.

Authors: *O. VAFAEVA[1], K. D. MURRAY[2], H.-J. CHENG[3], E. DIAZ[4];
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Abstract: The human brain comprises about 100 billion neurons precisely organized into networks by specific cell-cell contacts called synapses that give rise to cognitive phenomena such as emotion, learning and memory, and perception. Our lab has identified Synapse Differentiation Induced Gene 1 (SynDIG1), a novel transmembrane AMPAR-associated protein that regulates the maturation of excitatory synapse structure and function in an activity-dependent manner. However, the expression and distribution of SynDIG1 protein in the brain throughout life have not been well characterized.In this study, we define spatial and temporal patterns of SynDIG1 expression in the mouse brain throughout postnatal development. To analyze SynDIG1 protein distribution we used immunohistochemistry of free-floating sections with the knock-out confirmed monoclonal antibody raised against SynDIG1 in the mouse aged postnatal day 1 (P1) to 12-months-old (12M). We found that different brain regions show variation in the SynDIG1 expression onset. For example, at postnatal day 7 (P7) SynDIG1 shows significant immunoreactivity in the Purkinje cell layer in the cerebellum, while expression in the hippocampus and neocortex appeared at P14 and increased at P21. In the adult brain (6M), SynDIG1 signal is diffuse across the neocortex. Staining was also present in the subiculum and appeared strongly within the perinuclear region and the dendritic tree of the Purkinje cells of the cerebellum. Intriguingly, within the hippocampus, the most prominent SynDIG1 immunolabeling was found in the CA2 region. On a cellular level the SynDIG1 signal is punctate and most dense in cell soma around nucleus and extends to apical dendrites in CA2 neurons. We show that SynDIG1 immunoreactivity colocalization with the molecular marker of hippocampal CA2 region regulator of G protein signaling 14 (RGS14) is 95% ±1 . The same pattern is retained in tissue from 12M animals. The present results define the temporal patterns of SynDIG1 expression in different brain regions and suggest that SynDIG1 might have a differential role in the regulation of synaptic strength and maturation depending on where and when it is expressed throughout development.

Disclosures: O. Vafaeva: None. **K.D. Murray:** None. **H. Cheng:** None. **E. Diaz:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.05/D3

Topic: B.04. Synaptic Transmission

Support: NIH R00 MH118425
BBRF Young Investigator Award 30264

Title: An extracellular synaptic docking mechanism enabling AMPAR-dependent synaptic transmission and plasticity, and forms of memory.

Authors: *G. SANDOVAL, A. V. KOLLI, E. M. SAAVEDRA, J. DIAZ-ALONSO;
Anat. and Neurobio., Univ. of California, Irvine, Irvine, CA

Abstract: Regulation of AMPA receptor (AMPAR) abundance at synapses underlies synaptic plasticity mechanisms crucial for learning and memory, and AMPAR subsynaptic localization has recently been suggested to further regulate their activation. Transsynaptic nanocolumns have been observed linking postsynaptic AMPAR clusters with presynaptic glutamate release sites. Dynamic modulation of transsynaptic AMPAR alignment may impact synaptic plasticity, but the underlying molecular mechanisms remain unknown. Our laboratory and others recently uncovered the essential role of the AMPAR amino terminal domain (ATD) in AMPAR synaptic trafficking and long-term potentiation (LTP). The ATD makes up half of the protein size. Given its substantial protrusion into the synaptic cleft, it is conceivable that the AMPAR ATD contributes to AMPAR transsynaptic positioning through interactions with extracellular proteins forming an "extracellular AMPAR slot." The present study aims at probing the existence of such extracellular slot. Putative AMPAR ATD-interacting proteins identified in our proteomic assay included the astrocyte-secreted glycoprotein thrombospondin-1 and its receptor, the voltage-gated calcium channel subunit α2δ1. Here, we explore this putative tripartite protein interaction and its potential contribution to AMPAR trafficking, subsynaptic localization, and function. Our co-immunoprecipitation experiments, in a heterologous system, confirm a direct interaction between α2δ1 and the AMPAR ATD. In order to dissect the potential contribution of presynaptic α2δ1 to AMPAR docking and function, we generated Grik4-cre:α2δ1$^{f/f}$mice, in which α2δ1 is selectively deleted from CA3 pyramidal neurons, therefore absent presynaptically at CA3-CA1 synapses. Using slice electrophysiology, we found that CA1 pyramidal neurons in these mice have impaired AMPAR-mediated basal synaptic transmission ($n = 8\text{-}11$, $p < 0.01$) and LTP ($n = 7\text{-}11$, $p < 0.05$) relative to WT counterparts. Consistent with the specificity of our genetic manipulation, Grik4-cre:α2δ1$^{f/f}$ demonstrate a significant impairment in the CA1-dependent object location memory task, but no deficit in memory tasks involving other brain regions. Grik4-cre:α2δ1$^{f/f}$ mice also express normal anxiety- and despair-related behavior in the light/dark alternation and forced swim test paradigms. In summary, we present evidence showing that presynaptic deletion of α2δ1 affects basal AMPAR synaptic transmission, plasticity, and memory through its interaction with the AMPAR ATD. Our data supports the existence of an "extracellular slot" which contributes to AMPAR synaptic localization and function.

Disclosures: G. Sandoval: A. Employment/Salary (full or part-time):; University of California, Irvine. **A.V. Kolli:** A. Employment/Salary (full or part-time):; University of California, Irvine. **E.M. Saavedra:** A. Employment/Salary (full or part-time):; University of California, Irvine. **J. Diaz-Alonso:** A. Employment/Salary (full or part-time):; University of California, Irvine.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.06/D4

Topic: B.04. Synaptic Transmission

Support: 2023 UConn Surf Award
Spring 2023 IBACS Undergraduate Research Award

Title: C1ql3 facilitates cognitively challenging behavior

Authors: *T. RELIGA;
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Abstract: C1QL3 facilitates cognitively challenging behavior.
Trevor Religa[1] (trevor.religa@uconn.edu), Keaven Caro[1] (caro@uchc.edu), Shanawaz Alam[1] (shalam@uchc.edu), Maya Preibisz-Kamat[1] (maya.preibisz-kamat@uconn.edu), Mark Cristino[1] (mark.cristino@uconn.edu), Timothy Spellman[1] (tspellman@uchc.edu), David Martinelli[1] (davidmartinelli@uchc.edu)
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Synaptic adhesion molecules (SAMs) make a specialized cell-cell junction across the synaptic cleft, and various complexes have been shown to control synapse formation and plasticity. Dysfunction of SAMs have been implicated in neuropsychiatric disorders such as autism and schizophrenia. Complement component 1, Q subcomponent-like 3 (C1QL3) is a novel potential SAM that is promising due to the behavioral deficits observed in mice that are correlated with impaired synaptic maintenance *in vivo*. SAMs are associated with neuropsychiatric disorders that exhibit impaired attention set shifting, a behavior that requires a subject to ignore stimuli that was previously relevant and instead respond to a previously irrelevant sensory modality. Previous studies have demonstrated *C1ql3* expression in the prefrontal cortex (PFC), a brain region implicated in attention deficits and cognitive flexibility. To test if C1QL3 promotes attention and cognitive flexibility, we first tested *C1ql3* global KO mice in attentional set shifting behavior assays. To test if *C1ql3* expression specifically in the PFC is relevant, we created cKO mice by injecting AAV to express Cre into a *C1ql3* floxed line. We predict that both KO and cKO mice tested on attention set shifting tasks will have difficulty selectively attending to a target sensory modality while ignoring distracting modalities, and adapting behavior when attention rules change, suggesting impaired cognitive flexibility. As patients with autism, schizophrenia, and ADHD have also been observed to have deficiencies in attention set shifting, these results could build toward understanding a new potential pathological process contributing to the symptoms in these disorders and identify novel targets for modulating synaptic function.
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Disclosures: T. Religa: None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR321.07/D5

Topic: B.04. Synaptic Transmission

Title: Electron tomography reveals the organization and associations of transsynaptic complexes underpinning the coordination between the three synaptic compartments in cultured rat hippocampal synapses

Authors: *A. A. COLE[1], T. S. REESE[2];
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Abstract: The synapse is a complex ballet with the players, their roles, and their associations largely hidden by scale. To understand the synapse, the composition and distribution of components in each compartment has largely been analyzed and contextualized separately. Recently, advances in 3D light and electron microscopy techniques have dramatically revealed coordination between the presynaptic, postsynaptic, and cleft compartments. It is now clear that certain proteins align across the synapse and within the cleft. Here, 3D renderings visualize electron dense material from electron tomographic reconstructions of high-pressure frozen and freeze-substituted dissociated hippocampal rat neuronal cultures. In these renderings, nearly all cleft-spanning structures connect with a structure in either the pre-or postsynaptic compartment. More than half of all the abundant cleft-spanning structures connect to intracellular structures in both. These *full transsynaptic assemblies* can link with one another through shared intracellular structures. The resulting large clusters of intracellular structures grouped by linked transsynaptic assemblies are common around vesicles near the active zone membrane and align with groups of large structures with scaffolding morphology in the postsynaptic compartment. Here, we enumerate different types of assemblies, describe their associations, and map their distribution within the synapse. In our interpretation, the intracellular portion of linked assemblies form domains. The cleft components physically link these pre- and postsynaptic domains into nanocolumns. The mechanical forces underpinning this alignment may influence synaptic functions like endo- and exocytosis.

Disclosures: A.A. Cole: None. **T.S. Reese:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

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Topic: B.04. Synaptic Transmission

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NIH Grant F31-MH117920
NIH Grant R37-MH080046
NIH Grant R21-MH127822

Title: Differential protein nano-organization and the functional diversity of excitatory synapses

Authors: *A. D. LEVY[1], M. C. ANDERSON[2], P. A. DHARMASRI[2], S. R. METZBOWER[1], T. A. BLANPIED[1];
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Abstract: Information propagation and plasticity within neural circuits each rely on fine tuning of synaptic strength. Recent work has uncovered several key architectural elements of synaptic protein organization critical for this tuning. Within single synapses, presynaptic release proteins and postsynaptic scaffolds and AMPA receptors concentrate in ~80 nm nanoclusters (NCs). Acute disruption of an adhesion protein mediating NC alignment across the synaptic cleft (the "nanocolumn") reduces postsynaptic currents, directly demonstrating that nanostructure can regulate AMPAR activation. However, it is unknown whether nanocolumn architecture is conserved across diverse excitatory synapse types, or whether the plasticity-gating NMDA receptors are also located in the nanocolumn. We first used DNA-PAINT imaging to compare the organization of Munc13-1 and PSD-95 at excitatory synapses onto excitatory neurons (Ex->Ex) vs onto parvalbumin-expressing interneurons (Ex->PV) in rat hippocampal neuron cultures. While protein nanoclustering and alignment were generally conserved, the nano-organization of both Munc13-1 and PSD-95 were significantly impacted by postsynaptic cell identity. Further, the Munc13-1/PSD-95 trans-synaptic relationship was generally stronger with unique spatial features at Ex->PV synapses, consistent with the reported higher strength of these synapses. These results affirm the nanocolumn as a conserved structural motif, but suggest that differential protein nano-organization may contribute to functional diversity of excitatory synapses. NMDAR subtype expression varies across neuron types, and NMDAR location relative to the nanocolumn may modulate their activation and signaling. To map this relationship, we performed four target Exchange-PAINT of GluN2A, GluN2B, PSD-95 and Munc13-1 at Ex->Ex synapses. Surprisingly, both subunits were de-enriched from Munc13-1 NCs. However, GluN2 enrichment was dramatically higher around Munc13-1 NCs within nanocolumns than those with little alignment to PSD-95, suggesting that NMDAR subsynaptic positioning is governed by transsynaptic assembly of multiprotein ensembles. Together, these results suggest that protein nano-organization is a conserved architecture establishing synaptic strength and providing a measurable platform for synapse- and cell-type specific plasticity.

Disclosures: A.D. Levy: None. **M.C. Anderson:** None. **P.A. Dharmasri:** None. **S.R. Metzbower:** None. **T.A. Blanpied:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

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Topic: B.04. Synaptic Transmission

Support: F31NS129086
5R01MH119826-05
5R37MH080046-16

Title: Elucidating the dynamic role of PTPsigma in synaptic nano-organization and NMDA receptor function.

Authors: *E. M. DEMARCO[1,2], A. D. LEVY[2], S. R. METZBOWER[2], M. CONTRERAS[2], T. A. BLANPIED[2];
[1]Dept. of Physiol., Univ. of Maryland, Baltimore, Baltimore, MD; [2]Dept. of Physiol., Univ. of Maryland, Sch. of Med., Baltimore, MD

Abstract: Understanding the mechanisms by which neurons tune synaptic strength is a central goal in neuroscience, and recent work suggests the importance of controlling protein organization at individual synapses. Postsynaptic receptors as well as presynaptic release-relevant proteins concentrate into sub-synaptic regions of high local protein density called nanoclusters (NCs). NCs can be aligned across the synapse to position AMPA receptors (AMPARs) in opposition to evoked release sites to maximize their opportunity for activation. Our lab recently demonstrated that the optimal positioning of AMPARs is likely coordinated by the postsynaptic cell adhesion molecule (CAM) LRRTM2, as the acute disruption of its cleft interactions dispersed AMPARs from sites of evoked release and reduced the postsynaptic response. This suggests a novel role for synaptic CAMs in controlling synapse strength through the regulation of nanostructure and raises the question of whether NMDA receptors (NMDARs) are coordinated via a similar mechanism. An attractive model is that *presynaptic* CAMs linked to active zone (AZ) release machinery may convey structural information trans-synaptically to organize postsynaptic NMDARs. One candidate family for this are the LAR-RPTPs, which interact directly with the AZ protein Liprin-α and have several potential PSD-resident binding partners. Among the family members, PTPσ (*PTPRS*) and its trans-interactors have been associated with NMDAR dysfunction, well positioning PTPσ as a presynaptic CAM that may coordinate trans-synaptic alignment and fine tune NMDAR function. Here, we develop a novel approach to elucidate the ongoing functions of PTPσ in established synapses without compromising its earlier role in synapse formation. We engineered a series of PTPσ mutants containing extracellular proteolytic cleavage sites and transfected them into cultured hippocampal neurons. Using this approach, application of exogenous protease resulted in >70% loss of PTPσ extracellular domain after 1 hour. We then measured the effect of PTPσ cleavage on basal postsynaptic NMDAR function using GCaMP8f Ca^{2+} imaging. Using this assay, we detected acute changes in NMDAR-mediated synaptic responses in correlation with the loss of PTPσ cleft interactions. This result suggests that PTPσ's cleft interactions are required for normal NMDAR function on an acute time scale unobservable in knockout approaches. Altogether, elucidating the key players involved in coordinating nanostructure and maintaining trans-synaptic alignment will provide candidate molecules and new concepts for regulation of synaptic strength and plasticity.

Disclosures: **E.M. DeMarco:** None. **A.D. Levy:** None. **S.R. Metzbower:** None. **M. Contreras:** None. **T.A. Blanpied:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

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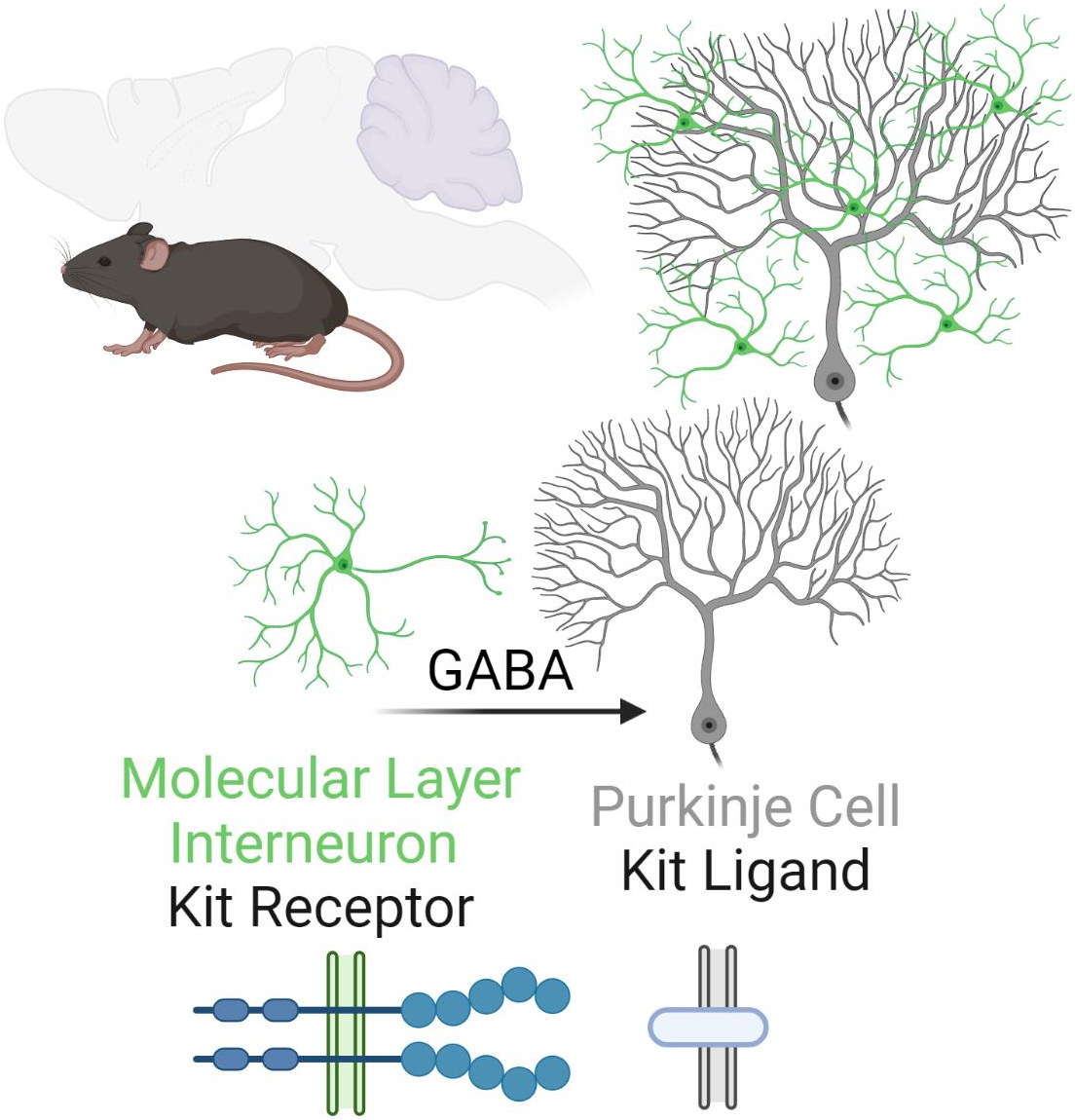
Topic: B.04. Synaptic Transmission

Support: NIH Grant K99/R00 MH110665
The Mall Family Foundation

Title: Synaptic Inhibition of Purkinje Cells Requires Kit Ligand and Kit Receptor Tyrosine Kinase Across the Lifespan

Authors: T. ZAMAN[1], D. VOGT[2], J. PROKOP[4], A. STAFFORD[3], G. SIMMS[3], ***M. R. WILLIAMS**[3];
[1]Pediatrics & Human Develop., Michigan State Univesity, Grand Rapids, MI; [2]Michigan State Univ., [3]Pediatrics & Human Develop., Michigan State Univ., Grand Rapids, MI; [4]Office of Res., Corewell Hlth., Grand Rapids, MI

Abstract: The cell-type specific expression of ligand/receptor and cell-adhesion molecules is a fundamental mechanism through which neurons regulate connectivity. Here we determine a functional relevance of the long-established mutually exclusive expression of the receptor tyrosine kinase Kit and the trans-membrane protein Kit Ligand by discrete populations of neurons in the mammalian brain. Kit is enriched in molecular layer interneurons (MLIs) of the cerebellar cortex (i.e., stellate and basket cells), while cerebellar Kit Ligand is selectively expressed by a target of their inhibition, Purkinje cells (PCs). By in vivo genetic manipulation spanning embryonic development through adulthood, we demonstrate that PC Kit Ligand and MLI Kit are required for, and capable of driving changes in, inhibition of PCs. We find that the knockout of KL or of Kit leads to a specific presynaptic defect. Without KL or Kit, MLIs are present in normal numbers and have unimpaired firing, however the ability of MLIs to translate depolarization into GABA release seems specifically impaired. We find that this functional defect is accompanied by structural differences at the MLI-PC synapse including smaller synaptic puncta and pinceaux, as well as reduced abundance of MLI axon terminal markers including PSD-95, Kv1.1, and Kv1.2. Collectively, these works in mice demonstrate that the Kit Ligand/Kit receptor dyad sustains mammalian central synapse function and suggest a rationale for the affiliation of Kit mutation with neurodevelopmental disorders.



Disclosures: **T. Zaman:** None. **D. Vogt:** None. **J. Prokop:** None. **A. Stafford:** None. **G. Simms:** None. **M.R. Williams:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

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Program #/Poster #: PSTR321.11/D8

Topic: B.04. Synaptic Transmission

Support: Roy J. Carver Charitable Trust

Title: Genetic deletion of GluD1 receptors alters midbrain dopamine neurons

Authors: *E. A. INGEBRETSEN[1], K. J. COCHRANE[1], N. L. O'PREY[2], S. C. GANTZ[1]; [1]Mol. Physiol. & Biophysics, The Univ. Of Iowa, Iowa City, IA; [2]Grinnell Col., Grinnell, IA

Abstract: The gene encoding the delta-1 glutamate receptor, *GRID1*, is classified as a strong candidate 'category 2' risk gene in autism spectrum disorder (ASD, SFARI gene database, accessed 02/17/2022). Diagnostic criteria for ASD include reduced social interaction and repetitive or inflexible behaviors, which may also present with hyperactivity, motor stereotypies, and bouts of aggression. In mouse models, deletion of the homologous gene, *Grid1*, results in many ASD-like features including reduced social interaction, hyperactivity and motor stereotypies. There is considerable evidence that some of the behavioral features of ASD, specifically motor behaviors and altered reward of social stimuli, are due to alterations in dopamine signaling in the brain. *Grid1* is expressed in midbrain dopamine neurons where it drives action potential firing (Benamer et al., 2018). But, there is a fundamental gap in knowledge of how loss of *Grid1* affects dopamine signaling. First, we evaluated behavior in male and female global *Grid1*-knock-out mice. We found that *Grid1*-knock-out mice were hyperactive when placed in an open field. They moved greater distances at higher velocities compared to age- and sex-matched wild-type mice. *Grid1*-knock-out mice also spent more time in the center of the open field compared to wild-type mice and displayed a different preference for social interaction; in all reproducing the findings of the Dravid group in male mice (Yadav et al., 2012). Then using whole-cell electrophysiological recordings from acute mouse brain slices, we assessed the morphology, electrical membrane properties, and action potential firing of substantia nigra dopamine neurons from *Grid1*-knock-out mice compared with wild type. The results show that loss of *Grid1* produces changes in midbrain dopamine neurons and dopamine-related behaviors. The consequence of these changes on somatodendritic dopamine D2 receptor-dependent synaptic transmission will be discussed.

Disclosures: E.A. Ingebretsen: None. **K.J. Cochrane:** None. **N.L. O'Prey:** None. **S.C. Gantz:** None.

Poster

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Program #/Poster #: PSTR321.12/D9

Topic: B.04. Synaptic Transmission

Support: NIH Grant R01NS118731

Title: Ultrastructural analysis of CGRP-positive terminals from the parabrachial region in the central amygdala of mice and monkeys

Authors: K. K. DALAL[1], D. CHOI[2], G. P. SHELKAR[3], S. DRAVID[3], *Y. SMITH[1];
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Abstract: Due to the high prevalence of persistent pain in American lives, there has been a strong need to better understand pain pathways to develop more effective pain management treatments. The connection from the parabrachial nucleus (PB) to the central amygdala, a main driver of pain behavior, is tightly regulated by glutamate Delta-1 receptors (GluD1). Unlike typical glutamate receptors, GluD1 is the postsynaptic component of a synaptogenic complex along with neurexin (Nrxn) and cerebellin (Cbln) that regulate the development, maintenance, and properties of inhibitory and excitatory synapses. GluD1 is heavily expressed post-synaptically at axo-somatic synapses formed by Calcitonin gene-related peptide (CGRP)-positive (CGRP+) terminals from the parabrachial region in the laterocapsular amygdala (CeLC). In addition to this dense pericellular innervation, CGRP(+) terminals are also diffusely expressed within the CeLC neuropil suggesting additional post-synaptic targets of PB-CeLC synapses. In this study, we used electron microscopy to further characterize the synaptic organization of this system in normal mice and monkeys. Furthermore, given evidence that the GluD1 signaling is altered in mice models of pain (Gandhi et al., 2021; Cells 10, 2644), we will determine if the morphology and synaptic organization of the CGRP(+) PB-CeLC terminals are disrupted in the Complete Freund's Adjuvant (CFA) pain mouse model. Data obtained so far from over 400 micrographs of CGRP(+) terminals in the CeLC tissue of 3 control and 3 CFA-treated mice indicate that PB-CeLC CGRP(+) terminals form a highly heterogeneous population that display a wide range of ultrastructural features (round, elongated or pleomorphic shapes, 1-3 um in diameter, variable density of synaptic vesicles, 1-5 mitochondria) and can be distinguished by the formation of structurally complex symmetric or asymmetric synapses with various post-synaptic targets (perikarya, dendrites and spines). Comparisons between control and CFA mice are ongoing. Data obtained so far from the analysis of over 150 CGRP(+) boutons in three rhesus monkeys revealed several common morphological features between mice and primates in terms of the PB-CeLC synaptic organization. Overall, the results from this study will set the stage for a deeper understanding of the synaptic microcircuits through which the PB-CeLC system mediates its effects on pain persistence and determine if disruption of this network is associated with the development of inflammatory pain.

Disclosures: K.K. Dalal: None. **D. Choi:** None. **G.P. Shelkar:** None. **S. Dravid:** None. **Y. Smith:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.13/D10

Topic: B.04. Synaptic Transmission

Support: NIH Grant R01MH116003
P51OD011132

Title: Ultrastructural localization of glutamate delta receptor 1 in the lateral habenula

Authors: *D. CHOI[1], J.-F. PARÉ[1], S. DRAVID[2], Y. SMITH[1];
[1]Emory Univ., Atlanta, GA; [2]Creighton Univ., Creighton Univ., Omaha, NE

Abstract: Glutamate delta receptors (GluDs) are synaptogenic molecules that bind to presynaptic neurexin-cerebellin dyads to form trans-synaptic complexes. These complexes regulate synapse organization as well as the transduction of presynaptic signals into distinct postsynaptic responses. GluD2 has been well-studied in the synaptic development of the cerebellar cortex, and GluD1 has been shown to have synaptogenic functions in regions such as the hippocampus, striatum, and central amygdala. GluD1 is strongly expressed in the lateral habenula (LHb), a subcortical structure that regulates negative reward prediction error as well as major monoaminergic systems. LHb dysfunction has been implicated in psychiatric disorders such as major depressive disorder and schizophrenia, both of which are associated with *GRID1*, the gene that encodes GluD1. As such, disruption in GluD1 synaptic signaling may contribute to LHb dysfunction and subsequently to psychiatric disorders associated with LHb dysfunction. Though LHb neurons are highly enriched in GluD1, its subcellular and subsynaptic localization has yet to be determined. Furthermore, elucidating which LHb afferents are connected to GluD1 will provide more information on the mechanism by which GluD1 mediates its synaptogenic effects. To address these issues, we combined immuno-electron microscopy (EM) and anterograde tracing methods. We have previously shown that LHb GluD1 is primarily expressed in dendritic profiles where it often aggregates at asymmetric synapses. Preliminary EM data from LHb tissue in three monkeys demonstrate a similar pattern of subcellular and subsynaptic localization of GluD1 in primates. To determine which LHb afferents express post-synaptic GluD1, we anterogradely labeled terminals from the lateral hypothalamus (LH), medial prefrontal cortex (mPFC), and entopeduncular nucleus (EPN) with AAV5-GFP and analyzed their relationships with GluD1 in rats. Consistent with the previous literature, both LH and EPN provided a much stronger input to LHb than mPFC. Preliminary results indicate that GluD1 is frequently expressed at LH synapses. Quantitative analysis of GluD1 expression at mPFC and EPN synapses is ongoing. Once data collection has been completed, the relative prevalence of LH, mPFC and EPN terminals associated with GluD1 will be compared. Future work will include 3D-EM reconstruction to determine if GluD1 knockout impacts the synaptic morphology of individual terminals associated with GluD1 in the LHb. Results of these studies will lay the foundation for functional analyses of the synaptogenic role of GluD1 at specific LHb afferents.

Disclosures: D. Choi: None. **J. Paré:** None. **S. Dravid:** None. **Y. Smith:** None.

Poster

PSTR321. Transsynaptic Ultrastructure Organization and Regulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR321.14/D11

Topic: B.04. Synaptic Transmission

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Department of Energy (contract no. DE-AC02-05CH11231)
CPRIT RP190682

Title: Designer molecules of the synaptic organizer MDGA1 reveal 3D conformational control of biological function

Authors: H. LEE[1], N. CHOFFLET[2], J. LIU[3], S. FAN[1], M. MACHIUS[1], G. REN[3], H. TAKAHASHI[2], ***G. RUDENKO**[1];
[1]Pharmacology/Toxicology and the Sealy Ctr. for Structural Biol., Univ. of Texas Med. Br., Galveston, TX; [2]Synapse Develop. and Plasticity, Inst. de Recherches Cliniques de Montréal, Montreal, QC, Canada; [3]Mol. Foundry, Lawrence Berkeley Natl. Lab., Berkeley, CA

Abstract: Synaptic adhesion and organizing molecules, also known as ‘synaptic organizers’, play an essential role in promoting synapse development. The synaptic organizers neuroligins (NLGNs) and neurexins (NRXNs) protrude into the synaptic cleft where they form trans-synaptic macromolecular bridges with each other specifying synaptic function. MDGA1 and MDGA2 (MAM domain-containing glycosylphosphatidylinositol anchor) block NRXN:NLGN trans-synaptic bridges by shielding NLGNs from NRXN binding, impacting the development of inhibitory versus excitatory synapses, respectively. MDGAs, NRXNs, and NLGNs are thought to impact the balance of excitation versus inhibition, affecting for example neural circuits critical for cognition and behavior. This trio of cell surface molecules is indeed implicated in many neuropsychiatric disorders including autism spectrum disorder and schizophrenia. MDGA1 contains a large, multi-domain extracellular region that adopts a striking triangular shape, both alone and in complex with NLGNs, in crystal structures. But it is not known if this unusual arrangement is required for biological function and binding to NLGNs. Here, we show that MDGA1 adopts not only compact, triangularly shaped molecules, but also extended 3D conformations. We engineer designer mutants that target strategic molecular elbows in MDGA1. We show that these designer molecules have an altered distribution of 3D conformations yet leave the binding affinity between soluble ectodomains of MDGA1 and NLGN2 essentially intact. However, these designer mutants trigger unique functional consequences, despite the mutations being located far away from the MDGA1:NLGN2 interaction site. Our data suggest that the 3D conformation of the MDGA1 ectodomain is critical for its function, especially within the confines of the synaptic cleft, and that the NLGN binding site located on the N-terminal Ig1-Ig2 domains of MDGA1 is not independent from the rest of the molecule. Furthermore, conformational changes to the MDGA1 ectodomain via strategic hinges may form a molecular mechanism to regulate MDGA1 action within the synaptic cleft.

Disclosures: **H. Lee:** None. **N. Chofflet:** None. **J. Liu:** None. **S. Fan:** None. **M. Machius:** None. **G. Ren:** None. **H. Takahashi:** None. **G. Rudenko:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.01/D12

Topic: B.09. Glial Mechanisms

Support: NHMRC 1138038
DoH (MRFF) 2009025

Title: Molecular Diversity of Astrocytes in the Primate Brain

Authors: ***L. TEO**[1], T. CHOW[1], K. ONISHI[2], T. A. HOANG[1], T. SHIMOGORI[2], J. A. BOURNE[3];
[1]Australian Regenerative Med. Inst., MONASH UNIVERSITY, Melbourne, Australia; [2]RIKEN, RIKEN, Saitama, Japan; [3]Natl. Inst. of Mental Hlth., NIMH, Bethesda, MD

Abstract: Astrocytes play diverse roles in the CNS that are likely underpinned by functional specializations. These range from maintaining homeostasis to active regulation of neural communication. However, the specific molecular signatures underlying astrocyte functional diversity still need to be clarified beyond anatomical and spatial phenotypes. Here, we investigated the gene expression of astrocytes in the mouse, nonhuman primate and human neocortex to identify molecular signatures that align with functional specializations.
We curated data from published and unpublished mouse, marmoset and human neocortical single-cell/nuclei RNAseq and spatial transcriptomic datasets. Data analysis focused on the broad categorization of astrocytes based on functional annotations, interspecies comparison, and spatial correlation.
Cortical astrocytes were categorized into 5 functionally distinct clusters that align with the known morphological (Protoplasmic, L1/Interlaminar, WM/Fibrous) and functional (Syt1/PSD95+, ST18/MBP+) categories. Protoplasmic clusters were primarily associated with typical astrocyte metabolism. Further specializations of protoplasmic astrocytes included a subpopulation of Syt1/PSD95+ astrocytes that enriched genes associated with post-synaptic density, calcium signaling, and glutamate exocytosis, consistent with bidirectional glutamate-dependent communication with neurones. ST18/MBP+ astrocytes were restricted to white matter but distinct from ITGB4/VCAN+ fibrous astrocytes and enriched perinodal astrocytes associated genes (NFASC, CNTN2, ANK3). ST18/MBP+ astrocytes also enriched myelin-associated genes (MAL, PLP1, MYRF), indicating involvement in myelin regulation or support. Primate interlaminar astrocytes exhibited gene expression associated with synaptic plasticity (SPARC, GPC6, TGFBs, NLGN1) and extracellular matrix (BCAN, VCAN, NCAN), and appeared to exhibit functional annotations distinct from rodent L1 astrocytes. This functional divergence and increased anatomical complexity likely arose in response to later evolutionary adaptations in

more complex brains.
Our data identified molecular signatures that aligns with and defines the anatomical, spatial and functional characteristics of astrocytes in the primate neocortex. These results provide new insights into their functional specializations and new adaptations that are likely driven by the increased complexity of the primate brain.

Disclosures: L. Teo: None. **T. Chow:** None. **K. Onishi:** None. **T.A. Hoang:** None. **T. Shimogori:** None. **J.A. Bourne:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.02/D13

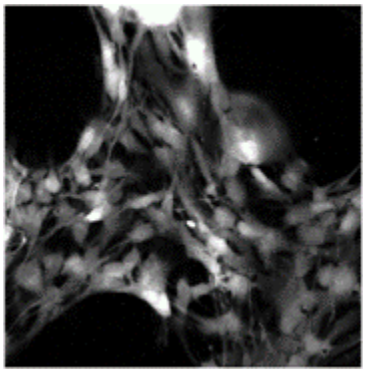
Topic: B.09. Glial Mechanisms

Support: AFOSR Grant 5281650

Title: Collective information and excitability in morphologically distinct astrocytes

Authors: *N. MENNNONA;
Univ. of Maryland, Col. Park, College Park, MD

Abstract: Astrocytes, a non-neural brain cell, regulate the information flow in the brain by coupling to neurons via the Tri-Partite synapse. Rather than quantifying the signaling properties of a single cell, we analyze the collective information flow of three main morphological phenotypes of astrocytes, (1) stellate, (2) polygonal, and (3) reactive. In terms of functional states, the phenotypes correspond to immature, mature, and injury prevention, respectively. Using data analytic measures to separately capture the activity of multiple cells in a FOV, we analyze the collective excitability measures of astrocytic networks. We find that while the speed of information content remains constant across conditions, the threshold for activity varies depending upon phenotype. These results suggest that astrocytes can be modelled as an excitable system.



Disclosures: N. Mennnona: None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.03/D14

Topic: B.09. Glial Mechanisms

Support: NIH Grant NS122157
Edna Ittner Pediatric Research

Title: Functional analysis of human interlaminar astrocytes following development in the mouse cortex

Authors: A. ANDING[1], M. BUERKOVETSKAYA[2], K. HOFFMAN[1], P. RAGUNATHAN[2], *A. DUNAEVSKY[2];
[2]Neurolog. Sci., [1]Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Interlaminar astrocytes (ILA) are primate-specific astrocytes that are located in the superficial layer of the cortex and project long processes that run perpendicular to the pial surface and traverse multiple layers of the cerebral cortex. Due to the lack of accessible experimental models, the functional properties of ILA and their role in regulating neuronal circuits remain unclear. We have previously shown that human interlaminar astrocytes can develop in humanized glial chimeric mice. Following engraftment of astrocytes differentiated from human-induced pluripotent stem cells (hiPSC) into the mouse cortex, hiPSC-astrocytes develop features characteristic of ILA observed in the human brain. Here we have extended this model to study the functional properties of ILA and to determine if they are altered in astrocytes derived from Fragile X Syndrome hiPSCs. Six months following engraftment, ILAs expressing the calcium sensor GCaMP6s were imaged in cortical brain slices. The spatiotemporal dynamics of calcium signals along the long interlaminar processes in response to purinergic and neuromodulatory stimulation were imaged with two-photon microscopy. In vivo calcium activity ILAs in awake head restrained mice are also examined by 2-photon imaging through a cranial window. Comparison between activity of ILAs derived from FXS and control hiPSCs is ongoing. Our hypothesis is that FXS ILAs have altered functional properties that contribute to FXS pathogenesis.

Disclosures: A. Anding: None. **M. Buerkovetskaya:** None. **K. Hoffman:** None. **P. Ragunathan:** None. **A. Dunaevsky:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.04/D15

Topic: B.09. Glial Mechanisms

Support: NSERC
Canada Research Chairs
Ontario Graduate Scholarships

Title: Neonatal estrogen induces male-like expression patterns of neocortical astroglia maturation markers in early postnatal development

Authors: *G. M. RURAK[1], A. GAHELRASOUL[1], J. STEAD[1], B. C. WOODSIDE[2], A. AGUILAR VALLES[1], G. COPPOLA[3], N. SALMASO[1];
[1]Dept. of Neurosci., Carleton Univ., Ottawa, ON, Canada; [2]Concordia Univ., Montreal, QC, Canada; [3]Dept. of Pathology, Yale Univ., New Haven, CT

Abstract: Astroglial cells have emerged as key regulators of various aspects of neurodevelopment, including building, maintaining, and regulating neuronal networks, synapse development and maturation, blood-brain barrier development. We previously used translating ribosome affinity purification with RNA sequencing (TRAPseq) and identified sex differences in neurodevelopmental patterns of the neocortical astroglial translatome during maturation from an "early" to a "late" phenotype such that males show faster maturation compared to females. Intriguingly, most sex differences in the astroglial translatome occur largely when circulating gonadal hormones are absent in the developing mouse brain. To investigate whether perinatal masculinizing hormones are responsible for sex differences in neocortical astroglial protein and gene expression, we administered estradiol benzoate to females pups 1-hour following birth and used immunohistochemistry and RT-qPCR to quantify astroglial markers of maturation and plasticity in the neocortex. We found females treated with estradiol benzoate had increased gene expression of makers associated with astroglial maturation, such as *Aldh1a1,*and decreased protein expression of markers associated with astroglial plasticity, such as vimentin, at postnatal day seven. These findings suggest that estradiol may be involved in the induction of early sex differences in neocortical astroglia and suggest potential functional implications that may lead to sex disparities in neurodevelopmental and psychiatric disorders. As astroglial cells play a crucial role in maintaining the structural and functional integrity of the brain, it is imperative to understand how gonadal hormones influence their differentiation and function. Further research is needed to fully understand the specific mechanisms that underlie the effects of gonadal hormones on astroglial cells and how these mechanisms contribute to sex differences in brain development and function.

Disclosures: G.M. Rurak: None. **A. Gahelrasoul:** None. **J. Stead:** None. **B.C. Woodside:** None. **A. Aguilar Valles:** None. **G. Coppola:** None. **N. Salmaso:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.05/D16

Topic: B.09. Glial Mechanisms

Support: Smith Grant

Title: Characterization of calcium clearance dynamics in hippocampal ex-vivo cultures

Authors: *G. K. JOHN, J. G. JACKSON;
Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Astrocytes respond to injury in the central nervous system by undergoing molecular and transcriptional changes that result in the gain or loss of homeostatic function, a process referred to as reactivity. This reactive phenotype is associated with dysregulation of calcium signaling within astrocytes, and has been implicated in several neurological disorders, including epilepsy, Alzheimer's disease, and stroke. Although various studies have explored the mechanisms by which calcium levels increase, there is a significant knowledge gap surrounding the termination of calcium signaling post-injury. Using live cell confocal microscopy, we investigated the dynamics of calcium clearance in astrocyte processes. To assess this, organotypic hippocampal cultures from post-natal C57BL/6 mice were transfected with a membrane tethered genetic calcium indicator (LCK-GCaMP6f) under an astrocyte specific promotor (GFA_{ABC1D}). By superfusing pharmacological inhibitors of known clearance pathways (PMCA, SERCA, NCX) during live cell imaging, we identified plasma membrane calcium ATPase (PMCA) as the primary mediator of calcium extrusion in naïve slices. PMCA inhibition resulted in significant changes in frequency of calcium events, spatial distribution, duration, and clearance rate, as compared to other clearance-related pathways. Inducing-astrocyte reactivity, either by 30 minutes of oxygen-glucose deprivation or 24-hour cytokine treatment (TNFa, IL1a, C1q), results in changes in calcium transient duration, propagation, and clearance rate. By pharmacologically isolating individual clearance pathways, we further characterized the contributions of PMCA, SERCA, and NCX to calcium clearance under these conditions.

Disclosures: G.K. John: None. **J.G. Jackson:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.06/D17

Topic: B.09. Glial Mechanisms

Title: Astrocytes mediate the effect of norepinephrine at excitatory synapses

Authors: *K. B. LEFTON, Y. WU, S. WALSH, R. MANNO, T. PAPOUIN;
Neurosci., Washington Univ. in St. Louis Neurosci. PhD Program, St. Louis, MO

Abstract: Astrocytes are known to respond to norepinephrine (NE) neuromodulation with a robust Ca^{2+} response, driving intense speculation to the circuit and behavioral relevance of astrocytes in NE-dependent processes. Surprisingly, the significance of this responsiveness to the circuit effects of NE neuromodulation, however, has remained unaddressed. In the cortex and hippocampus, NE induces a profound change in circuit connectivity, in part via its well-documented dampening of synaptic efficacy. Here we report that NE modulates synaptic networks by signaling entirely through astrocytes. Indeed, we find that scavenging endogenous adenosine or blocking adenosine A1 receptors (A1R) abolishes the effect of NE on excitatory synapses in adult (P100) mouse hippocampal slices. In the hippocampus, A1Rs are predominantly expressed pre-synaptically and are negatively coupled to release probability. Consistently, we find that the effect of NE on synaptic transmission is mediated by a decrease in pre-synaptic release probability. Furthermore, we find that NE applications are without effect on synaptic strength in slices obtained from mice lacking the ATP-adenosine converting enzyme, CD73. Together, this indicates that, rather than a direct effect, NE modulation of synaptic efficacy leverages ATP-adenosine signaling. Astrocytes are a major source of extracellular ATP, and astrocyte-derived ATP release is Ca^{2+}-dependent. Therefore, we sought out to test directly the role played by astrocytes in the effect of NE on synaptic function. We find that interfering with astrocytic Ca^{2+} activity using CalEx, iBARK, and pharmacological approaches entirely abolishes the effect of NE on synapses. We further show that knocking out alpha1a noradrenergic receptors from astrocytes entirely blocks both the effect of NE on astrocyte Ca^{2+} activity and the inhibitory effect of NE on synapses. Combined, our findings fuel the notion that NE neuromodulation remodels synaptic networks via an entirely indirect, astrocyte-dependent mechanism rather than by acting directly through neuronal noradrenergic receptors.

Disclosures: K.B. Lefton: None. **Y. Wu:** None. **S. Walsh:** None. **R. Manno:** None. **T. Papouin:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Topic: B.09. Glial Mechanisms

Support: HKRGC-GRF 14102221
HKRGC-GRF 14115821
HKRGC-GRF 14113522
CRF-C4012-22GF

Title: Plasticity of cortical astrocytes during motor learning

Authors: Y. ZHENG[1], ***S. WANG**[1], W. YUNG[2], Y. KE[1];
[1]The Chinese Univ. of Hong Kong, HONG KONG, China; [2]Neurosci., City Univ. of Hong Kong, Kowloon, Hong Kong

Abstract: Ample evidence in recent years have confirmed the active participation of astrocytes in modulating learning and memory formation. However, how astrocytes are involved in the process of motor learning is still unclear. Here, we investigated the role of astrocytes in terms of their structural and functional plasticity during a lever-pushing task. We first demonstrated morphological changes of astrocytes upon motor learning. Additionally, we monitored calcium dynamics in primary motor cortex (M1) astrocytes and dissected the spatiotemporal distribution of Ca2+ signals in M1 astrocytes throughout the task training. Our results revealed clear temporal patterns of astrocytic Ca2+ activities concentrating around movement-window as learning progressed. Chemogenetic manipulations of Ca2+ activities in M1 astrocytes suppressed learning of the lever task. Together, we have provided evidence that astrocytic calcium dynamics play an active role during motor learning.

Disclosures: Y. Zheng: None. **S. Wang:** None. **W. Yung:** None. **Y. Ke:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR322.08/D19

Topic: B.09. Glial Mechanisms

Support: R01MH107886 to KMF
1F31MH118822-01A1 to LRT
National Scholarship Programme of the Slovak Republic, SAIA, n.o., and Tatra bank foundation grant to MP
RFAG042189 to FS
1F31NS118970-01A1 to TEB

Title: The astrocytic glutamate transporter GLT-1 is essential for the memory-enhancing effects of 17-beta estradiol in ovariectomized mice.

Authors: *L. TAXIER[1], M. PILLEROVA[2], T. BRANYAN[3], F. SOHRABJI[4], K. FRICK[5]; [1]Univ. of North Carolina-Chapel Hill, Chapel Hill, NC; [2]Inst. of Mol. Biomedicine, Med. Faculty, Comenius University, Bratislava, Slovakia, Bratislava, Slovakia; [3]Texas A&M Univ., Bryan, TX; [4]Neurosci. and Exptl. Therapeut., Texas A&M Univ. Syst. Hlth. Scien Neurosci. and Exptl. Therapeut., Bryan, TX; [5]Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The mnemonic benefits of 17-beta estradiol (E2) depend upon rapid activation of cell signaling cascades in the dorsal hippocampus (DH), presumably within pyramidal neurons. Although the presence of estrogen receptors on hippocampal astrocytes suggests that these cells are targets for estrogenic action, a role for DH astrocytes in promoting the procognitive effects of E2 has yet to be demonstrated. Here, we aimed to 1) test the hypothesis that the astrocytic glutamate transporter, GLT-1, is required for estrogenic enhancement of object recognition and object placement memory consolidation, and 2) evaluate whether E2 initiates cell signaling

events, including rapid phosphorylation of extracellular signal regulated kinase (ERK) and Akt, in DH astrocytes. Ovariectomized female mice were bilaterally cannulated into the DH or the dorsal third ventricle (ICV). Post-training infusion of the GLT-1 inhibitor dihydrokainic acid (DHK) dose-dependently impaired memory consolidation for a previously seen object or spatial location. Next, mice were infused with vehicle or DHK into the DH, and vehicle or E2 ICV. The memory-enhancing effects of E2 were blocked by DH DHK infusion in both tasks. In DH astrocytes isolated via magnetic-activated cell sorting, E2 increased rapid phosphorylation of p42 ERK and Akt proteins, an effect that was blocked by DH infusion of DHK. These results demonstrate that DH astrocytic glutamate transport is required for object recognition and spatial memory consolidation, and for the memory-enhancing effects of E2 in both tasks. Furthermore, DH astrocytic GLT-1 activity is required for E2 to rapidly activate p42 ERK and Akt signaling in DH astrocytes. Together, these findings suggest a critical role for DH astrocytic GLT-1 activity in the memory-enhancing effects of E2.

Disclosures: L. Taxier: None. **M. Pillerova:** None. **T. Branyan:** None. **F. Sohrabji:** None. **K. Frick:** Other; Estrigenix Therapeutics, Inc.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR322.09/D20

Topic: B.09. Glial Mechanisms

Support: NIH Grant R21NS097913
NIH Grant R21NS108508
NIH Grant R21NS120315
NIH Grant R01NS121542

Title: Cerebellar astrocytes encode and regulate reward-associated behavior

Authors: *C. LI[1], W. LI[2];
[1]UAB Neurosci. Grad. Programs, Birmingham, AL; [2]Neurobio., UAB, Birmingham, AL

Abstract: The cerebellum, a brain structure renowned for its involvement in motor coordination and balance, has recently emerged as a key player in cognitive and emotional processing. Astrocytes, a type of glial cells, have been shown to actively contribute to higher-order brain functions by regulating the extracellular environment, providing metabolic support to neurons, and modulating synaptic transmission. Despite these advancements, the implications of cerebellar astrocytes in cognitive processes remain unknown. In this study, we employed fiber photometry to capture astrocytic population activities in mice expressing the glial Ca^{2+} sensor GCaMP6f during a reward task. The task involved a 30-minute period where mice had to enter the trigger zone to initiate a trial, followed by the reward zone to trigger water ejection and receive sucrose water reward. Our preliminary data revealed that Ca^{2+} dynamics in cerebellar

cortex astrocytes were correlated with reward and exhibited two distinct patterns in different astrocytic domains, referred to as type I and type II astrocytes. Type I astrocytes displayed rapid activation of Ca^{2+} signals when trained mice entered the trigger or reward zone. In contrast, type II astrocytes exhibited a gradual and robust rise in Ca^{2+} as the animals approached the reward. This Ca^{2+} enhancement was followed by a marked decrease when the animals licked water. Additionally, we recorded astrocytic activities in different regions of the deep cerebellar nuclei (DCN). The medial DCN signals exhibited a mixture of type I and type II signals, while Ca^{2+} dynamics in the lateral DCN showed a reverse trend compared to type II astrocytic activities. To investigate single astrocytic activities, we employed miniscope Ca^{2+} imaging, confirming the presence of different astrocyte subtypes. To modulate astrocyte activities, we selectively activate the Gi or Gq pathways of astrocytes in specific regions using chemogenetic tools, targeting type I and type II astrocytes. Activation of both Gq and Gi pathways resulted in decreased Ca^{2+} transients, leading to observable changes in reward behaviors, including fewer activated trials during the 30-minute period and shorter licking time. Overall, our study provides compelling evidence that cerebellar astrocytes actively encode and regulate reward behaviors, shedding light on their important role in the reward circuitry.

Disclosures: C. Li: None. **W. Li:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.10/D21

Topic: B.09. Glial Mechanisms

Title: Astrocytic NMDARs in the dorsal striatum controls fine motor skills

Authors: *S. SABNIS, S. DRAVID;
Creighton, omaha, NE

Abstract: Astrocytic NMDARs in the dorsal striatum controls fine motor skills Sabnis Siddhesh[1], Dravid Shashank[1] [1]Creighton University, Department of Pharmacology and Neuroscience
Astrocytes are known to provide trophic support, maintain homeostasis, regulate synapse and have been implicated in various neuropsychiatric and neurological disorders like Parkinson's, schizophrenia, autism, and others. Astrocytes are known to control behaviors like hyperactivity, goal-directed behavior, behavioral flexibility, reward-seeking behavior, motor behavior. Astrocytes express numerous receptors for glutamate, purines, neuroactive amino acids, catecholamines. The expression and function of astrocytic N-Methyl D-Aspartate receptors (NMDARs) have remained enigmatic for several decades. Recent studies using novel tools have unequivocally demonstrated astrocytic NMDAR expression and roles in behavior control. However, there is still limited information regarding their ability to regulate different circuits regulating behavior. We deleted astrocytic GluN1, an obligatory subunit using tamoxifen

injections (75mg/kg for 5 days intraperitonially) in AldhCreGluN1$^{flox/flox}$ mice model and studied changes in their behavior. Mice with deletion of astrocytic GluN1 caused impairment in fine motor skills. Fine motor behavior includes the tasks of walking on a fine beam, removing sticky paper from its hind paw and walking through a bridge. Dorsal striatum is involved in the regulating the fine motor skills thus we deleted the astrocytic GluN1 from the dorsal striatum of GluN1$^{flox/flox}$ mice using adeno-associated-virus (AAVs) and subjected these mice to the fine motor tests. We found that deletion of astrocytic GluN1 from dorsal striatum also showed deficit in fine motor skills implying that the astrocytic NMDARs from the dorsal striatum are important for controlling fine motor skills. In the ongoing studies we are examining the mechanism by which these astrocytes control the fine motor skills.

Disclosures: S. Sabnis: None. **S. Dravid:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.11/D22

Topic: B.09. Glial Mechanisms

Support: NSERC R5476A06
Canada First Research Excellence Fund R5476A02

Title: Impairments in spatial pattern separation following dentate gyrus astrocyte perturbation are rescued by D-serine

Authors: *P. A. S. SHEPPARD, O. R. GHOSH-SWABY, A. M. CROOKS, V. F. PRADO, M. A. PRADO, T. J. BUSSEY, L. M. SAKSIDA;
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Abstract: Pattern separation - the cognitive process of keeping similar or overlapping memories distinct from one another - is essential for accurate memory recall. Computational models and both clinical and pre-clinical research highlight the dentate gyrus (DG) region of the hippocampus as critical to this process. As astrocytes release numerous neuroactive compounds known to be involved in pattern separation (e.g. brain-derived neurotrophic factor) and modulate synaptic plasticity, we recently began investigating the role of these non-neuronal brain cells in spatial pattern separation. Here, we used two techniques to alter DG astrocyte activity. We employed Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) to selectively activate Gi signaling in DG astrocytes of GLAST- and Aldh1l1-CreERT2 mice performing a Spontaneous Location Recognition task. Gi activation by intra-DG administration of clozapine N-oxide (CNO) in DREADD-expressing mice impaired pattern separation, indicated by an inability to discriminate similar object locations. Next, we knocked down astrocytes in the DG via intra-DG L-a-aminoadipate (L-AAA) administration, which similarly resulted in impaired pattern separation. Following this, intra-DG or systemic treatment with D-

serine (NMDAR co-agonist) improved pattern separation. When administered low doses of D-serine, pattern separation was restored in mice impaired by astrocyte Gi activation or L-AAA-induced knockdown. Finally, in mice fed a high-fat, high-sugar diet that has previously been shown to disrupt hippocampal astrocyte function, pattern separation was impaired, but restored with acute, systemic D-serine treatment. Collectively, these data suggest normal astrocyte functioning in the DG is required for pattern separation and may involve D-serine release.

Disclosures: **P.A.S. Sheppard:** None. **O.R. Ghosh-Swaby:** None. **A.M. Crooks:** None. **V.F. Prado:** None. **M.A. Prado:** None. **T.J. Bussey:** Other; Campden Instruments. **L.M. Saksida:** Other; Campden Instruments.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.12/D23

Topic: B.09. Glial Mechanisms

Support: MH106490
NS118747
FRAXA Fellowship

Title: Selective deletion of astroglial miR-128-3p alters astroglial molecular maturation and cognitive behaviors

Authors: *K. E. REYNOLDS, A. PANG, C. CUNNINGHAM, R. JARVIS, M. MAJID, M. BERTOLIO, Y. YANG;
Dept. of Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Astroglial cells play active and diverse roles in modulating synaptogenesis and synaptic functions, especially during postnatal development. Astroglia undergo postnatal maturation in which unique morphological features are established and functional proteins are induced. In the CNS, microRNAs (miRs) have been shown to play key roles in the specification of neural progenitor cells and synaptic regulation. Specific miRs have also been identified to regulate oligodendrocyte and microglia developmental maturation. Surprisingly, specific miRs involved in astroglial development, especially postnatal molecular and morphological maturation, are essentially unknown. In the current study, we set out to investigate the role of miRs in regulating postnatal astroglial molecular maturation. By acutely isolating cortical astroglia at P7 (immature) and P23 (mature) ages from BAC Aldh1l1-eGFP mice and performing miR microarray, we first identified a number of miRs that are developmentally regulated in cortical astroglia. To identify mRNA targets of these developmentally changed miRs in astroglia, we then developed a Python-based and TargetScan-dependent miR binding analysis program, M2 Binding (M2B), to streamline the analysis of miR binding sites on mRNAs that are expressed during postnatal maturation of cortical astroglia. We also performed miR binding site

analysis on mRNAs that are selectively or preferentially expressed in astroglia over other CNS cell types based on relative FPKM values (fold change >4). Our bioinformatic analysis found that a subset of developmentally regulated miRs can individually and simultaneously bind to multiple (>5) astroglial functional mRNAs. In particular, brain-enriched miR-128-3p is predicted to simultaneously bind more than 8 mRNAs that encode important astroglial functional proteins such as xCT, GAT1, and mGluR5. Direct transfection of miR-128-3p is sufficient to significantly decrease xCT and mGluR5 protein levels in astrocyte cultures, which can be fully rescued by miR-128-3p specific antisense (A/S). We further generated astroglial miR-128-3p conditional knockout (cKO) mice (Slc1a3-CreER^{+}miR-128-3p$^{f/f}$) and performed behavioral tests that assess hyperactivity, stereotypy, sociability, sensory sensitivity, and fear-conditioned learning and memory. Interestingly, delayed memory extinction and reduced stereotypic marble burying behavior were observed in astroglial miR-128-3p cKO mice compared to 4-OHT-injected CreER^{-} littermate controls. Whole-genome transcriptome and proteome analysis in miR-128-3p-deleted cortical astroglia is currently ongoing.

Disclosures: K.E. Reynolds: None. **A. Pang:** None. **C. Cunningham:** None. **R. Jarvis:** None. **M. Majid:** None. **M. Bertolio:** None. **Y. Yang:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.13/D24

Topic: A.01. Neurogenesis and Gliogenesis

Support: Lafayette Parish Medical Society Endowed Professorship
UL Lafayette GSO
Undergraduate Minigrant

Title: Loss of fgfr1 signaling in the central nervous system results in impaired maternal behaviors

Authors: *J. A. STAGRAY[1], A. N. CHISTOSERDOV[2], T. STEVENS[3], A. FAUL[3], P. WALLS[2], J. P. FISER[3], J. RICHARD[3], K. M. SMITH[4];
[1]Envrn. and Evolutionary Biol., [3]Biol., [2]Univ. of Louisiana at Lafayette, Lafayette, LA; [4]Univ. of Louisiana At Lafayette, Univ. of Louisiana At Lafayette, Lafayette, LA

Abstract: Rodent models demonstrate that maternal rearing has influences upon endocrine function, cognitive development, and emotional responses. Mice are more susceptible to underlying health predisposition induction as a result of “poor” maternal behaviors. Offspring reared by mothers with poor maternal caring have increased production of stress and anxiety-associated behaviors through alterations in stress-response hormonal pathways associated with glucocorticoid receptor signaling. Some of these changes are linked to epigenetic modification of stress-pathway genes. Ultimately, these “poor” maternal behaviors lead to increasingly

"stressed" adult progeny. Stress-associated behaviors can ultimately affect how adult progeny parent their own pups later in life.
Fibroblast Growth Factor Receptor 1 (FGFR1) is an important tyrosine kinase receptor involved in development of several cell type subpopulations within the Central Nervous System (CNS). FGFR is important for stem cell division and maintenance, neurogenesis, and in astrocyte-neuron interactions. We employed the Nestin-Cre-mediated deletion of the *Fgfr1* gene which targets neural stem cells in CNS, including tanycytes near the hypothalamus third ventricle (3V). Tanycytes help relay the hormonal signals that allow the hypothalamus to regulate physiology through hormone secretion. Our previous studies demonstrated Fgfr1 is strongly expressed in tanycytes at both embryonic and adult time points. Additionally, when comparing Nestin-Cre (+) mice to Nestin-Cre (-) littermates, (+) mice demonstrate altered tanycyte morphology and proliferation capabilities. Furthermore, Fgf2 expression in tanycytes is increased in lactating rodents.
Our experiences in mating this mouse line suggested that (+) female mice exhibit more "poor" maternal behaviors compared to (-) females, with greater pup loss. Here, we assessed 1st time *Fgfr1*$^{Flox/Flox}$ control mothers to *Fgfr1*$^{Flox/Flox}$*; Nestin-Cre+* mothers for Nesting, Exploring, Foraging, and Self-grooming maternal behavioral traits. Mothers "nurturing" abilities by measuring pup retrieval tests, nest-making scores, litters pup milk-spot scores, and pup survival rates to reproductive adulthood. Progeny from both maternal groups were aged to reproductive ages and subjected to elevated + maze test, tail suspension test, and fur quality scoring. Our data indicates (+) mothers exhibit "worse" maternal traits. Future studies will characterize if *(+) mothers* display altered levels of maternal hormones (prolactin and oxytocin).

Disclosures: **J.A. Stagray:** None. **A.N. Chistoserdov:** None. **T. Stevens:** None. **A. Faul:** None. **P. Walls:** None. **J.P. Fiser:** None. **J. Richard:** None. **K.M. Smith:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

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Topic: B.09. Glial Mechanisms

Support: R01 NS051445

Title: Nrf2 Regulates Basal Glutathione Production in Astrocytes via Constitutive Transcriptional Control of Glutamate Cysteine Ligase and xCT

Authors: *J. HE, S. J. HEWETT;
Program in Neurosci., Syracuse Univ., Syracuse, NY

Abstract: Glutathione (GSH) is the most abundant non-protein thiol antioxidant in the central nervous system with the largest reservoir found in astrocytes. The biosynthesis of GSH occurs in the cytosol of cells and involves two steps: the conjugation of cysteine and glutamate to form γ-

glutamylcysteine - catalyzed by glutamate cysteine ligase (GCL) - followed by the addition of glycine - catalyzed by glutathione synthetase (GSS) - to produce γ-L-glutamyl-L-cysteinylglycine (GSH). Additionally, the glutamate/cystine antiporter, System x_c^-, is critical for providing cells with imported cystine that is rapidly reduced to cysteine intracellularly for its incorporation into GSH. Oxidative stress is known to upregulate System x_c^- and GSH synthetic enzymes with Nrf2 acting as a critical transcription factor. However, whether Nrf2 controls constitutive biosynthesis of GSH synthesizing enzymes and System xc- has never been explored. To answer this question, Nrf2 wild-type ($Nrf2^{+/+}$) and null mutant ($Nrf2^{-/-}$) astrocytes were cultured from cerebral cortices of mouse pups derived from $Nrf2^{+/-}$ heterozygous breeding pairs. Fourteen to 29 days later, the concentration of intracellular and extracellular GSH (GSH + GSSG) was measured. Data show that $Nrf2^{-/-}$ astrocytes have significantly less intracellular ($p < 0.0001$) and extracellular ($p = 0.017$) GSH when compared to levels measured in $Nrf2^{+/+}$ cells. Concomitant with this reduction, we found by qRT-PCR that $Nrf2^{-/-}$ astrocytes had a significant decrease in the basal mRNA levels of the glutathione synthesizing enzyme, GCL (both the catalytic and modifier subunits; $p =0.047$ and 0.006, respectively) but not GSS ($p =0.572$). Additionally, mRNA for xCT, the substrate specific light chain of System x_c^-, was also reduced in $Nrf2^{-/-}$ astrocytes ($p <0.0001$). Taken together, our findings provide evidence supporting the involvement of Nrf2 in the basal production of GSH in astrocytes, most likely through constitutive transcriptional regulation of GCL and xCT.

Disclosures: J. He: None. **S.J. Hewett:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Topic: B.09. Glial Mechanisms

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PA Dept. of Health, SAP4100088542
NSF IOS-1921065
NIH NINDS R01NS118562

Title: The transcriptional signature of astrocyte-like glia across Drosophila melanogaster late-stage metamorphosis

Authors: *J. SAUNDERS[1], Y. KURMANGALIYEV[3], A. D. R. GARCIA[1,4], C. R. VON REYN[2,4];
[1]Dept. of Biol., [2]Sch. of Biomed. Engineering, Sci. and Hlth. Systems, Drexel Univ., Philadelphia, PA; [3]Dept. of Biol., Brandeis Univ., Waltham, MA; [4]Dept. of Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Astrocyte development coincides with the formation and refinement of developing neural circuits and their key roles in synapse formation and function are now well recognized. Nevertheless, the mechanisms driving the production and maturation of astrocytes and how these processes cooperate with circuit development is not well understood. Here, we examine the morphological and transcriptional development of astrocytes in the *Drosophila melanogaster* visual system during metamorphosis, the period during which the embryonic nervous system is deconstructed, and the adult nervous system is established. Like vertebrate astrocytes, *Drosophila* astrocytes associate closely with synapses and their morphogenesis coincides with synaptic partner matching and circuit refinement. We first examined the timing of astrocyte invasion into the synaptic neuropil across multiple time points during metamorphosis. We find astrocytes begin to invade the optic lobe neuropil at 60h after puparium formation (APF), coincident with synaptogenesis. There is a progressive increase in neuropil occupation by astrocyte processes until 96h APF, during which synapses are formed and refined. Interestingly, the density of astrocyte processes is greater in the lobula complex compared to the optic glomeruli, suggesting differential occupation of specific neuropil by astrocyte processes. To understand the molecular mechanisms that drive astrocyte development and maturation, we investigated their transcriptional profiles across multiple time points between 48h and 96h APF. We analyzed glial clusters in a recently published sc-RNAseq dataset and identified 2 clusters corresponding to putative astrocytes. These clusters exhibit high levels of *Eaat1* and *Gat* expression, well-established genes that are expressed specifically in astrocytes. Differential gene expression analysis revealed unique transcriptional profiles, with a progressive increase in the number of genes with significantly higher expression in astrocytes as compared to other glia at each time point. This suggests that as astrocytes mature during metamorphosis, they exhibit increasingly divergent gene expression programs that distinguish them from other glial cell classes. Ongoing studies are investigating the role of candidate genes in driving astrocyte development and how these processes cooperate with developing synapses. Overall, this work provides novel insight into the genetic mechanisms regulating astrocyte development, providing a foundation to understand the role of astrocytes in circuit development.

Disclosures: J. Saunders: None. **Y. Kurmangaliyev:** None. **A.D.R. Garcia:** None. **C.R. von Reyn:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR322.16/D27

Topic: B.09. Glial Mechanisms

Support: JSPS International Research Fellow 19F197728
RIKEN Center for Brain Science
Okinawa Institute of Science and Technology

Title: A role for APP and APP-like proteins in astrocyte development

Authors: M. SAINT-MARTIN[1], ***Y. GODA**[2];
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Abstract: The amyloid precursor protein (APP), whose proteolytic cleavage gives rise to amyloid-β peptide, has been extensively studied for its role in Alzheimer's disease, but its physiological function is not fully understood. In neurons, APP and its two homologs, the amyloid precursor-like protein 1 (APLP1) and 2 (APLP2) are found in the synaptic compartment and promote synaptogenesis (Schilling et al., 2017). In addition, APP has been found to be present in astrocytes (Haass et al, 1991; LeBlanc et al., 1991) but its role in these cells remains largely unknown. Here we have investigated the expression of APP, APLP1 and APLP2 in mouse astrocytes and their possible function. We show that APP, APLP1 and APLP2 are expressed in astrocytes in primary hippocampal cultures and in 3 week-old mouse hippocampus and neocortex. Compromising the expression level of APP, APLP1 or APLP2 by shRNA-mediated knockdown in astrocytes differentially alters the extent of morphological elaboration of cultured hippocampal astrocytes compared to control scrambled shRNA. Furthermore, neonatal sinus vein injection of AAV shRNA vector in newborn mice which results in a significant knockdown of APP in 3 week-old mouse brain astrocytes, decreases the neuropil infiltration volume of astrocytes, a measure of astrocyte process complexity, by ~20% compared to control scrambled shRNA in hippocampal and cortical astrocytes. This reduction can be rescued by co-expressing knockdown-resistant APP. Our results suggest a role for astrocyte APP in the elaboration of astrocyte process morphology in the developing brain, which may impact neuronal circuit functions.

Disclosures: M. Saint-Martin: None. **Y. Goda:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR322.17/D28

Topic: B.09. Glial Mechanisms

Support: NIH Grant HD076892
Ministry of Education of Singapore (MOE2018-T2-2-103)

Title: Gfap is a master regulator of astrocyte organelles

Authors: *L. KONG[1], B. WANG[1], D. XIONG[2], L. FIELDS[1], M. CROCKETT[1], X. LI[1], Y. TAO[1], J. GRAHAM[1], R. SHANKAR[1], K. XU[1], A. AUDHYA[1], L. LI[1], S.-C. ZHANG[1,2];
[1]UW-Madison, Madison, WI; [2]Duke-NUS Med. Sch., Singapore, Singapore

Abstract: Glial fibrillary acidic protein (GFAP) is an intermediate filament and a common astrocyte marker. Mutations in the GFAP gene cause Alexander Disease (AxD), a rare yet fatal neurodegenerative disease. GFAP upregulation is one of the most characteristic features in brain

injuries and neurodegenerative diseases. Yet, what GFAP does and how GFAP alterations contribute to CNS disorders remain vastly unknown. We profiled GFAP associated proteins in human pluripotent stem cell (hPSC)-derived astrocytes, using TurboID mediated proximity labeling and mass spectrometry. We found that GFAP associates with proteins relating to multiple organelles, including ER, Golgi, mitochondria, lysosomes and lipid droplets. Using immunofluorescence, live cell imaging and electron microscopy, we found that GFAP forms direct contacts with these organelles and that GFAP knock-out (KO) alters their morphologies and distributions. Concomitantly, their functions are altered due to GFAP KO and/or AxD mutations. These results suggest that GFAP is a master regulator of organellar morphology, distribution and function in astrocytes. Alteration in GFAP likely results in functional abnormalities of astrocytes and their interactions with surrounding cells, contributing to CNS disorders.

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Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

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Topic: B.09. Glial Mechanisms

Support: NRF-2019R1A5A2026045
NRF-2019M3C7A1031905

Title: Circadian clock controls ER calcium response in primary mouse astrocyte culture

Authors: *J. RYU[1,2], K.-W. SHIM[3], H. ROH[1,4], J.-H. LEE[5], E. KIM[1,2];
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Abstract: The circadian clock, an internal time-keeping system generates approximately 24-hour (circadian) rhythms in physiology and behavior. Disruption of the circadian rhythm is frequently observed in neurodegenerative diseases and has been found to accelerate their progression, indicating a causative and pathogenetic role of the circadian clock. Astrocytes play a crucial role in maintaining brain homeostasis. Hence, it is reasonable to hypothesize that the Circadian clock regulate crucial astrocyte functions, the dysfunctions of which impacts on brain diseases. To investigate the circadian regulation of astrocyte function, we performed circadian transcriptome

analysis using primary astrocyte cultures from wild type (n=3) and Bmal1$^{-/-}$ mice (n=2). The Metacycle and Biocycle algorithms were employed to identify 412 transcripts (2.3%) that exhibited circadian rhythmicity. Gene ontology analysis of these rhythmic genes revealed their involvement in the metal ion homeostasis process. Thus, we examined whether intracellular Ca^{2+} response is regulated by the circadian clock via live imaging of ER Ca^{2+} response with the G-CEPIAer indicator. Upon ATP treatment, we observed rapid decrease in ER Ca^{2+}, and the extent was higher at circadian time (CT) 22 compared to CT34. The increases in cytosolic Ca^{2+} following ATP treatment exhibited a circadian response consistent with the ER Ca^{2+} response. However, mitochondrial Ca^{2+} response remained consistent across different CTs. In the Bmal1$^{-/-}$ astrocyte culture, ATP- induced ER Ca^{2+} response was not different at both CT22 and CT34. Furthermore, we discovered that the levels of ER Calcium release channel, inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) levels were higher at CT22 than at CT34, which is responsible for CT-dependent differential ER Ca2+ release. Intracellular Ca^{2+} serves as a key mediator of astrocyte functions including gliotransmission, intercellular communications, and vascular function. Therefore, our results suggest that circadian clock modulate astrocyte functions in a timely manner by gating ER calcium response according to different times of the day.

Disclosures: **J. Ryu:** None. **K. Shim:** None. **H. Roh:** None. **J. Lee:** None. **E. Kim:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

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Program #/Poster #: PSTR322.19/D30

Topic: B.09. Glial Mechanisms

Title: Core clock gene BMAL1 regulates lipid droplet accumulation and oxidative stress responses in astrocytes.

Authors: *Y. CHEN, C. MCKEE, E. MUSIEK;
Washington Univ. in St. Louis Neurosci. PhD Program, St. Louis, MO

Abstract: Circadian clock genes play important roles in metabolic regulation in the periphery, but little is known about their roles in metabolism in the brain, particularly in glia. Our group previously reported that astrocyte-specific deletion of the core circadian clock gene BMAL1 leads to cell-autonomous activation and oxidative stress response. Here, we found that *Bmal1* knockdown in cultured astrocytes induces profound lipid droplet accumulation, possibly via its downstream transcription factor, REV-ERBα. Lipid droplets are lipid-storage organelles that perform versatile functions in cells, including sequestering free fatty acids and protection against oxidative stress. However, the specific function of lipid droplets in astrocytes remains elusive. We found that *Bmal1* knockdown in astrocytes improves survival in response to oxidative stress caused by hydrogen peroxide treatment. We hypothesized that induction of lipid droplets might be the mechanism by which *Bmal1* knockdown protects astrocytes against oxidative stress. We

observed that treating astrocytes with oleic acid induced lipid droplets, which was also protective against hydrogen peroxide. Surprisingly, a combination of oleic acid treatment and lipid droplet inhibitor further improves astrocyte survival, suggesting a lipid-droplet-independent pathway of fatty acid metabolism may be responsible for sustaining astrocytes under hydrogen peroxide toxicity. BMAL1 regulates expression of numerous lipid metabolic genes in astrocytes, and we are working to identify the downstream factors leading to lipid droplet accumulation caused by *Bmal1* knockdown and elucidate the function of lipid droplets in astrocytes. These studies will provide a deeper understanding of the relationship between the molecular clock, astrocyte metabolism, and brain redox homeostasis.

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Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

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Topic: B.09. Glial Mechanisms

Support: NRF Grant 2021R1A2C3007164

Title: Identification of Thalamic Subnuclei-Dependent Astrocyte Morphology

Authors: *C. PARK, G. HA, Y. CHO, E. CHEONG;
Yonsei Univ., Seoul, Korea, Republic of

Abstract: Astrocytes, one of the glial cell types, play a critical role in maintaining homeostasis in the brain. Furthermore, Astrocytes are known to be heterogeneous, exhibiting different regional-specific functions and morphology. The thalamus, an important region for sensory information processing, has been implicated in the regulation of sensory discriminability through the modulation of tonic GABA by astrocytes. However, the characteristics of Astrocyte morphology have not yet been unexplored in the thalamus. Furthermore, the tool was limited in observing the three-dimensional morphology of a single astrocyte until the fine process. In this study, we characterized the astrocyte morphology in different nuclei of the thalamus through dimensional by imaging figures. In order to see the entire area of the single astrocyte, the cell body and process were visualized in different colors so that they could be viewed. we achieved specific expression of tdTomato in astrocytes, enabling us to visually distinguish their soma and main processes. We utilized a membrane tethering virus to visualize fine processes, thus enabling comprehensive visualization of astrocyte territory. We anticipate that the characterization of thalamic astrocyte morphology will provide not only clues to their functional diversity but also implications for dysfunction.

Disclosures: C. Park: None. **G. Ha:** None. **Y. Cho:** None. **E. Cheong:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR322.21/D32

Topic: B.09. Glial Mechanisms

Support: 2017R1D1A1B05028221
HI20C0206

Title: Lysosomal stress-induced TFEB activation requires zinc release from lysosomes

Authors: *B.-R. SEO[1], J. CHOI[1], Y. YOON[2], J.-Y. KOH[3];
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Abstract: Transcription factor EB (TFEB) is the master switch for the activation of the CLEAR network genes that encode proteins involved in the catabolism of waste proteins and organelles. As such, TFEB activation and upregulation of the CLEAR network proteins may be an important reaction to lysosomal stress. Several reports showed that calcium release from stressed or dysfunctional lysosomes may play a key role in the activation of TFEB and its translocation to nuclei. Since we previously showed key roles of zinc in the maintenance of lysosomal function, we examined whether lysosomal zinc also plays a role in TFEB activation under lysosomal stress in cultured cortical astrocytes. To induce lysosomal stress in cultured cortical astrocytes, we used BafA1, a potent and selective inhibitor of vATPase. After 1 hr of BafA1 treatment, lysosomal pH was increased and endogenous TFEB was found to be translocated from cytosol to nuclei. Concomitantly, staining to BafA1 treated astrocytes with fluorescence dyes for zinc showed that while zinc fluorescence in lysosomes decreased, zinc levels in the cytosol increased. In contrast, fluo-8 staining did not detect changes in calcium fluorescence. Consistently, addition of zinc chelator TPEN completely blocked endogenous TFEB translocation upon BafA1 treatment/lysosomal stress. In previous reports, TRPML1 channels may be the main route for release of zinc and calcium from lysosomes. To activate TRPML1 channels in an astrocyte, we used TRPML1 activator ML-SA1 or 5. TRPML1 activator was increased cytosolic zinc levels and activated TFEB translocation in a TPEN-blockable fashion. We find increase in zinc signals upon TRPML1 activation by ML-SA1 or 5. Also, fluo-8 staining detect mild increase in calcium signals upon TRPML1 activation by ML-SA1 or 5. Our result suggests that under lysosomal stress, release of zinc rather than calcium from lysosomes to the cytosol via TRPML1 channels may be required to activate TFEB. Hence lysosomal zinc may act as a key ionic mediator to deliver lysosomal stress signals via TFEB to the nucleus.

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Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

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Topic: B.09. Glial Mechanisms

Support: NIH Grant R01NS119243

Title: Microglial P2RY12-dependent regulation of astrocytic features

Authors: *A. O. LOPEZ, U. B. EYO;
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Abstract: P2RY12 is a G protein-coupled receptor (GPCR) exclusively expressed by microglia in the brain parenchyma. It has been extensively used as a marker for homeostatic microglia and their ramified morphology that allows constant patrolling of the nervous tissue in the healthy brain. This continuous scanning behavior promotes microglial processes interactions with neurons and all the other CNS cells, resulting in microglial steady-state functions intimately involved in brain homeostasis. In our lab's preliminary findings, we show using a genetic approach with P2RY12-deficient mice that P2RY12 regulates microglial density and others have shown an altered morphology in P2RY12-deficient microglia. Additionally, in P2RY12-deficient mice, we find that microglia display fewer cell body interactions with capillaries compared to such interactions in wild-type mice, with a consequent increase in cerebral blood flow. This suggests that microglial P2RY12 could be involved in mechanisms underlying cerebral blood flow regulation. This novel microglia vasculature interaction led us to hypothesize that microglial P2RY12 may regulate other cell activity in the CNS, particularly, those associated with the neurovascular unit, such as astrocytes. Several aspects of the brain physiology are actively regulated by astroglia cells, one of the most enriched and diverse cell type in the CNS. Beyond their essential role in neurotrophic support and synapses, they have also been identified as important regulators of vessel tone and cerebral blow flow, which made us decide to first delineate roles for P2RY12 in regulating astrocytic structure and function. To address this matter, we have performed preliminary histological staining and observed altered astrocytic features, including increased cortical density of S100B-positive astrocytes. Moreover, staining of glial fibrillary acidic protein (GFAP) also showed altered features of cortical astrocytes, an indicator of increased astrocyte reactivity in this region of the brain. These exciting results suggest that microglial P2RY12 could be regulating astrocytic physiology.

Disclosures: A.O. Lopez: None. **U.B. Eyo:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.23/D34

Topic: B.09. Glial Mechanisms

Support: NIH grant M280001-NIH232

Title: Atypical astrocytes in the aging mouse cortex are associated with areas of blood brain barrier leakage and disrupted glutamate homeostasis

Authors: *M. SOMMER[1], M. ARMBRUSTER[2], P. TIRJA[1], R. GARIEPY[3], S. NASKAR[4], M. J. MCCONNELL[5], K. ARKUN[6], S. ROBEL[7], C. MUÑOZ-BALLESTER[8], C. G. DULLA[9]; [1]Neurosci., [2]Tufts Univ., Boston, MA; [3]Tufts Univ. Grad. Program In Neurosci., Boston, MA; [4]Northwestern Univ., Chicago, IL; [5]Lieber Inst. for Brain Develop., Baltimore, MD; [6]Pathology, Tufts Med. Ctr., Boston, MA; [7]Dept. of Neurobio., Univ. of Alabama In Birmingham, Birmingham, AL; [8]Univ. of Alabama at Birmingham, Birmingham, AL; [9]Neurosci., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Physiological network function is dependent on support from astrocytes, but as the brain ages, that support declines. We report a novel phenotype of non-reactive astrocytes in the aging cortex that express low levels of key astrocytic proteins, including excitatory amino acid transporters EAAT1/EAAT2 and the inwardly rectifying K+ channel Kir4.1, which are necessary for a healthy brain. A similar phenotype was previously documented following mild traumatic brain injury and referred to as atypical astrocytes (AtAs), but these have not yet been reported in normal aging or in human tissue. Using WT mice at various ages (p3-2 years), immunohistochemical analysis shows progressively more astrocytes lose protein expression of typical astrocytic markers as animals age. In cortex as early as P15, this protein loss can be seen in single or clusters of astrocytes while bordering cells will retain robust immunolabeling. Linear mixed modelling confirmed significant regional and age effects (≥3 mice/age; in age groups over P21 ≥3 mice per sex). AtAs were more likely found in the retrosplenial (RSC) or the prefrontal cortices (PFC); the somatosensory cortex (SSC) less so and rarely in the hippocampus. Like the cell-by-cell protein loss, functional glutamate analysis in 1 year old mice is consistent with random EAAT loss in regions AtAs are common. Glutamate uncaging in slice identified a subset of astrocytes with diminished EAAT activity; most of this subset was found in the RSC, while SSC cells had uniform glutamate conductance. In addition, iGluSnFR imaging shows focal slowing of glutamate uptake, more often observed in the RSC while the SSC had mostly homogeneous glutamate uptake speeds. While they appear to have largely normal morphology, nuclei in AtAs are significantly smaller and more elliptical, consistent with cellular senescence. AtAs also express βgal, a marker of senescence. As AtAs have Sox9 expression and <1% are GFAP+, they are non-reactive astrocytes. They are negative for the neuronal marker NeuN, oligodendrocyte marker MBP and OPC marker NG2. Appearing often near major blood vessels, AtAs have diminished endfeet proteins and are found in areas with age-related BBB disturbance, quantified with IV injection of cadaverine-488. A completed study of AtA prevalence in human cortical regions also finds aging-related increases in AtA abundance. Future studies aim to investigate the molecular underpinnings of AtA generation and their functional consequences.

Disclosures: **M. Sommer:** None. **M. Armbruster:** None. **P. Tirja:** None. **R. Gariepy:** None. **S. Naskar:** None. **M.J. McConnell:** None. **K. Arkun:** None. **S. Robel:** None. **C. Muñoz-Ballester:** None. **C.G. Dulla:** 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.; Bridge Bio.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.24/D35

Topic: B.09. Glial Mechanisms

Support: Deutsche Forschungsgemeinschaft (DFG), Research Unit 1757 "Synapses under Stress", Ro2327/13-2.
National Institutes of Health through grant number R01NS130916

Title: Modeling the Impact of Astrocytic NBCe1 on Ischemia-induced Astrocytic Na+ Loading and ATP Depletion in Mouse Neocortex

Authors: *P. THAPALIYA[1], K. EVERAERTS[2], N. PAPE[2], E. ROUSSA[3], C. ROSE[2], G. ULLAH[1];
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Abstract: Ischemia leads to increased sodium concentration in astrocytes, disrupting ionic balance and causing cellular damage. Astrocytes have high levels of NBCe1, an electrogenic sodium-bicarbonate cotransporter that regulates intracellular pH and operates near its reversal potential. We investigated how NBCe1 functions during transient energy deprivation using mathematical simulation of astrocytic pH, sodium (Na+), and ATP in mouse neocortical slices. Metabolic inhibition to mimic ischemic conditions caused temporary acidosis, increased Na+ levels, and decreased ATP levels in astrocytes. Blocking NBCe1 intensified astrocytic acidosis during ischemia, while reducing Na+ accumulation and ATP loss. Similar results were observed in NBCe1-deficient mice compared to wild-type. Fluorescence imaging confirmed these findings. In conclusion, our data demonstrate that transient energy failure activates NBCe1 inwardly in astrocytes, mitigating astrocytic acidosis during ischemia but leading to increased Na+ influx and decreased cellular ATP.

Disclosures: P. Thapaliya: None. **K. Everaerts:** None. **N. Pape:** None. **E. Roussa:** None. **C. Rose:** None. **G. Ullah:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.25/D37

Topic: B.09. Glial Mechanisms

Support: National Cancer Institute (NCI) Grant CA179563
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ational Cancer Institute (NCI) Grant CA232103
NIH Common Fund Grant CA179563

Title: Visualizing Glioblastoma-Astrocyte cross-talk mediated by extracellular vesicles in a transgenic mouse model

Authors: *L. NIELAND[1], Y. SAMAHA[1], E. R. ABELS[3], E. ABU HAIDAR[1], M. L. BROEKMAN[3], X. BREAKEFIELD[2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Massachusetts Gen. Hosp., Charlestown, MA; [3]Leiden Univ. Med. Ctr., Leiden, Netherlands

Abstract: Astrocytes, the most abundant glial cells of the brain, regulate glutamate and ion homeostasis and metabolism. Astrocytes interact with many different cell types in their surrounding through different communication strategies. One route of interaction is through signaling mediated by extracellular vesicles (EVs). Astrocytes react to environmental factors and become activated upon neural stress such as inflammation of the brain or in a neuropathological context. Glioblastoma (GB) is the most aggressive primary tumor of the brain. Upon tumor growth, astrocytes get activated in the tumor micro-environment (TME) and shape a glial border surrounding the tumor. By using a new transgenic AstroGreen mouse model designed to track astrocyte-derived EVs in the mouse brain, with expression of membrane-targeted mNeonGreen fused to hsCD81 only in GFAP expressing astrocytes, we show EV mediated cross-talk between GB cells and astrocytes *in vivo.* In this study we visualize direct uptake of astrocyte-derived EVs by GB cells and GB secreted palmitoylated EVs being taken-up by astrocytes in a tumor-bearing transgenic mouse model. These findings are crucial not only for understanding glioblastoma-astrocyte communication but could also aid in studying astrocyte-derived EVs in a health or diseased brain.

Disclosures: L. Nieland: None. **Y. Samaha:** None. **E.R. Abels:** None. **E. Abu Haidar:** None. **M.L. Broekman:** None. **X. Breakefield:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.26/D38

Topic: B.09. Glial Mechanisms

Support: MOE RG34/20
NRF2017-NRF-ISF002-2676

Title: Extracellular vesicle based transport of RNA from the pineal to the meninges

Authors: *S. J. JESUTHASAN[1], K. W. B. CHEOW[2], R. GHOSH[2];
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Abstract: The pineal gland is a well-established regulator of the body through the release of the hormone melatonin. Unexpectedly, it was recently found that the zebrafish pineal gland also secretes extracellular vesicles that are internalized by a subpopulation of meningeal fibroblasts. Aside from a potential role in the removal of waste from pineal photoreceptors, the significance of this secretion is unknown. Here, we provide evidence that these vesicles transport mRNA, which is then expressed in the meninges. The cells that release the vesicles are glial cells that express the agouti related peptide AgRP2 (or Asip2b). Expression of the medium fluorescent timer (medium FT), which switches from blue to red fluorescence, in the AgRP2 glial cells leads to expression of young protein both in the pineal and meninges, consistent with the presence of medium FT mRNA in the meninges. Expression of eGFP-CD63 in the AgRP2 glia led to the presence of the transgene mRNA in the meninges, as detected by hybridization chain reaction. To test whether meningeal expression could be caused by a leaky AgPR2 promoter, we used intersectional genetics. The *rpe65a* promoter was used to drive Cre in the pineal AgRP2 cells, in fish that also carried the *UAS:loxP-eGFP-loxP-mCherry* and *AgRP2:GAL4* transgenes. Expression of mCherry was detected in the meninges, independent of eGFP, consistent with export from the pineal. To identify endogenous mRNAs that are exported, we have compared the transcriptome of the pineal with the zebrafish meninges. This data establishes a novel pathway by which the pineal can influence gene expression in the meninges, a structure with multiple roles in brain development and homeostasis.

Disclosures: S.J. Jesuthasan: None. **K.W.B. Cheow:** None. **R. Ghosh:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.27

Topic: B.09. Glial Mechanisms

Support: Grant-in-Aid for Scientific Research (B) from Japan Society For The Promotion Of Science KAKENHI
PRESTO from Japan Science and Technology Agency

Title: Proximity labeling of specific cell-surface in vivo

Authors: *T. TAKANO;
Keio Univ., Tokyo, Japan

Abstract: In the mouse central nervous system, each neuron is thought to be connected to up to 10,000 other neurons. In addition, perisynaptic astrocyte processes (PAPs) are an integral part of central nervous system synapses. Depending on the type of pre-, post-synaptic neurons and PAPs, synapses are formed at specific subcellular locations and contain different sets of

neurotransmitter receptors, enabling synapse-specific functions. However, it remains largely unknown how such synapse-specific characteristics are regulated mainly because the information about molecules constituting each type of synapse is scarce. Here, we developed *in vivo* cell-surface proximity-dependent biotinylation (BioID) approaches, TurboID-surface and Split-TurboID, to comprehensively understand the molecular composition between astrocytes and neuronal synapses. These proteomic approaches have discovered a novel molecular framework for understanding the tripartite synaptic cleft that arbitrates neuronal circuit formation and function. Thus, these cutting-edge approaches to studying synaptic connectivity will provide new insights into the development of specific neurocircuits in the brain.

Disclosures: T. Takano: None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.28/D39

Topic: B.09. Glial Mechanisms

Support: NRF-2020R1A6A1A03043283
NRF-2019M3D1A1078943
NRF-2023K1A4A3A02057280
20021987 funded by MOTIE, Korea

Title: Effects of 660-nm LED photobiomodulation on drebrin expression pattern and astrocyte migration

Authors: *S. YOON, S.-Y. CHANG, M. LEE, J.-C. AHN;
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Abstract: Photobiomodulation (PBM) is a therapeutic tool that uses red or near-infrared light in medical applications. It's applications in both central (CNS) and peripheral nervous system (PNS) are widely studied. Among glial cells, astrocytes are known to be activated in injured or damaged brains. Astrocytic cell migration is crucial for maintaining homeostasis in the brain. Our previous study showed that PBM led to astrocyte proliferation and differentiation, but the effects on migration has not been investigated. The aim of this study was to evaluate the effect of PBM on astrocyte migration, drebrin (DBN) expression and cytoplasmic morphology using primary cultured rat astrocyte. We applied a 660-nm light-emitting diode (LED) with fluence of 6, 12 and 18 J/cm^2. PBM effects on astrocyte migration were analyzed by two different migration assays (scratch assay and transwell assay). We used immunofluorescence microscopy for visualizing DBN and glial-fibrillary acidic protein (GFAP) and analysis of DBN expression and astrocyte cytoplasmic morphology. Both scratch assay and transwell assay showed significant difference in astrocyte migration following PBM irradiation. With these specific fluence conditions, differences in DBN expression and cell morphology were revealed. PBM

could increase the astrocyte migration by altering the cell morphology and DBN expression pattern.

Disclosures: **S. Yoon:** None. **S. Chang:** None. **M. Lee:** None. **J. Ahn:** None.

Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.29/D40

Topic: B.09. Glial Mechanisms

Support: K99 NS126642-01
DRG 2329-18

Title: How do astrocytes remodel neuronal circuits?

Authors: *Y. KANG, A. J. JEFFERSON, R. Y. DE LA TORRE, A. E. SHEEHAN, M. R. FREEMAN;
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Abstract: Pruning neuronal connections and eliminating superfluous neurons are required in the mature brain to generate optimized neuronal circuits. Although it is well established that neurons and glia coordinate neural circuit refinement, the molecular mechanisms underlying this process remain poorly defined. To define the cellular and molecular mechanisms by which glia help drive the refinement of neuronal circuits during development, we used the *Drosophila* larval nervous system, which goes through extensive neuronal remodeling during metamorphosis. We conducted two large-scale screens to identify new glial molecules required for the elimination of neuronal debris. First, we performed a large-scale *in vivo* RNAi screen for single glial genes required for glial pruning of a defined population of neurons (vCrz+ neurons). In this screen, we focused on the vast majority of secreted, transmembrane, or signaling molecules encoded in the *Drosophila* genome. Second, we transcriptionally profiled glia during neuronal remodeling, identified genes upregulated in glia during remodeling, and then screened them for regulators of neuronal remodeling. Through these screens, we identified Tweek, a highly conserved molecule, functions during glial phagocytosis of pruned neurons. Tweek is an enormous protein (565 kDa) that is highly conserved from yeast to humans, with no clear molecular function. Interestingly, mutations in *tweek*'s human homolog *KIAA1109* cause a rare autosomal neurological disease. We show that Tweek localized in the plasma membrane (PM) and also to the endoplasmic reticulum (ER), leading to a possibility that Tweek is a novel ER-PM contact protein. Using structural prediction models, we were able to predict Tweek forms a structure similar to a previously described class of endoplasmic reticulum (ER) contact proteins which form tube-like structures and function as lipid transfer proteins. Our current model is that Tweek transfers lipids from the ER to the PM to support efficient glial engulfment of neuronal debris. Indeed, using the ER-PM contact marker dMAPPER, we identified that dMAPPER is present in *Drosophila* astrocytes

during phagocytosis, but lost in *tweek* knockdown. Our work provides exciting new insights into basic biology of lipid transfer proteins during glial phagocytosis.

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Poster

PSTR322. Astrocytes: Physiology and Molecular Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR322.30/D41

Topic: B.09. Glial Mechanisms

Support: European Research Council NEURO-PATTERNS 647725
Horizon 2020 ICT DEEPER
NIH Brain Initiative U19 NS107464
NIH Brain Initiative R01 NS109961
NIH Brain Initiative R01 NS108410

Title: Astra: a deep learning algorithm for fast semantic segmentation of large-scale astrocytic networks

Authors: J. BONATO[1,4,5], ***S. CURRELI**[2,3,1], S. ROMANZI[1,3,6], S. PANZERI[5,1], T. FELLIN[3,1]; [1]Neural Coding Lab., [3]Optical Approaches to Brain Function Lab., [2]Inst. Italiano di Tecnologia, Genova, Italy; [4]Dept. of Pharm. and Biotech., Univ. of Bologna, Bologna, Italy; [5]Dept. of Excellence for Neural Information Processing, Ctr. for Mol. Neurobio. (ZMNH, Univ. Med. Ctr. Hamburg-Eppendorf (UKE), Hamburg, Germany; [6]Univ. of Genova, Genova, Italy

Abstract: Variations in the concentration of intracellular calcium are a fundamental functional feature of astrocytes, the most abundant type of glial cell in the mammalian brain. Astrocytic calcium dynamics can be captured using two-photon microscopy, are localized in anatomically restricted subcellular portions of the astrocytic cell, and can be correlated across astrocytes. However, current image analysis tools used to determine the subcellular regions of astrocytes displaying calcium dynamics are time-consuming and heavily rely on user-defined parameters. Thus, current methods show limited reproducibility and prevent scalability to large datasets and fields-of-views. Here, we developed Astrocytic calcium Spatio-Temporal Rapid Analysis (ASTRA), a novel approach combining deep learning with image feature engineering for fast and automated semantic segmentation of astrocytic two-photon fluorescence imaging recordings. When tested on multiple two-photon microscopy datasets, ASTRA rapidly detected and segmented astrocytic cell somata and processes obtaining near-human performance. Moreover, ASTRA outperformed current state-of-the-art algorithms for the analysis of astrocytic and neuronal calcium data, and it generalized well across indicators and acquisition parameters. Finally, we applied ASTRA to two-photon mesoscopic imaging of hundreds of astrocytes in awake mice and found large-scale redundant and synergistic interactions in distributed astrocytic

networks. Together, these results show that ASTRA is a powerful tool enabling rapid large-scale reproducible investigation of astrocytic morphology and function.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.01/D42

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

Support: NIH grant P01AG14449
NIH grant RF1AG061566
NIH grant RF1AG081286
BrightFocus Foundation CA2018010
Barrow Neurological Foundation
Fein Foundation
Arizona Alzheimer’s Consortium
NIH grant P30AG066511

Title: Default mode network splicing protein alterations during the onset of Alzheimer’s disease

Authors: *E. MUFSON[1], M. NADEEM[1], B. HE[1], M. MALEK-AHMADI[2], C. HALES[3], S. E. PEREZ[1];
[1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Banner Alzheimer's Inst., Glendale, AZ; [3]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Default mode network (DMN) is an episodic memory connectome comprised, in part by the frontal (FC), precuneus (PreC) and posterior cingulate (PCC) cortex that display, amyloid and tau pathology and disconnection early in the onset of Alzheimer’s disease (AD). The PreC hub appears the most resilient to AD pathology suggesting differential states of neuronal vulnerability within the DMN in AD. Although the cellular mechanisms that underlie this differential pathobiology remain obscure, alterations in nuclear splicing proteins are potential candidates. Here, we examined the role that RNA splicing proteins play in the differential pathogenesis seen in components of the DMN during AD progression. In this regard, quantification of phospho-RNA polymerase II (pS5,2 and pS5-RNA pol II), splicing factor 2 (SRSF2), small nuclear ribonucleoproteins U1-70K and U1A, heterogenous ribonucleoproteins A2/B1 (hnRNPA2/B1) splicing markers and tau isoforms (3Rtau and 4Rtau) were performed by immunoblotting of frozen FC, PreC and PCC tissue samples obtained from non-cognitive impairment (NCI), mild cognitive impairment (MCI), and mild to moderate AD subjects from the Rush Religious Orders Study. In the FC, we found a significant upregulation of hnRNPA2B1 and pS5-RNA pol II protein levels in NCI compared to AD, while FC 3Rtau and 4Rtau levels

were downregulated in NCI compared to AD. PreC snRNPs U1-70K protein were significantly upregulated in AD compared to both NCI and MCI, and PreC pS5-RNA pol II protein levels were significantly increased in AD than NCI. PreC 3Rtau levels were higher in NCI compared to MCI and AD, while 4Rtau levels were significantly downregulated in NCI compared to AD. PCC hnRNPA2B1 and SRSF2 protein levels were significantly upregulated in AD compared to NCI. PCC 3Rtau protein levels were significantly upregulated in MCI compared to NCI, whereas NCI 4Rtau was downregulated compared to AD and MCI. Correlational analysis revealed that FC pS5-RNA pol II protein values were positively associated with episodic memory and global cognition. FC 4Rtau negatively correlated with episodic memory and 3Rtau with global cognition across clinical groups. PreC U1-70K protein levels correlated with MMSE, global cognition, episodic and semantic memory, and perceptual speed, while pS5-RNA pol II values associated only with MMSE and global cognition. PCC hnRNPA2B1 and SRSF2 values negatively correlated with MMSE, global cognition, episodic memory, and perceptual speed across the clinical groups. These findings suggest that mRNA splicing protein alterations contribute differentially to pathobiology of the DMN connectome and select cognitive deficits in AD.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.02/D43

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

Support: AG014449
AG017617
AG072599
AG074004
AG077103
T32AG052909

Title: Gene expression profiles of frontal cortex pyramidal neurons across the Alzheimer's disease spectrum.

Authors: *A. LABUZA[1,2], M. J. ALLDRED[1,2], H. PIDIKITI[1], A. HEGUY[3], P. D. COLEMAN[6], E. J. MUFSON[7], S. D. GINSBERG[1,2,4,5];
[1]Nathan S Kline Inst., Orangeburg, NY; [2]Psychiatry, [3]Genome Technol. Ctr., [4]Neurosci. & Physiol., [5]NYU Neurosci. Inst., New York Univ. Grossman Sch. of Med., New York, NY; [6]ASU-Banner Neurodegenerative Res. Ctr., ASU-Banner Neurodegenerative Res. Ctr., Tempe, AZ; [7]Departments of Translational Neurosci. and Neurol., Barrow Neurolog. Inst., Phoenix, AZ

Abstract: Alzheimer’s disease (AD) is an irreversible, age-related neurodegenerative disorder affecting an estimated 6.7 million Americans, and yet the underlying cause of AD is not understood. Current FDA approved treatments only slow the progression of disease but do not arrest or prevent the onset or progression of AD. Therefore, it is imperative to identify molecular and cellular mechanism(s) underlying AD, which would lead to novel therapeutics. Currently there are several approaches under development to evaluate changes at the transcriptomic, proteomic, and metabolic levels associated with AD. RNA sequencing (RNA-seq) provides an index of expressed genes as well as noncoding RNAs (ncRNAs) within a given cellular population. However, a limitation is that bulk-tissue resolution masks complex alterations occurring across different cell types. Here, we applied single population RNA-seq using laser capture microdissection (LCM) to isolate Nissl-stained layer III or layer V pyramidal neurons from the frontal cortex (BA9) from postmortem human brain tissue. Samples are taken from subjects across the AD spectrum ranging from no cognitive impairment (NCI, n=8), through mild cognitive impairment (MCI, n=5), ending in fulminant AD (n=7). A total of 600-900 Nissl-stained pyramidal neurons from each lamina were collected via LCM, RNA was isolated, converted to RNA-seq cDNA libraries, and analyzed on the Illumina NovaSeq platform at an average sequencing depth of 63 million reads per sample. Preliminary bioinformatic pathway analyses including IPA, KEGG, and GO were used to identify changes in differently expressed genes (DEGs) and canonical pathways between the AD and age-matched control cases. Total DEGs ranged from 580 (MCI versus NCI in layer III pyramidal neurons) to 3230 (AD versus NCI in layer III pyramidal neurons). Pathway analysis revealed several pathways were altered with disease progression, including pathways labeled “Alzheimer's disease” and “neurodegenerative diseases” as positive controls. Other pathways of interest included oxidative phosphorylation, mitochondrial dysfunction, and immune responses. We found differences in DEGs and pathways via bioinformatic inquiry across the AD spectrum from early to late stage. DEG and pathway changes seen exclusively between NCI and MCI can be targets for early intervention, while changes seen in early and late stages can be considered targets for biomarker analysis. Through this unbiased pathway analysis, we expect to identify and understand mechanistic changes in vulnerable pyramidal neurons that are integral to cortical circuitry and inform novel therapeutic strategies.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.03/D44

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

Support: NIH grant P01AG14449
NIH grant RF1AG061566
NIH grant RF1AG081286

BrightFocus Foundation CA2018010
Barrow Neurological Foundation
Fein Foundation
Arizona Alzheimer's Consortium

Title: Lipid dysregulation in the frontal cortex of elderly non-demented and Alzheimer's disease cases: a mass spectrometry imaging study

Authors: *M. MORENO-RODRIGUEZ[1], S. E. PEREZ[1], J. MARTINEZ-GARDEAZABAL[2], I. MANUEL[2], M. MALEK-AHMADI[3], R. RODRIGUEZ-PUERTAS[2], E. J. MUFSON[1]; [1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Univ. of the Basque Country, Leioa, Spain; [3]Banner Alzheimer's Inst., Glendale, AZ

Abstract: Alzheimer's disease (AD) is an irreversible neurodegenerative disorder, which underlying pathogenesis remains largely a mystery. In the last several years, it has been suggested that neurolipids, which originate from membrane lipid precursors that include endocannabinoids (eCB) and sphingosine 1 phosphate (S1P) and possess agonistic or neuromodulatory properties within the gray (GM) and white matter (WM) are dysregulated in the frontal cortex (FC) in AD. Although the development of mass spectrometry, which provided a faster and more accurate detection of lipids in brain, alterations that occur in the AD cortex compared to healthy controls remain an active area of research. In the present study, we investigated eCB and S1P neurolipid-based signaling in relation to the lipidome in FC samples obtained from people who died with an antemortem clinical diagnosis of no cognitive impairment (NCI, n = 5; 86.27 ± 4.8 years), mild cognitive impairment (MCI, n = 5; 83.32 ± 7.4 years) and mild/moderate AD (mAD, n = 5; 92.04 ± 5.4 years) from the Rush Religious Orders Study cohort using autoradiography and matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI) to localize the distribution of these lipids within the FC GM and WM. In addition, we performed a comparative MALDI-MSI analysis using tissue from younger healthy controls (mean age 68 ± 7, n = 5). Specifically, [^{35}S] GTPgS autoradiography activity-mediated by CB_1, $S1P_1$ and M_2/M_4 muscarinic acetylcholine $G_{i/0}$ coupled receptors (CB_1R, $S1P_1R$ and M_2/M_4 mAChRs) and MALDI-MSI were used to determine receptor activity and positive/negative ionization lipid levels, respectively, in FC WM and GM. Quantitative analysis revealed an upregulation of M_2/M_4 mAChRs in FC layers V-VI in MCI compared to NCI. Cortical layer V-VI CB_1R activity was also increased in mAD compared to NCI. Conversely, WM $S1P_1R$ activity was significantly downregulated in mAD compared to NCI. MALDI-MSI analysis showed upregulation of WM docosahexaenoic acid enriched phosphatidic acid (PA-DHA) and diacylglycerol 36:1 in MCI and AD compared to NCI, while WM arachidonic acid enriched phosphatidylinositol (PI-AA) were downregulated in mAD compared to NCI. In addition, WM PA-DHA and PI-AA showed an upregulation in the older compared to younger NCI cases. Correlation analysis revealed that alteration in WM PI-AA correlated with $S1P_1R$ activity and with perceptual speed performance across the clinical groups. Together these data suggest that WM lipids, eCB and S1P neurolipid systems alterations are related to myelin dysfunction in the early stages of AD, resulting in connectome disruption.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.04/D45

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

Support: AS-PG-21-008

Title: BONCAT analysis of amyloid-beta-induced increases in protein synthesis

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Abstract: Synapse loss is one of the pathological signs associated with cognitive dysfunction manifested in Alzheimer’s disease (AD), and oligomeric amyloid beta (oAβ) is thought to initiate synapse loss in AD. We have modelled this early synaptotoxcity in primary cortical neurons (DIV 27) by exposing the nanomolar concentration of oAβ. Our preliminary data demonstrate a reduction in the synaptic markers, synaptotagmin and PSD-95, 1 day and 5 days after oAβ treatment, respectively. This confirms that in our model system synapses are more vulnerable to damage when exposed to oAβ. Protein synthesis is the main event that regulates synapse homeostasis and function, and alteration in this process results in abnormal synaptic plasticity, especially in AD. Therefore, we aimed to investigate the role of oAβ in altering protein synthesis. To assess de novo protein synthesis, we used Bioorthogonal Noncanonical Amino Acid Tagging (BONCAT), which involves the incorporation of a noncanonical amino acid into newly synthesized proteins. Rat primary neurons were treated with 100 nM oAβ and saline for control for 1 day (n=4). Cells were then incubated with L-azidohomoalanine (AHA), an artificial amino acid of L-methionine, in methionine-free media 4 hours prior to cell harvest. Following cell lysis, the AHA-labelled proteins were biotinylated with a biotin-alkyne tag via a click chemistry reaction. Western blot analysis revealed that there is a significant increase in *de novo* protein synthesis 1 day after Aβ treatment when compared to the non-treated samples ($t(6)=2.541$, $p<0.05$). These results confirm earlier work using puromycin labelling of protein synthesis. However, BONCAT is an optimal method to study protein turnover because all proteins share the same pool of amino acids which makes it difficult to separate the *de novo* synthesized proteins, and BONCAT specifically allows detection of the newly translated proteomes by labelling them with an azide-bearing methionine-surrogate without interfering with the native biochemical processes of newly formed peptides. Future studies will include performing quantitative proteomics analysis with BONCAT-labelled samples using tandem mass tag (TMT) labelling to further identify the affected proteomes by Aβ toxicity that kills synapses. Understanding the dysregulation in protein synthesis in the presence of Aβ will help us prevent the detrimental effects of Aβ in synapse degeneration before the onset of AD.

Disclosures: S. Lee: None. **M. Sumiya:** None. **P. Giese:** None.

Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.05/D46

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

Support: NIH Grant P01AG14449
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Fein Foundation
Arizona Alzheimer’s Consortium

Title: Default mode network splicing protein alterations in Down syndrome with Alzheimer’s disease-related dementia

Authors: *S. E. PEREZ[1], M. NADEEM[1], B. HE[1], M. MALEK-AHMADI[2], E. J. MUFSON[1]; [1]Barrow Neurolog. Inst., Phoenix, AZ; [2]Banner Alzheimer's Inst., Glendale, AZ

Abstract: Down syndrome (DS) is the largest population with a genetic predisposition to develop Alzheimer’s disease (AD) type of dementia, and by the fourth decade of life exhibit tau containing neurofibrillary tangles (NFTs) and Aβ plaques, which increase with age. However, only about 70% develop dementia. We recently reported that DS with AD-related dementia (DSD+) display a greater number of *NFTs* consisting of a more advanced tau pathology compared to DS people without dementia (DSD-) in the frontal cortex (FC), a component of the default mode memory network (DMN), suggesting that differences in tau pathobiology are linked to DS dementia. Another hub of the DMN episodic memory connectome is the precuneus (PreC), which appears resilient to extensive tau pathology suggesting differential neuronal vulnerability within the DMN. Although the cellular mechanisms that underlie the differences in tau pathology between DSD+ and DSD- and DMN regional vulnerability remain unknown. Recent observations suggest that RNA splicing proteins play a role in tau pathogenesis in AD and DS. Here, we quantified the level of several tau-related splicing proteins (i.e., phospho-RNA polymerase II (pS5,2 and pS5-RNA pol II), serine/arginine splicing factor 2 (SRSF2), small nuclear ribonucleoproteins U1-70K and U1A, heterogenous ribonucleoproteins A2/B1 (hnRNPA2/B1), and kinase CLK1 involved in phosphorylation of SR splicing factors) as well as tau isoforms (3Rtau and 4Rtau) by immunoblotting of frozen FC and PreC tissue samples obtained from DSD+ and DSD-. In the FC we found a significant upregulation of hnRNPA2B1, pS5,2-RNA pol II and 4Rtau in DSD+ compared to DSD-, while FC U1-70K protein levels were downregulated in DSD+ compared to DSD-. Conversely, only the 3Rtau protein levels were significantly upregulated in the PreC in DSD+. Correlational analysis revealed that FC U1A protein values were positively correlated with CLK1, and 4Rtau correlated with Braak staging across clinical groups. PreC U1-70K were associated with hnRNPA2/B1 and 3Rtau correlated

with Braak staging. Comparison between the two DMN hubs revealed an upregulation of the CLK1 protein levels in FC compared to PreC in DSD-, while in DSD+ FC SRSF2 and 4Rtau protein levels were upregulated. FC pS5,2-RNA pol II and U1-70K were downregulated compared to PreC. These findings suggest that tau-related splicing protein alterations are greater in the FC in DSD+ and contribute differentially to DMN regional pathobiology in DS.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.06/D47

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

Support: AG014449
AG017617
AG072599
AG074004
AG077103

Title: Frontal cortex pyramidal neurons from Layer III and Layer V exhibit a neurotoxic phenotype in aged individuals with Down syndrome

Authors: *M. J. ALLDRED[1,2], H. PIDIKITI[1], A. HEGUY[3], P. ROUSSOS[5], G. E. HOFFMANN[5], S. D. GINSBERG[1,2,4];
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Abstract: Down syndrome (DS) is the most prevalent genetic abnormality, occurring in ~1/700 live births caused by triplication of human chromosome 21 (HSA21). DS individuals have a multitude of phenotypic changes in peripheral systems and most notably, are cognitively impaired, with deficits in learning, language and memory acquisition and consolidation. Further, individuals with DS develop Alzheimer’s disease (AD) pathology during early middle-age (mid-30’s), although the underlying mechanism(s) driving this pathology onset are not well understood. Contributing to cognitive impairment in DS, morphological deficits are present in cortical lamination and distribution, with Layer III (L3) and Layer V (L5) pyramidal neurons showing reduced proliferation and a disorganized laminar structure. As such, examining cortical L3 and L5 pyramidal neurons in DS individuals with AD pathology may elucidate alternate driver mechanisms of disease onset. We examined DS and age-matched control (CTR) brains from individuals without intellectual disabilities or dementia using postmortem frontal cortex

(BA9) via single population low input RNA-sequencing. We specifically targeted L3 and L5 pyramidal neurons to understand circuitry-based alterations in DS/AD individuals to elucidate mechanistic drivers of disease pathology. We show convergent gene expression changes in L3 and L5 pyramidal neurons which may underlie cognitive deficits and degenerative pathology associated with aging in the DS brain. We pinpoint alterations in HSA21 gene expression as well as a multitude of convergent differentially expressed genes (DEGs) and relevant pathways via bioinformatic inquiry in the DS brains that underlie mechanisms of degeneration. Distinctly, convergent gene expression reveals a neurotoxic phenotype, with multiple canonical pathways of dysregulation and disease functions linked to the convergent DEGs, including downregulation of the CLEAR signaling pathway and significantly increased activation of the neuroinflammation signaling pathway. We identify several target genes including mitogen activated protein kinase 1 and 3 (MAPK1/3), calcium voltage-gated channel subunit alpha1 A (CACNA1A) and superoxide dismutase 1 (SOD1), which exhibit overlap in multiple pathways and disease functions and/or display dysfunctional protein-protein network interactions for further examination. Novel targets identified by our single population approach in L3 and L5 pyramidal neurons may be drivers of the disease mechanism and therefore may be therapeutic candidates for amelioration of degenerative pathology that targets memory and executive function circuits in DS/AD.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.07/D48

Topic: C.03. Parkinson’s Disease

Support: PF-RCE-1948
F30AG07461801

Title: Rna- and Atac-sequencing reveals a unique $CD83^{+}$ microglial population focally depleted in Parkinson’s disease

Authors: *Z. CHATILA[1], A. YADAV[2], J. MARES[2], X. E. FLOWERS[3], T. D. YUN[2], A. F. TEICH[7], M. RASHID[2], R. TALCOFF[2], Z. PELLY[4], P. L. DE JAGER[5], Y. ZHANG[4], R. COSTA[8], E. AREA GOMEZ[9], G. J. MARTINS[8], R. ALCALAY[10], J. G. VONSATTEL[2], V. MENON[2], E. M. BRADSHAW[2], S. PRZEDBORSKI[6];
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Seattle, WA; [9]Dept. of Biomedicine, Ctr. de Investigaciones biologicas “Margarita Salas”. CSIC., Madrid, Spain; [10]Tel Aviv Sourasky Med. Ctr., Tel Aviv, Israel

Abstract: Morphologically activated microglia are a pathological hallmark of Parkinson’s disease (PD). However, our comprehension of microglial involvement in PD remains poor, hampered by our currently limited understanding of microglial molecular phenotypes and diversity in this disease. To define the heterogeneity of these innate immune cells in PD, we performed single nucleus RNA- and ATAC-sequencing on postmortem human samples, focusing on the substantia nigra pars compacta (SN) of 19 donors with sporadic PD and 14 non-PD controls. This midbrain region was selected as it represents a prototypic brain area that is most affected in PD. In addition to the SN, we also sequenced nuclei from three other brain regions from the same cohort of PD donors, the ventral tegmental area (VTA), the substantia inominata (SI), and the rostral hypothalamus (HypoTs), which are differentially affected in this disease with varying degrees of neurodegeneration. We identified thirteen microglial subpopulations, one perivascular macrophage population, and one monocyte population, and characterized their transcriptional and chromatin profiles. Remarkably, we discovered a unique *CD83* and *HIF1A*-expressing microglial subpopulation with a distinct chromatin state specifically depleted in the PD SN. These microglia are enriched in transcripts associated with antigen presentation and heat-shock proteins, as well as PD susceptibility genes. In non-disease brain tissues, this subpopulation exhibits regional specificity to the brainstem, an early target of PD neurodegeneration, suggesting that its depletion is intricately connected to the disease state in this region. These findings provide insights into microglial subpopulation-specific effects which open novel avenues for targeted neuroimmune interventions in PD.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.08/D49

Topic: C.06. Neuromuscular Diseases

Support: NIH IRP ZIA-HD008966

Title: Revealing pathways of motor neuron resilience or vulnerability in ALS using single cell transcriptomics

Authors: M. ALKASLASI[1], J. WLASCHIN[2], H. SILBERBERG[1], P. LEE[1], E. CLARK[1], ***C. LE PICHON**[3];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder in which spinal motor neurons (MNs) progressively degenerate, leading first to paralysis and eventually to death. While many genetic risk factors have been linked to ALS, the pathways leading to MN degeneration or resilience remain unclear. The $SOD1^{G93A}$ mouse is the best characterized model of ALS, and recapitulates the progressive paralysis seen in patients. In this model like in patients, specific MN subtypes were shown to be differentially vulnerable to degeneration, with alpha MNs being more vulnerable than gamma MNs, and with differential vulnerability among alpha MN subtypes. However, these studies were limited by methods to differentiate between these cell types. Our recent establishment of the transcriptomic map of adult MNs by single nucleus RNA sequencing (snRNAseq) revealed novel markers for these MN types that we can now use to quantify the surviving subtypes.The germline SOD1 mutation is expressed widely throughout the body and during development. We sought to determine whether alterations in the transcriptome of MNs caused by this mutation are apparent at early stages, and what profiles define the most resilient MNs at late stages of disease. We performed single cell transcriptomics of motor neurons of lumbar spinal cord from pre-symptomatic until late stages of disease in male and female $SOD1^{G93A}$ mice to identify transcriptional states traversed by these neurons during disease progression. These transcriptional states are characterized by groups of genes, or gene modules, that are up or down-regulated together to produce specific cellular states. By identifying and analyzing these modules of co-regulated genes, we are identifying important pathways of vulnerability or resilience in MNs. We will test whether blocking candidates of selective vulnerability, or enhancing the expression of candidate resilience factors can protect MNs.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.09/D50

Topic: C.06. Neuromuscular Diseases

Support: PDF Grant PF-RCE-1948

Title: Subcortical region-specific astrocyte diversity using single cell genomics

Authors: *A. YADAV[1,2], Z. CHATILA[2], X. E. FLOWERS[3], T. D. YUN[3], A. F. TEICH[6], P. L. DE JAGER[4], E. AREA GOMEZ[7], G. J. MARTINS[5], R. ALCALAY[3], J. G. VONSATTEL[3], S. E. PRZEDBORSKI[3], E. M. BRADSHAW[3], V. MENON[3];

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Abstract: Astrocytes are a prominent glial cell type in the central nervous system and play critical roles in maintaining brain homeostasis and neuronal health. However, our understanding of their involvement and molecular changes in Parkinson's disease (PD) remains limited. In order to shed light on astrocyte involvement in PD neurodegeneration, we applied a single-nucleus profiling approach to four selected brain regions that are known to be differentially affected in PD using human postmortem samples from 19 sporadic PD and 14 non-PD control (NPC) donors. After integrating the datasets to identify the major brain cell types, we further interrogated the astrocyte population in order to identify disease-specific gene expression signatures in the substantia nigra (SN), as well as regional heterogeneity across astrocyte subpopulations in the PD brain. We found signatures of stress response to be upregulated in astrocytes in SN in PD compared to NPC donors, and identified multiple subpopulations of astrocytes enriched in the SN in PD compared to the SN of NPC donors. One of these enriched clusters has higher expression of genes associated with a reactive astrocyte phenotype, including *GFAP* and *C3*. We also found another SN-PD enriched population expressing CCL2, suggesting a potential role for this astrocytic subpopulation in recruiting infiltrating immune cells to the SN in PD. This latter population also expresses *CD44*, which has been associated with fibrous astrocytic morphology in other brain regions. These astrocytes enriched in the SN in PD may contribute to immune-mediated pathogenic events in this disease. Our ongoing studies aim to integrate our single nucleus RNA-seq data with paired ATAC-seq data to generate a multimodal signature of astrocyte molecular phenotypes in PD, which ultimately may be used for target prioritization and therapeutic studies.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.10/D51

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

Support: R35NS097370
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R01NS127913
R21MH122239
R25NS065745
The Lieber Institute for Brain Development
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation
“La Caixa" Foundation

Title: Unveiling the Glia Diversity and Molecular Signatures across Human Hippocampal Postnatal Lifespan and in Alzheimer’s Disease

Authors: *Y. SU[1,2], Y. ZHOU[1], M. BENNETT[1], S. LI[1], M. CORDON[1], L. LU[1], S. HUH[1], D. JIMENEZ-CYRUS[1], B. KENNEDY[1], S. KESSLER[1], A. VIAENE[1], I. HELBIG[1], X. GU[3], J. KLEINMAN[4], T. HYDE[4], D. WEINBERGER[4,3], D. NAUEN[2], H. SONG[1], G.-L. MING[1]; [1]Univ. of Pennsylvania, Philadelphia, PA; [2]Johns Hopkins Univ., Baltimore, MD; [3]Nantong Unviersity, Nantong, China; [4]Lieber Inst., Baltimore, MD

Abstract: The molecular diversity of glial cells in the human hippocampus and their temporal dynamics over the lifespan remain largely unknown. Here we performed single-nucleus RNA sequencing to generate a transcriptome atlas of the human hippocampus across the postnatal lifespan. Detailed analyses of astrocytes, oligodendrocyte lineages, and microglia identified subpopulations with distinct molecular signatures and revealed their association with specific physiological functions, age-dependent changes in abundance, and disease relevance, as well as the molecular features change over ages. We further characterized the spatiotemporal heterogeneity of GFAP-enriched astrocyte subpopulations in hippocampal formation using immunohistology. Leveraging glia subpopulation classifications as a reference map, we revealed the diversity of glial cells differentiated from human pluripotent stem cells and identified dysregulated genes and pathological processes in specific glia subpopulations in Alzheimer’s disease (AD). Together, our study significantly extends our understanding of human glial cell diversity, population dynamics across the postnatal lifespan, and dysregulation in AD, and provides a reference atlas for stem cell-based glia differentiation.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.11/D52

Topic:

Support: P01 AG014449
P30 AG072931
P30 AG072976

Title: Posterior cingulate cortex microRNA alterations in cognitive resilience, mild cognitive impairment, and Alzheimer's disease

Authors: *S. E. COUNTS[1,2], J. S. BECK[1], B. MALONEY[3], M. M. AHMADI[4], S. D. GINSBERG[5,6], E. J. MUFSON[7], D. LAHIRI[3,8];
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Abstract: MicroRNA (miRNA) dysregulation is linked to Alzheimer's disease (AD) pathophysiology. We recently reported a role for human miR-298 and miR-20b in AD risk and amyloid and tau metabolism. To examine the extent to which miRNAs are dysregulated during the earliest stages of AD, we quantified miRNA transcript levels in postmortem samples of posterior cingulate cortex (PCC), a default mode network (DMN) hub that underlies autobiographical memory, in Rush ROS/MAP participants who came to autopsy with a diagnosis of a) no cognitive impairment and low pathology (NCI-LP, Braak stage I/II, n = 12), b) NCI with high pathology (NCI-HP, Braak stage IV, representing cognitive resilience, n = 8), c) mild cognitive impairment (MCI, n = 10), or d) dementia due to AD (n = 9). We performed Illumina-based sequencing of total RNA and analyzed trimmed reads with a size of 15-31 bases. Differential expression analysis (FDR ≤ 0.04) revealed 42 miRNAs significantly dysregulated among the NCI (independent of Braak stage), MCI, and AD groups. Two miRNAs, miR-99a (FDR-adjusted p = 0.01) and miR-664b (p = 0.005), were downregulated in AD vs NCI and MCI, while miR-30a (p = 0.006), miR-374a (p = 0.004), and miR-501 (p = 0.005) were significantly upregulated in MCI vs. NCI and AD. Three miRNAs were significantly downregulated in NCI-HP vs. NCI-LP: miR-103a (p= 0.04), miR-211 (p = 0.03), and miR-4443 (p = 0.03). These miRNAs may operate in resilience-related pathways. Correlation analysis revealed that decreasing PCC miR-664b levels were associated with poorer performance on antemortem tests of episodic memory (r = 0.41, p = 0.009), semantic memory (r = 0.44, p = 0.004), and visuospatial ability (r = 0.45, p = 0.004). Notably, ordinal linear regression modeling identified six miRNAs - miR-32, -3560, -6500, -101, -183, and -3570 - that modified AD risk, with age as a significant co-variate. Taken together, these preliminary data may identify potential pathogenic or protective miRNA-related mechanisms contributing to PCC and DMN function during the progression of AD, which may inform biomarker and intervention strategies. Ongoing analysis will potentially identify functional pathway enrichment and miRNA-specific targets.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.12/D53

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

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NIH Grant P30 AG072976

Title: A novel cellular pathway in which human microRNA-298 regulates cytokines, amyloid, and tau (miR-CAT) proteins

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Abstract: Disease modification in Alzheimer’s disease (AD) presents a major unmet problem. Our goal is to understand how biochemical changes lead to neuropathological features of AD and related dementias (ADRD). We hypothesized that disruption of small non-coding microRNAs (miRNA), which regulate multiple biological and pathological processes via mRNA stability, could contribute to ADRD. AD is marked by neurofibrillary tangles mainly composed of hyperphosphorylated tau (or MAPT) protein, neuritic plaques comprising aggregates of amyloid β (Aβ) peptides, and neuroinflammation by proinflammatory cytokines. We recently showed that human miR-298 lowered the expression of APP, BACE1, and a specific tau isoform (PMC8758483; PMC8866491). Herein, we report a novel cellular network in which miR298 regulates cytokines, amyloid, and tau (miR-CAT) proteins using well-characterized human brain samples and human-derived cell cultures. We obtained autopsy brain tissues from non-cognitively impaired (NCI) and AD subjects. We measured levels of miR-298, AD-related mRNAs and proteins in cortical samples from these subjects. For mechanistic studies, we transfected the human astrocyte cell line U373-MG with either mock, miR-298 mimic, its antagomiR, a combination of both, or a negative control mimic. Elevated brain miR-298 associated with a reduced risk of AD. Subject age and *APOE* genotype altered this association. miR-298 stimulated several pro-inflammatory cytokines and chemokines: IL-α, IL-β, IL-6, and TNF-α. While miR-298 treatment induced IL-6 secretion, it also reduced levels of APP pathway proteins, including BACE1, and tau. RNAseq revealed that miR-298 transfection reduced AD-related gene cluster mRNA expression, including APP, BACE1, MAPT, and the tau-related kinase GKS3β. The induction of multiple pro-inflammatory cytokines may be due to the combined regulation of miRNA activity and upstream signaling pathways. Hence, miR-298 may act as a "control switch" regulating AD and inflammation-related genes. We suggest that miR-

298 could be therapeutic, such as when high proinflammatory cytokines would be necessary for neuroprotection, along with a simultaneous reduction of APP, BACE1, and tau protein levels. Supported by NIH grants.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.13/D54

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

Support: NIH Grant U24AG061340

Title: Bridging the gap: advancing aging & dementia research through the open-access AD Knowledge Portal and Agora

Authors: *A. KALLAHER, A. VANDER LINDEN, Z. LEANZA, J. S. BRITTON, L. HEATH, J. BECK, A. PENA, W. L. POEHLMAN, R. H. YAXLEY, V. BAHAM, J. SCANLAN, J. HENDRICKSON, J. C. WILEY, K. LEAL, J. MALENFANT, A. K. GREENWOOD;
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Abstract: The AD Knowledge Portal (https://adknowledgeportal.org) and Agora (https://agora.adknowledgeportal.org) are open resources developed to support Alzheimer's disease researchers in accessing and sharing valuable information and data generated within the National Institute on Aging's (NIA) translational research portfolio. The AD Knowledge Portal serves as a freely accessible and open platform aiming to accelerate AD research and optimize therapeutic discoveries. It adheres to FAIR (Findable, Accessible, Interoperable, and Reproducible) principles, ensuring transparency and wide utilization of resources and data. Researchers can explore curated and contextualized data, conduct independent experiments to test hypotheses, and prioritize novel mechanisms for therapeutic advancements. By contributing multi-omics data from both human and non-human sources, researchers ensure proper attribution and compliance with data governance and ethical guidelines. The secure Synapse data-sharing platform, supported by the NIH, and a user-friendly data portal form the foundation of this collaborative endeavor. The AD Portal currently hosts resources from nine research consortia and over 50 grants, providing data from a diverse range of modalities, including genomics, transcriptomics, imaging, proteomics, metabolomics, and more. Additionally, the portal offers access to experimental mouse models, computational tools, publications, and summarized evidence for potential AD targets through integrated results explorers. Agora is a publicly available platform designed to expedite AD research and optimize therapeutic discoveries. It

facilitates information sharing about potential AD therapeutic targets through data visualizations, summary evidence, and targeted validation study results. Researchers can browse a comprehensive list of over 600 targets nominated by the NIA's Accelerating Medicines Partnership in AD (AMP-AD) consortium, the Target Enablement to Accelerate Therapy Development for AD (TREAT-AD) centers, and the broader AD research community. Agora provides interactive tools, data visualizations, and concise summaries to enhance the accessibility and understanding of this information for researchers in the field of AD. Together, the AD Knowledge Portal and Agora serve as accessible hubs for researchers seeking data, tools, and results to study AD and related dementias.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.14/D55

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

Support: NIH R01 AG073826

Title: Relief based analyses on recent human postmortem hippocampal datasets elucidate interactions among Rbp1 and other proteins that differentiate Alzheimer’s Disease from age-matched controls.

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Abstract: Alzheimer’s Disease (AD) is a neurodegenerative disorder and the most common type of dementia, leading to cognitive impairment and memory loss. Despite increased efforts to understand and treat AD, the molecular mechanisms remain unclear, creating obstacles to developing effective treatment strategies. Over the last decade, efforts to investigate these mechanisms using transcriptomic (RNAseq) data, have been focused on statistical algorithms like Differential Expression Analysis, to measure the differences in the expression of individual genes. However, these algorithms do not consider statistical interactions between proteins and possible links that could provide insight into the mechanisms of AD. In this work, we review the different algorithms to infer statistical interactions in transcriptomic data, basing our work on popular Machine Learning ideas, like the Relief Based Algorithms (RBA) and Sequential Forward Search (Urbanowicz, 2018), and applied them to the dataset from van Rooij and colleagues (2019). The data consists of the RNAseq expression of 14564 genes from the

hippocampus of 28 brains (18 AD and 10 age-matched controls). After applying RBA, computing the feature importance, and thresholding the importance, we found a group of 100 genes. Most of the genes in the selections have not been reported in the AD literature, and the ones reported are mainly associated with general neurological diseases, and not reported within an AD pathway. The gene with most importance in our analysis was Rbp1 (retinol binding protein) a gene that is critical for transport of vitamin A (retinol) from liver to peripheral tissue, including brain. We show different molecular networks involving Rbp1, including functional links with the Wnt signaling cluster, the potassium voltage-gated channel cluster and some proteins that are also included in Cancer pathways. Our analysis places increased importance on the role of vitamin A homeostasis in AD pathology. We conclude that our methods are successful in finding differences between two groups, and in inferring Gene Regulatory Networks that could be crucial for the understanding and modeling of AD pathways. Finally, we are aiming to validate our findings using the MayoHippocampus dataset.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

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Program #/Poster #: PSTR323.15/D56

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

Support: R01AG063175
R01MH116281
R01AG081374

Title: Allele-specific open chromatin mapping in human iPSC-derived microglia prioritizes functional GWAS risk variants for Alzheimer’s disease and implicates a novel role of PICALM

Authors: *A. KOZLOVA[1], A. SUDWARTS[2,3], S. ZHANG[1,4], H. ZHANG[1], X. SUN[5], B. JAMISON[1], W. WOOD[1], S. SENGUPTA[1], Z. PANG[6], A. SANDERS[1,4], X. HE[5], G. THINAKARAN[2,3], J. DUAN[1,4];
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Abstract: Genome-wide association studies (GWAS) of Alzheimer’s disease (AD) have identified 75 risk loci. However, the causal variants/genes in AD remain elusive. We have developed an approach to map functional GWAS risk variants that affect gene expression by comparing the differential allelic chromatin accessibility, i.e., allele-specific open chromatin (ASoC), in human induced pluripotent stem cell (hiPSC)-derived neurons (iNs). By performing

ASoC mapping in hiPSC-derived microglia (iMG) of 38 donor lines, we identified 72,518 ASoC SNPs that were significantly enriched for human brain microglia expression QTLs. The ASoC SNPs showed much stronger enrichment for GWAS risk of AD in iMG (P=1.4×10^{-33}) than in iNs (P=0.013~8.9×10^{-6}) or hiPSC-derived astrocytes (iAstro) (P=1.3×10^{-5}). In total, 32 AD GWAS risk SNPs at 20 AD risk loci showed ASoC in iMG. One of the ASoC SNPs, rs10792832, was located inside an iMG-specific open chromatin peak ~87 kb upstream of *PICALM* (*Phosphatidylinositol Binding Clathrin Assembly Protein*), a gene whose function in microglia has not been well studied. To establish the risk allele of rs10792832 with its functional impact, we performed CRISPR/Cas9 editing of rs10792832 on hiPSC lines (from homozygous G/G to A/A; G = risk allele). We found that the risk allele was associated with reduced *PICALM* expression in iMGs but not in iAstro. Consistent with the transcriptional effect of the rs10792832, we also found a decrease in *PICALM* levels in the brains of patients with AD. We next showed that iMGs carrying the rs10792832 risk allele exhibited a compromised capability to phagocytose myelin and Aβ, which can be mimicked by CRISPRoff-induced reduction of *PICALM*. Interestingly, we found that lipid droplet accumulation was increased in iMG carrying the rs10792832 risk allele. To further explore how the AD risk rs10792832 may affect *PICALM* expression, we analyzed the transcription factor (TF) binding at the SNP site and found that the rs10792832 disrupted the TF-binding motif of PU1 that is encoded by another strong AD risk gene *SPI1*. Our chromatin immunoprecipitation (ChIP)-qPCR further showed that the rs10792832 risk allele was associated with ~50% reduction of PU1 binding in iMG. These data suggest that this AD risk variant of *PICALM* may confer disease risk by altering the chromatin accessibility to PU1, thereby causing dysfunctional myelin and Aβ phagocytic activity and lipid metabolism in microglia. Our study provides a framework for prioritizing functional AD GWAS risk variants that affect chromatin accessibility in hiPSC-derived microglia, and sheds novel mechanistic insight on how an AD GWAS risk variant of *PICALM* confers disease risk in microglia.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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

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Michael J. Fox Foundation for Parkinson's Research

Title: Cerebral gray- and white matter monogalactosyl diglyceride levels rise with the progression of Alzheimer's disease

Authors: *J. K. BLUSZTAJN[1], N. AYTAN[1], T. RAJENDIRAN[2], T. J. MELLOTT[1], T. SONI[2], C. F. BURANT[2], G. E. SERRANO[3], T. G. BEACH[3], H. LIN[4], T. D. STEIN[1];
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Abstract: Multiple studies have reported brain lipidomic abnormalities in Alzheimer's disease (AD) that affect glycerophospholipids, sphingolipids and fatty acids. However, there is no consensus in the field regarding the nature of these abnormalities, and it is not clear if they relate to disease progression. Monogalactosyl diglycerides (MGDG) are a class of lipids which have only recently been detected in the human brain. We measured these compounds by ultrahigh performance liquid chromatography tandem mass spectrometry in postmortem dorsolateral prefrontal cortex gray matter and subcortical corona radiata white matter samples derived from three cohorts of participants: the Framingham Heart Study, the Boston University Alzheimer's Disease Research Center and the Arizona Study of Aging and Neurodegenerative Disorders/Brain and Body Donation Program (total n=288). We detected 40 molecular species of MGDG (including diacyl and alkyl/acyl compounds) in these samples and found that the levels of 29 of them, as well as total MGDG levels, are positively associated with AD-related traits including pathologically confirmed AD diagnosis, clinical dementia rating, Braak and Braak stage, neuritic plaque score, phospho-Tau AT8 immunostaining density, levels of phospho-Tau396 and levels of Aβ40. Increased MGDG levels were present in both gray and white matter, indicating that they are widespread and likely associated with myelin-producing oligodendrocytes - the principal cell type of white matter. Overall, our data implicate the MGDG metabolic defect as a central correlate of clinical and pathological progression in AD.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.17/D58

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

Support: NIH Grant 1RO1-AG057895
Duke - Bass Connections Grant

Title: The neuroprotective effects of exercise against menopause-induced alterations in forebrain metabolites in an Alzheimer's disease mouse model

Authors: J. WILLIAMS-DORIA[1], E. A. FINCH[3], C. GRANT[4], E. WEN[5], C. COLTON[6], ***C. L. WILLIAMS**[2];
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Abstract: Two-thirds of Americans with Alzheimer's Disease (AD) are women. Sex differences in biology—notably, the gradual loss of ovarian hormones during the perimenopausal transition—are thought to be critical factors contributing to women's greater AD risk. Accumulating evidence suggests that exercise is a powerful lifestyle strategy for maintaining brain health and staving off cognitive decline associated with aging and AD, and that exercise and estrogens influence overlapping networks and functional pathways in the brain. Here, we asked whether long-term exercise training following ovarian senescence attenuates cognitive decline and improves AD- and menopause-mediated changes in forebrain metabolite levels in the CVN-AD mouse model (*APPSwDI*$^{+/+}$*/mNos2*$^{-/-}$), which exhibits age-related increases in pathological and cognitive hallmarks of AD. A gradual loss of ovarian function was induced in half of the mice by treating with 4-vinylcyclohexene diepoxide (VCD) from 9 to 12 weeks of age (WOA); this accelerates the natural human-like process of follicular atresia that occurs during the menopausal transition. The exercise intervention for CVN-AD mice consisted of both voluntary wheel running and forced treadmill training from 24 to 36 WOA; NOS2$^{-/-}$ control mice (VCD-treated and oil-treated) remained sedentary. Novel object recognition tests at 36 WOA revealed that exercise improved short-term memory in both oil- and VCD-treated CVN-AD mice. To investigate the effects of AD, VCD-treatment, and exercise training on forebrain metabolic function, we used an untargeted Quant 500 metabolomics panel to measure forebrain metabolites and overrepresentation analysis using the MetaboAnalyst platform to assess affected pathways. This analysis showed that metabolites in 6 pathways were significantly different between untreated sedentary CVN-AD and NOS$^{-/-}$ mice and that VCD treatment affected metabolites in 9 pathways. Exercise training of CVN-AD mice mitigated some metabolic changes caused by VCD-treatment. In both oil- and VCD-treated mice, exercise also modulated the adenosine and pentose phosphate pathways, which are involved in mitochondrial bioenergetics. Furthermore, VCD-treatment induced changes in the arginine and proline metabolic pathway in all experimental groups (sedentary and exercised CVN-AD and sedentary NOS$^{-/-}$ mice). Importantly these pathways have been implicated in the progression of AD. Together, these findings suggest that ovarian hormones play a crucial role in regulating brain metabolism and that exercise may influence brain metabolic pathways shared by AD and menopause to reduce the increased risk for AD in postmenopausal women.

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Poster

PSTR323. Alzheimer's, Parkinson's and ALS: Genomics and Other Omics Approaches

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR323.18/D59

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

Support: NS115161
NS129878
GM121310

Title: Single Nucleus RNA-sequencing reveals neuronal and glial defects in 5XFAD mouse model

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Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder, characterized by the presence of abnormal amyloid plaques and tau protein deposition, leading to neuroinflammation and neurodegeneration. 5XFAD mouse model overexpressing mutant human amyloid beta precursor protein (APP) and presenilin 1 (PS1), has been widely employed to study mechanisms of AD pathogenesis as it reproduces abnormally accumulated amyloid plaques. In this study, we conducted single nucleus RNA-sequencing (snRNA-seq) on the medial prefrontal cortex of 13-month-old 5XFAD mice. Our analysis revealed a significant loss in somatostatin and parvalbumin inhibitory neurons, in 5XFAD mice compared to their age-matched control. Our results further demonstrated that excitatory neurons in 5XFAD mice exhibited diminished expressions in immediate early genes, such as *Fos, Homer1, Egr4* and *Junb*, suggesting their reduced activity. Additionally, analysis of markers for Disease Associated Astrocytes (DAA) demonstrated a significant increase in the presence of DAA subtypes signatures, specifically those associated with A1 astrocytes, A2 astrocytes and PAN-reactive astrocytes in 5XFAD mice. Microglia in 5XFAD mice also exhibited a Disease Associated Microglia (DAM) phenotype, evidenced by a significant increase in the DAM score and reduced expression of homeostatic genes, potentially exacerbating the disease progression. To assess the relevance of our findings, we compared our list of differentially expressed genes (DEGs) with those from human AD studies, revealing over 60% overlapped DEGs across all the major cell types except for the inhibitory neurons. Our findings support that 5XFAD mouse model most likely recapitulates cellular and circuitry pathogenic changes in human AD brains. In conclusion, our snRNA-seq study in 5xFAD mouse model provides valuable insights into the complex pathogenic alterations associated with amyloid plaques in AD progression.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.01/D60

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

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Title: Immunomodulation restores proteostasis in macrophages via OPN-UCHL1 axis and preserves synaptic integrity in the brains of AD-model mice

Authors: *D.-T. FUCHS[1], A. RENTSENDORJ[1], K. RAEDSCHELDERS[1], H. SHI[1], B. GAIRE[1], J. SHEYN[1], J. DOUSTAR[1], T. TORBATI[2], Y. KORONYO[1], K. BLACK[1], J. E. VAN EYK[1], M. KORONYO-HAMAOUI[1];
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Abstract: Background and methods: Osteopontin (OPN; encodes by Spp1 gene) is a matricellular immunomodulatory cytokine highly expressed by macrophages in response to brain injury. We previously revealed that glatiramer acetate (GA) stimulation of bone marrow-derived macrophages (BMMΦ), induced OPN expression and promoted an anti-inflammatory, pro-healing phenotype. In contrast, OPN inhibition triggered a pro-inflammatory phenotype. We further found that GA immunomodulation amplified OPN expression in macrophages that infiltrated the brains of transgenic $APP_{SWE}/PS1_{dE9}$ mouse models of Alzheimer's Disease (ADtg mice). However, the role of OPN in macrophage phenotypic shifts is undefined. To gain a mechanistic understanding of OPN suppression versus induction in macrophages, we applied a quantitative global proteome profiling via a mass spectrometry (MS) analysis in OPN-knockout (OPN^{KO}) compared to OPN-primed GA-treated or wild-type (WT) BMMΦ. Proteomic results were validated by IHC, immunoprecipitation, and Western blot assays. To further investigate the beneficial mechanism of GA immunization in ADtg mice, the expression of cerebral OPN and infiltrating macrophages in GA-immunized ADtg mice were measured by immunohistochemistry (IHC). **Results:** We identified over 630 differentially expressed proteins (DEPs) in OPN^{KO} and GA stimulated macrophages as compared to WT cells. One of the topmost DEP downregulated in OPN^{KO} and upregulated by OPN-primed GA-stimulated macrophages was the ubiquitin C-terminal hydrolase L1 (UCHL1), a neuron-specific protein and a key component of the ubiquitin-proteasome system (UPS). *In-vitro* assays revealed an OPN-

dependent expression of UCHL1 in macrophages. OPN affected the UPS system and was critical for macrophage proteostasis and activation, and survival. We confirmed *in vivo* that UCHL1 colocalizes within OPN-expressing $Iba1^{+}CD45^{high}$ infiltrating monocytes surrounding cortical Aβ plaques in ADtg mice. Further, quantification of UCHL1 revealed a 55% and 66% loss in the hippocampi and cortices of ADtg mice compared to WT mice. Interestingly, GA immunomodulation restored UCHL1 neuronal expression along with reduced Aβ plaque burden and microgliosis in ADtg mice. Moreover, increased UCHL1 expression levels strongly correlated with the post-synaptic marker PSD95. **Conclusions**: OPN-UCHL1 axis is essential for macrophage homeostatic balance via regulation of cell viability and protein synthesis and turnover, implying its potential in immune-based therapy. GA immunomodulation restored UCHL1 expression in the hippocampus and cortex of ADtg mice and was associated with synaptic preservation.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.02/D61

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

Support: NIH grant R01 AG075998
Alzheimer's association grant AARG-NTF-21-84658

Title: Intranasal Chlamydia pneumoniae Infection Induces Neuroinflammation and Cognitive Decline in Mice

Authors: *L. SUBEDI[1,2], D. MARTINON[2], J.-P. VIT[3], D.-T. FUCHS[3], M. LANE[2], M. MITA[2], S. CHEN[2], B. GAIRE[3], Y. KORONYO[3], M. ARDITI[2], M. KORONYO-HAMAOUI[3,6,4], T. CROTHER[2,5];
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Abstract: Background: Alzheimer’s disease (AD) is a progressive irreversible dementia characterized by deposition of beta-amyloid protein and hyperphosphorylation of tau forming neurofibrillary tangles, and neurodegeneration. An emerging theory posits that infections could be one of the triggering factors in AD development and progression. Multiple lines of evidence have linked *Chlamydia pneumoniae* (Cp), a gram-negative obligate intracellular bacterium with AD. Cp has been detected in the post-mortem brain tissues of AD patients, however, pathomechanisms associated with Cp in AD remain unknown.Methods: Transgenic $APP_{SWE}/PS1_{\Delta E9}$ (ADtg) mice and non-tg wildtype (WT) littermates were infected with Cp

intranasally and sacrificed either 1 week or 6 months after infection. Mice were perfused with saline, and the brains were harvested for subsequent analysis, including PCR, flow cytometry, immunofluorescence, and live Cp growth. Neurobehavioral functions were assessed through open field, visual-stimuli X maze, and Barnes maze tests. Primary microglia and astrocytes were infected with Cp and cytokine and chemokines production was measured by ELISA.Results: We found Cp in the olfactory bulb and brain of mice 7 days after infection and the infiltration of inflammatory cells into the brain. Cp infection resulted in microglial activation with increased expression of MHCII and CD206. Immunohistochemical analysis also revealed the activation of microglia and astrocytes in the Cp-infected mouse brain. In addition, Cp infection led to increased mRNA expression of neuroinflammatory mediators such as cytokines and chemokines. Primary astrocytes and microglia cultures could sustain Cp growth and produce proinflammatory cytokines and chemokines in response to infection. Live Cp in the brain was also observed 7 months after infection and the Cp load was higher in ADtg mice compared with WT. Cp infection resulted in significant cognitive decline in both WT as well as ADtg mice together with increased AD-related neuropathology.Conclusion: Intranasal Cp infection leads to brain colonization and activated glia cells. Both primary astrocytes and microglia support Cp growth and produce neuroinflammatory cytokines. Long-term Cp infection promotes neuroinflammation and cognitive decline in mice along with increased Aβ deposits. Cognitive impairment in Cp-infected ADtg and WT mice as well as increased Aβ in ADtg mice suggest that Cp infection may play an important pathological role in AD development.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

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Topic:

Support: NIH R01 AG062500
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Title: The retinoblastoma binding protein 7 (Rbbp7), which protects against tau acetylation and subsequent hyperphosphorylation, is reduced in Alzheimer's disease

Authors: *J. M. JUDD[1], W. WINSLOW[1], G. E. SERRANO[2], T. G. BEACH[2], I. S. PIRAS[3], M. J. HUENTLEMAN[3], R. VELAZQUEZ[1];
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Abstract: Accumulation of amyloid-β plaques and neurofibrillary tangles are key neuropathologies of Alzheimer's disease (AD). Aberrant chromatin remodeling and epigenetic dysfunction contribute to pathogenic gene expression in AD. Notably, work highlights that dysregulation of epigenetic proteins may contribute to pathological tau. Recently, we identified the Retinoblastoma Binding Protein 7 (Rbbp7), which chaperones chromatin-remodeling proteins to their nuclear targets, mediating epigenetic modification, as a potential target for AD interventions. Our previously published data show that Rbbp7 mRNA is down regulated in AD patient brains compared to age-matched controls (CON). Additionally, Rbbp7 mRNA is negatively correlated with Braak stage (a measure of tau pathology), and positively correlates with brain weight. Further supporting a role in neurodegeneration, Rbbp7 is downregulated in the PS19 mouse model of tauopathies, and its genetic overexpression in hippocampal (Hp) CA1 reduced tau acetylation, phosphorylation, and neuronal death. The goal of the present study is to better understand the relationship between protein level of Rbbp7 and AD pathology and determine whether utilizing the AAV/PHP.eB serotype to overexpress Rbbp7 throughout the brain reduces tau pathology more broadly in PS19 mouse. We obtained frontal cortical brain samples from severe AD (AD-Sev, n = 10), moderate AD (AD-Mod, n = 12), and CON (n = 9) human cases (balanced for sex) from Banner Sun Health's Brain and Body Donation Program, that included cognitive scores (Mini-Mental State Examination (MMSE)), brain weight, plaque density, and Braak stage. Rbbp7 protein levels are significantly reduced in AD-Mod and AD-Sev compared to CON. A significant positive correlation shows that higher Rbbp7 protein levels are associated with better cognitive scores and higher post-mortem brain weight. There were significant negative correlations between Rbbp7 levels and both CERAD neuritic plaque density and Braak stage, consistent with our previous report. In mice, we retro-orbitally injected an AAV/PHP.eB to upregulate Rbbp7 globally in PS19 and NonTg mice at 3.5 months, prior to tau pathogenesis. Tissue was collected at 8.5 months. We found significantly elevated levels of Rbbp7 protein in the cortex and Hp that was associated with a significant reduction in phosphorylated Tau at Thr 181 and Ser 396. Multi-omics analysis using ATAC- and RNA-seq to assess epigenetic and transcriptomic changes of Hp tissue are ongoing. Collectively, these results expand on our previous work showing that reduced Rbbp7 contributes to AD pathogenesis and that rescuing its levels reduces tau pathologies.

Disclosures: **J.M. Judd:** None. **W. Winslow:** None. **G.E. Serrano:** None. **T.G. Beach:** None. **I.S. Piras:** None. **M.J. Huentleman:** None. **R. Velazquez:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.04/D63

Topic:

Support: NIH R01AG059627
NIH R01AG062500

Title: Thirteen weeks of glyphosate exposure at early adulthood followed by cessation is sufficient to exacerbate neuroinflammation, amyloid-β, and tau pathology in the 3xTg-AD mouse model of Alzheimer's disease.

Authors: *H. LEON[1], W. WINSLOW[1], S. K. BARTHOLOMEW[1], M. N. MARTINEZ[2,4], K. V. PATHAK[3,4], R. SHARMA[3,4], P. PIRROTTE[2,4], R. VELAZQUEZ[1,5];
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Abstract: Alzheimer's disease (AD) is a prevalent and growing problem in the US that affects one in nine people over the age of 65. Lifestyle factors, including toxic exposures, have been shown to significantly contribute to the development of AD. Our work has previously shown that sub-chronic glyphosate exposure leads to a dose dependent accumulation of glyphosate and elevations in the levels of pro-inflammatory cytokine Tumor Necrosis Factor alpha (TNFα) in the brains of C57BL/6J mice. Additionally, we found that primary cortical neurons derived from the APP/PS1 mouse model of AD show elevated amyloid-β (Aβ) after glyphosate exposure *in vitro*. Whether chronic exposure followed by cessation of glyphosate exacerbates AD-like pathology late in life *in vivo* has yet to be determined. Here, we examined the effects of exposure using chemically pure glyphosate (N-(phosphonomethyl)-glycine from Sigma Aldrich) for thirteen weeks followed by a cessation period of 6 months in both 3xTg-AD, a mouse model of AD, and NonTg mice. Mice were orally gavaged with either a vehicle, 50, or 500 mg/kg dose of glyphosate every day starting at 4.5 months of age, followed by cessation from ~7.5 to 13.5 months. Blood and brain tissue were collected at euthanasia for mass spectrometry and neuropathological analysis. While we did not detect glyphosate in cortical (Ctx) tissue of mice after 6 months of cessation, we detected aminomethylphosphonic acid, the major metabolite of glyphosate, in the Ctx and blood plasma of mice exposed to glyphosate, 6 months after exposure. The levels of TNFα were significantly elevated in the Ctx in a dose-dependent manner in both NonTg and 3xTg-AD mice, with AD mice showing greater elevations. We also found significantly higher levels of soluble $A\beta_{42}$ and insoluble $A\beta_{40}$ in the hippocampus (Hp) of the 500mg/kg group, and elevated levels of insoluble $A\beta_{42}$ in the Hp and Ctx of both the 50mg/kg and 500mg/kg mice compared to the vehicle group. We observed elevated levels of the amyloid precursor protein cleaved product C99 in the 50mg/kg and 500 mg/kg groups, illustrating increased production of Aβ. Lastly, we found that the levels of soluble phosphorylated Tau at Serine 396 (pTau Ser396) were significantly elevated in the Hp and Ctx of both the 50 and 500mg/kg 3xTg-AD groups, while insoluble pTau Ser396 levels were elevated in the Hp and Ctx of only the 500mg/kg 3xTg-AD mice. Collectively, our results offer tantalizing evidence tying in glyphosate exposure with hallmark mechanisms associated with AD-like pathologies, demonstrating that exposure to this herbicide may be detrimental to health and therefore warrants further studies to determine its impact in the general population.

Disclosures: H. Leon: None. **W. Winslow:** None. **S.K. Bartholomew:** None. **M.N. Martinez:** None. **K.V. Pathak:** None. **R. Sharma:** None. **P. Pirrotte:** None. **R. Velazquez:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.05/D64

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

Support: NIH Grant AG064239

Title: Dap12 deficiency increases resilience to tau toxicity by modulating oligodendrocyte state and myelination in tauopathy mice despite elevated tau inclusions

Authors: *H. CHEN[1], F. LI[2], Q. GUO[4], M. WONG[2], C. LOPEZ-LEE[2,3], B. LIU[2,3], G. CARLING[2,3], Y. HUANG[2], F. YU[2], N. FOXE[2], Q. MA[4], H. FU[5], L. GAN[2], W. LUO[2]; [1]Cornell University: Weill Cornell Med. Col., Long Island City, NY; [3]Weill Cornell Neurosci. Grad. Program, [2]Weill Cornell Med., New York, NY; [4]Dept. of Biomed. Informatics, [5]Dept. of Neurosci., Ohio State Univ., Columbus, OH

Abstract: Alzheimer’s disease (AD) involves the accumulation of pathogenic tau and white matter loss, in addition to grey matter degeneration. The mechanisms underpinning tau toxicities in AD and other tauopathy disorders are not yet fully understood. Microglia activate immune responses and crosstalk with other brain cells, including oligodendrocytes, which make myelin to support neuronal functions. Dap12 (DNAX-activation protein 12, or TYROBP) is a crucial adaptor that mediates signaling from surface immune receptors such as TREM2, an AD risk gene. Dap12-deficient mice become resistant to tau toxicity with reduced brain inflammation and improved cognition in a heterozygote P301S tauopathy mouse model, despite elevated tau pathology. How deleting Dap12 increases resilience to tau toxicity in tauopathy brain remains unclear. Using single-nucleus RNA sequencing analysis, we observed that tau induces unique clusters in microglia and oligodendrocytes. Tau specific microglia (MG) clusters resemble DAM-like proinflammatory signatures. Interestingly, tau-dependent oligodendrocyte (OL) cluster expresses markers of previously identified intermediate oligodendrocytes (iOli). Spatially resolved transcriptomics in human AD brain showed a significant association of AT8-positive tau with the expression levels of iOli gene set, suggesting a disturbed transcriptomic state in OL related to tauopathy. Strikingly, Dap12 deletion completely blocked tau-inducible OL clusters. Meanwhile, loss of Dap12 in tau mouse brains restored homeostatic microglial population, significantly reducing disease associated microglial population and suppressing inflammatory signaling. Removing Dap12 also restored the expression of genes related to myelination and prevented myelin loss in the brains of mice with tauopathy. On the other hand, deficiency of Dap12 interfered tau processing by cultured microglia and exacerbated tau pathology in tauopathy mouse brain.Taken together, our study demonstrates that tauopathy disturbs the transcriptomic state of oligodendrocytes through the activation of microglial Dap12-dependent signaling. Our results suggest that Dap12 plays a multifaceted role in regulating microglial response to tauopathy. In addition to its pivotal role in mediating tau toxicity on oligodendrocytes, Dap12-dependent signaling is also required for microglia-dependent tau metabolism through tau processing. Our study unveils a new mechanism for myelin loss and

neurodegeneration in AD, whereby toxic tau shifts the state of oligodendrocytes and disrupts myelination through activating Dap12-dependent microglial signaling.

Disclosures: **H. Chen:** None. **F. Li:** None. **Q. Guo:** None. **M. Wong:** None. **C. Lopez-Lee:** None. **B. Liu:** None. **G. Carling:** None. **Y. Huang:** None. **F. Yu:** None. **N. Foxe:** None. **Q. Ma:** None. **H. Fu:** None. **L. Gan:** None. **W. Luo:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.06/D65

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

Support: NIA funding

Title: Low dose dextran sodium sulfate as a model for systemic chronic inflammation and its impact on alzheimer’s disease progression in 5xfad mice

Authors: *A. TRUMBO[1], E. TOMMER[4], F. PAN[1], K. BANEGAS-MORALES[1], N. FERNANDO[5], H.-Y. SHIH[5], Y. ZHANG[2], E. LEHRMANN[2], A. ORNELAS LOREDO[1], D. SARANTOPOULOU[1], J. SEN[3], R. SEN[1];
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Abstract: The prevalence of Alzheimer’s disease (AD) has increased over the past twenty years, resulting in one-third of the elderly presenting with AD. There are genetic and environmental risk factors for AD. Humanized mouse models that express human genes associated with AD have been used in research. Despite the genes used in these models being present in humans since birth, the onset of neurodegeneration in these individuals occurs later in life. Thus, environmental factors play a large role in the onset of neurodegeneration. Aging correlates with microbiome dysbiosis, gut leakage, and consequently systemic inflammation. To investigate how systemic inflammation, initiated by a leaky gut, impacts neurodegeneration, we established a model of chronic low-grade inflammation and applied it to the 5XFAD AD mouse model. Ingenuity pathway analysis of microarray data indicated that low-dose dextran sodium sulfate (DSS) given continuously in drinking water to young C57B6J mice recapitulates aspects of the inflammation present in 2-year-old mice. The larger number of upregulated inflammatory pathways in male mice supports pilot study observations of males’ susceptibility to DSS. Flow cytometry of female brain tissue showed increased infiltration of $CD8^+$ T cells in DSS-treated wild-type mice compared to non-treated, and no difference between DSS-treated and non-treated 5XFAD mice. Both DSS and non-treated 5XFAD mice had higher numbers of $CD8^+$ T cells compared to the wild-type mice. 5XFAD is a rapidly progressing disease model, thus DSS’s lack of effect in these mice may indicate a threshold for $CD8^+$ T cell infiltration prior to 6 months of

age. Work is ongoing to establish sex differences in DSS-treated 5XFAD mice, the impact of DSS on the amyloid beta burden, and cytokine levels in brain tissues, plasma, and cerebral spinal fluid. These findings have established a sustainable model of low-grade systemic inflammation and will give insight into its role in AD and neurodegeneration.

Disclosures: A. Trumbo: None. **E. Tommer:** None. **F. Pan:** None. **K. Banegas-Morales:** None. **N. Fernando:** None. **H. Shih:** None. **Y. Zhang:** None. **E. Lehrmann:** None. **A. Ornelas Loredo:** None. **D. Sarantopoulou:** None. **J. Sen:** None. **R. Sen:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.07/D66

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

Support: The City University of New York (Graduate Center, CNC program)
NIH R01 grant # 1R01AG057555

Title: Assessing Atractylenolide III as a Potential Treatment for Alzheimer’s Disease in the TgF344-AD Rat Model

Authors: *A. STEELE[1,3], M. FIGUEIREDO-PEREIRA[2,3], P. ROCKWELL[2], P. SERRANO[1,3]; [1]Dept. of Psychology, [2]Dept. of Biol., CUNY Hunter Col., New York, NY; [3]CUNY Neurosci. Collaborative, Biol., CUNY Grad. Ctr., New York, NY

Abstract: Alzheimer's Disease (AD) is a debilitating neurodegenerative disease characterized by amyloid beta (Aβ) plaque accumulation, hyperphosphorylation of tau, and neuroinflammation, which ultimately results in cognitive decline. AD’s prognosis is projected to reach 13 million people by 2050. Despite the prevalence of the disease, limited treatments have been produced due to many treatments focusing on a one-drug-one-target approach. In silico analysis of known drugs has highlighted Atractylenolide III (ATC III) as having repurposing potential as a therapeutic treatment for AD. ATC III has been demonstrated to decrease inflammation and improve cognition across different neurodegenerative and neuroinflammatory models. However, the role of ATC III treatment on learning and molecular pathology in AD remains unknown. We hypothesize that ATC III modulates molecular pathways and the innate immune system to reduce Aβ plaque burden, which in turn results in improved learning. We utilized the TgF344 transgenic rat model of AD to investigate the effects of ATC III on cognition and molecular pathology. The TgF344-AD rat model processes the APP Swedish mutation and the presenilin 2 exon 9 deletion resulting in Aβ plaque formation, neurofibrillary tangles, and neuroinflammation leading to age-dependent cognitive decline. Findings indicate that ATC III treatment improves hippocampal dependent learning using the active Place Avoidance Task in 11 month transgenic male rats by significantly decreasing the percent of time spent in the shock zone ($p<0.05$). Furthermore, RNA sequencing has identified transcriptomic changes following long term ATC

III treatment including the upregulation of Brain Derived Neurotrophic Factor (BDNF), which is highly implicated in learning and memory. These results contribute to elucidating the mechanism of ATC III and how it mitigates the cognitive decline in our AD rat model.

Disclosures: A. Steele: None. **M. Figueiredo-Pereira:** None. **P. Rockwell:** None. **P. Serrano:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.08/D67

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

Support: NIH grant RF1AG068400
BrightFocus Foundation A20201166F

Title: Differential effect of hexokinase 2 gene dosage on microglial activation and Alzheimer's disease progression

Authors: *J. CODOCEDO[1], C. MERA REINA[2], P. B. LIN[2], S. PUNTAMBEKAR[4], B. CASALI[2], N. JURY GARFE[5], P. MARTINEZ[3], C. A. LASAGNA-REEVES[6], G. E. LANDRETH[7];
[1]Dept. of Anatomy, Cell Biol. & Physiol., [3]Dept. of Anat. Cell Biol. & Physiol., [2]Indiana Univ., Indianapolis, IN; [4]Indiana University-Purdue University, Indianapolis, Indianapolis, IN; [5]Anat. and cell biology, Stark Neurosciences Res. Inst., Indianapolis, IN; [6]Stark Neurosciences Res. Inst., [7]Stark Neurosci. Res. Institute, NB214C, Indiana Univ. Sch. of Med., Indianapolis, IN

Abstract: Microgliosis and neuroinflammation are hallmarks of Alzheimer’s disease (AD). Pro-inflammatory microglia meet their increased energy demand by reprogramming metabolism, specifically, switching to favor glycolysis over oxidative phosphorylation, suggesting that modulations of microglial metabolism might be of therapeutic benefit for treating AD. We report that in the brains of 5xFAD mice and postmortem brains of AD patients, we found a significant increase in the levels of Hexokinase 2 (HK2), an enzyme that supports inflammatory responses by increasing glycolysis. Additionally, the mitochondrial binding of HK2 have been reported to regulate inflammation, by preventing mitochondrial dysfunction and reactive oxygen species production.To directly dissect the role of HK2 in the control of microglial immune response during AD progression, we generated mice that have a conditional deletion of one or two copies of HK2 gene in microglial cells by crossing 5xFAD;CX3CR1-Cre^{ERT2} mice with mice harboring a floxed HK2 allele.We induced the microglial deletion of one or both HK2 alleles (termed 5xFAD;HK2$^{Fl/wt}$ or 5xFAD;HK2$^{Fl/Fl}$ respectably) by tamoxifen (TAM) i.p. injections at 2 months of age, a period at which amyloid deposition and microgliosis begin. Mice harboring the Cx3cr1-CreERT2 cassette but lacking the LoxP sites in HK2 were treated with TAM and used as controls (5xFAD;HK2$^{wt/wt}$). Three months later (5 months of age), we evaluated AD

pathogenesis. Similarly, we evaluated the acute effect of its pharmacological inhibition with Lonidamine. To ascertain the biological process affected by HK2 inhibition in AD, we performed transcriptomic analysis of the cortical samples with NanoString.Here we report, that HK2 antagonism selectively affects microglial phenotypes and disease progression in a gene-dose dependent manner. Paradoxically, its complete loss exacerbates inflammation, while its haploinsufficiency display reduced pathology and improved cognition in the 5XFAD mice. We propose that the partial antagonism of HK2, is effective in decrease inflammation, by targeting specifically its energetic role without secondary side-effects on mitochondria.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.09/D68

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

Title: Immunoneutralization of TNFSF10 improves immune response and attenuates neuroinflammation in a mouse model of Alzheimer’s disease

Authors: A. CANTONE[1], C. BURGALETTO[1,3], *G. DI BENEDETTO[1], R. CALTABIANO[2], C. BELLANCA[1], E. AUGELLO[1], R. BERNARDINI[1], G. CANTARELLA[1];
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Abstract: Alzheimer’s disease (AD) is the most common cause of dementia worldwide, associated with accumulation of the anomalous protein amyloid-β and hyperphosphorylation of the tau protein in the brain. Neuroinflammation has increasingly been regarded to as a crucial factor contributing to AD pathogenesis. Brain inflammation in AD appears associated with chronically activated CNS-resident innate immunocytes, as well as with increased leukocyte re-trafficking across the blood-brain barrier. TNFSF10, a proinflammatory cytokine belonging to the TNF superfamily, is substantially expressed in the AD brain, where it energetically modulates the immune response. TNFSF10, in fact, is abundantly released by activated glia, CNS-infiltrating macrophages and injured neurons, finally acting as a cell death signal. Previous data have highlighted that TNFSF10 fuels inflammation by recruiting peripheral Treg cells in the 3xTg-AD mice brain, therefore restraining the counteracting efficacy of the latter on the accumulation of anomalous proteins. Consistently, immunoneutralization of TNFSF10 blunts spleen and hippocampal inflammation. With such background, changes in both splenic and brain infiltrating Treg cells and resident monocytes were studied by means of flow cytometry in 3xTg-AD mice undergone a treatment with an anti-TNFSF10 monoclonal antibody. Moreover, the amount of splenic exhausted $CD4^+PD1^+$/ $CD8^+PD1^+$ T cells was measured in the same animals.

The number of $CD4^+CD25^+FOXP3^+$ cells out of the total $CD45^+CD4^+$ gated infiltrating T cells was significantly decreased in both the brain and the spleen of 3xTg-AD mice treated with the anti-TNFSF10. Flow cytometry also indicated that the anti-TNFSF10 treatment implied reduced number of $CD11b^+LY6C^{high}P2RY12^-$ proinflammatory monocytes. In the same line, immunofluorescence experiments on the brain showed a decreased expression of the inflammatory marker CD86, inversely related to the increased levels of the anti-inflammatory marker CD206, suggesting a switch of microglia towards its neuroprotective phenotype. Finally, immunoneutralization of TNFSF10 resulted in lower exhausted $CD4^+PD1^+$/ $CD8^+PD1^+$ T cell count, pointing out that, eventually, the anti-TNFSF10 treatment positively resolves the impairment of the immune function associated with neurodegeneration. In conclusion, it appears plausible to hypothesize that TNFSF10 system-targeted treatments effectively restrain overshooting CNS inflammation through a re-balancing effect on the immune response, so to attenuate progression of pathological processes underpinning uncontrolled progressive neurodegeneration in AD.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.10/D69

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

Support: NIH RF1AG072727
NIH P20GM113123
NIH U54GM128729

Title: Increased fluoride concentration in water elevated Aβ plaque load in App^{NL-G-F} mice

Authors: *K. JEROME, A. MCINTEE, S. NOOKALA, C. COMBS;
Biomed. Sci., Univ. of North Dakota, Grand Forks, ND

Abstract: The relationship between microbiome changes and Alzheimer's Disease (AD) has been examined in numerous studies. Although much of the focus has been on intestinal microbiome changes, it has become clear that the oral cavity microbiome composition may also be altered during disease and influence changes in the brain. We previously demonstrated oral dysbiosis in the *App^{NL-G-F}* mouse model of AD. To determine whether oral dysbiosis influences brain aspects of disease, we continued to use the *App^{NL-G-F}* mice and provided dietary intervention of fluoridated water to affect the oral dysbiosis and presumably alter brain histologic changes associated with AD. We compared male and female *App^{NL-G-F}* mice to littermate control wild type C57BL/6J mice. The mice were randomly assigned to either a highly fluoridated water

treatment group or a control water group. Mice in the fluoride treatment were provided water with a fluoride concentration of 4ppm, the highest FDA-approved fluoride concentration in municipal water in the US, from weaning to 6 months of age while mice in the control group were provided standard 1ppm fluoridated water. The brains and mandibles were collected upon study completion. Mandibles were stained using caries-detecting dye for cavity counting quantitation and left hemisphere brain sections were immunostained using anti-Aβ, GFAP, and Iba1 antibodies to quantify Aβ plaque load, astrogliosis, and microgliosis. Fluoride treatment increased the number of cavities in wild type male and *App*$^{NL\text{-}G\text{-}F}$ female mice but had no significant effect on wild type female or *App*$^{NL\text{-}G\text{-}F}$ male mice. Additionally, fluoride treatment had no effect on male astrogliosis but increased astrogliosis in wild type female mice. Microgliosis was increased in both wild type male and female mice in the fluoride-treated group compared to controls but had no effect on *App*$^{NL\text{-}G\text{-}F}$ mice. Finally, the fluoride treatment significantly increased Aβ plaque load in both male and female *App*$^{NL\text{-}G\text{-}F}$ mice. These results suggest that manipulating oral dysbiosis through higher fluoride consumption in water has a negative effect on AD-associated pathology.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.11/D70

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

Support: Interdisciplinary Training in Pathobiology and Rehabilitation Medicine
2T32HD071866-06
1K99AG078400-01
AG066489

Title: Neuroinflammatory protein networks associate with altered decision making, and impaired executive functions in the TgF344 Alzheimer’s Disease rat model

Authors: M. A. MCCUISTON[1], N. L. JACKSON[2], L. L. MCMAHON[2], *C. M. HERNANDEZ[1];
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Abstract: The relative weight that individuals give to rewards and “costs” (such as delay to reward delivery) in making decisions varies significantly across the population and disease states. One aspect of decision making involves weighing the relative benefits and costs associated with immediate versus delayed outcomes. This aspect of decision making is often referred to as temporal discounting (or intertemporal choice) and can be assessed on tasks in which subjects are required to choose between small, immediate rewards and larger rewards

delivered after varying delays. Furthermore, intact executive functioning and motivation are foundational to the decision-making process. Recent literature shows there is a great deal of variability in the temporal discounting phenotype between individuals with mild cognitive impairment, frontal temporal dementia, and Alzheimer's Disease (AD), with some showing no differences relative to healthy controls and others showing altered rates of temporal discounting. Even beyond these disorders, individual differences in choice behavior (either maladaptive or normative) in young adults predict a variety of life outcomes, including educational success and socioeconomic status. The current study used a behavioral and molecular approach to determine the effect of genotype on phenotypic differences in temporal discounting, motivation, and executive function and neuroinflammation in the TgF344AD (TgAD) rat model of AD. Young adult (6-7 mo) wild-type (WT; n=6) and TgAD (n=6) were trained on a several operant tasks including temporal discounting (decision making), progressive ratio (motivation), set shifting (cognitive flexibility), and delayed response (working memory) tasks. Basolateral amygdala (BLA), Prelimbic Cortex, and Nucleus Accumbens tissue was then isolated and processed for use in a multiplex ELISA to assess markers of inflammation. Results suggest TgAD rats showed a greater preference for the large, delayed reward relative to WT, however, this preference did not translate to a greater number of total rewards earned. Additionally, TgAD rats were less motivated to obtain the larger reward, were less cognitively flexible, and showed impaired working memory relative to WT controls. Additionally, AD-associated markers of inflammation were higher in the BLA of TgAD rats relative to WT. Interestingly, young TgAD rats show a similar decision-making and cognitive phenotype as old rats, and these data suggest AD pathology may manifest as maladaptive decision making in addition to impaired executive functions early in life.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.12/E1

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

Support: NIH Grant P30GM127211
NIH Grant R21AG066865
NIH Grant T32AG057461
NIH Grant P30AG072946
NIH Grant UF1NS125488

Title: $Cd4^+$ and $cd8^+$ effector and memory t cells are associated with neurofilament light chain in an aging based community cohort

Authors: ***E. D. WINFORD**[1], J. LUTSHUMBA[2], D. M. WILCOCK[3], G. A. JICHA[2], B. S. NIKOLAJCZYK[4], A. M. STOWE[2], A. D. BACHSTETTER[1];

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Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder that is the leading cause of dementia worldwide. Multiple factors contribute to AD's progression, including neuroinflammation and a dysregulated neuro-immune response. Recent studies have highlighted the importance of the adaptive immune response during the progression of AD, more specifically, the T cell response and how it is associated with cognition. Studies have also shown the importance of AD-related plasma biomarkers and how they aid in diagnosing AD and its severity. However, it is still unclear how the T cell response is correlated with AD-related plasma biomarkers. Therefore, we tested the hypothesis that effector and memory T cell populations correlate with AD-related biomarkers. Methods: Study participants from a University of Kentucky Alzheimer's Disease Research Center (UK-ADRC) community-based cohort of aging and dementia were used to test our hypothesis. Participants underwent physical examination, neurological examination, medical history, cognitive testing, and blood collection to determine plasma markers and isolate peripheral blood mononuclear cells (PBMCs). There were 84 participants, 44 women and 40 men. PBMCs were collected and processed for flow cytometry. All samples were stained with a viability dye (Ghost dye 710) and immune cell markers CD3, CD4, CD8, CCR7, and CD45RA. Gating and analysis were done in FlowJo v10. Multiple linear regression models adjusted for age and sex were used to determine the relationship between plasma markers for Aβ42/Aβ40 ratio, total tau, Neurofilament Light chain (NF-L), Glial Fibrillary Acidic Protein (GFAP), and effector and memory T cells populations such as $CD4^+$ and $CD8^+$ central memory T cells (T_{CM}), Naïve T cells, effector memory T cells (T_{EM}), and effector memory $CD45RA^+$ T cells (T_{EMRA}). These data analyses were completed in JMP Pro v16. Results: We did not find an association between Aβ42/Aβ40, total tau, GFAP, and any of our $CD4^+$ effector or memory T cell populations. However, we did find a positive correlation between NF-L and the percentage of $CD4^+$ T_{CM} cells ($p=.022$; $R^2=.32$). When we looked at our $CD8^+$ effector and memory T cell population, we also did not find any association between Aβ42/Aβ40, total tau, GFAP, and effector or memory T cell population. We did find a positive correlation between NF-L and $CD8^+$ $_{TEMRA}$ cells ($p=0.021$; $R^2=.32$) and a negative correlation between NF-L and $CD8^+$ naïve cells ($p=.030$; $R2=.31$). These findings suggest that both the CD4+ and CD8+ memory and effector T cells response during AD may be a response to neuronal injury (NF-L).

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.13/E2

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

Support: Alzheimer's Association International Research Zenith Fellows Award

Title: Inhibition of MIF's nuclease activity reduces plaque burden and improves learning and memory deficit in the 5xFAD mice model

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Abstract: Increased dsDNA breaks, PARP1 hyperactivation, and PARylation of nuclear proteins are observed in Alzheimer's disease (AD) and are consistent with Poly (ADP-ribose) polymerase 1 (PARP-1)-dependent cell death (PARthanatos). MIF, an atypical cytokine with tautomerase activity, acquires pathological gain of function as a nuclease to execute parthanatos. However, the role of parthanatic cell death and MIF in AD pathophysiology is unknown. We observed elevated MIF protein levels in the hippocampus of aged 5xFAD (N=4) but not in wild-type (WT) mice. Next, we crossed MIF^{E22Q} (lacks nuclease activity) and MIF^{P2G} (lacks tautomerase activity) mice with 5xFAD mice to assess the relative contribution of MIF's nuclease or tautomerase activity in AD. Via immunohistochemical analysis, we found ablation of MIF nuclease activity, but not tautomerase activity, reduces amyloid beta (Aβ) load (6E10 antibody for filamentous Aβ labeling) in the cortex and hippocampus at 8 months of age (N=6). In addition to reduced Aβ load, dense core plaques labeled by Thioflavin S [ThioS] were significantly reduced in 5xFAD MIF^{E22Q} mice. Nucleic acid (NA) containing plaques are found in the human AD brain and various β-amyloidosis models and is implicated in reactive gliosis. We observed a striking reduction in plaque NA deposition (acridine orange and 6E10 dual stain) and gliosis of 5xFAD MIF^{E22Q} mice compared to 5xFAD and 5xFAD MIF^{P2G} mice. Both gliosis and Aβ load can inversely influence neuronal health and brain function. Therefore, we evaluated the performance of 8-month-old 5xFAD, 5xFAD $MIF^{E22Q,}$ and 5xFAD MIF^{P2G} mice in the Morris water maze (MWM) (N>=10, each genotype) to assess spatial learning and memory. We found that WT littermate controls and 5xFAD MIF^{E22Q} mice showed similar escape latency, whereas 5xFAD and 5xFAD MIF^{P2G} took longer to identify the platform on day 5 of the MWM test. Furthermore, the probe trial on the 6^{th} day confirmed unimpaired spatial memory in 5xFAD MIF^{E22Q} mice. Our findings suggest that parthanatos is involved in AD pathology, and MIF nuclease inhibition can potentially inhibit neurodegeneration in AD.

Disclosures: **M. Roy:** A. Employment/Salary (full or part-time):; Neuroregeneration and Stem Cell Programs, Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. **V. Dawson:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. **T. Dawson:** A. Employment/Salary (full or part-time):; Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.14/E3

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

Support: NIH R01AG048993
NIH P20GM113123
NIH P20GM103442
NIH U54GM128729
NIH RF1AG069378
NIH RF1AG072727

Title: Splenectomy modifies amyloid plaque load, microglial phenotype, and cytokine levels in male mouse models of Alzheimer's Disease

Authors: *B. SAHU, M. SOHRABI THOMPSON, A. M. FLODEN, A. MCINTEE, G. MANOCHA, S. NOOKALA, C. K. COMBS;
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the brain accumulation of amyloid beta (Aβ) plaques, neurofibrillary tangles, neuronal death, robust gliosis, and neuroinflammation, affecting around 35 million individuals worldwide. Unfortunately, current therapeutic options are limited in efficacy, requiring a further understanding of the disease. Numerous studies support the idea that peripheral immune dysfunction likely influences immune cell phenotype in the brain. As the secondary lymphoid organ for the circulatory system, the spleen constantly monitors antigens to modulate innate and adaptive immune responses. Since splenic control of immune cell phenotype is associated with both pro and anti-inflammatory effects in the brain in paradigms such as stroke and traumatic brain injury, we hypothesized that a similar spleen-to-brain communication is relevant in AD. To test this idea, splenocytes from male and female C57BL/6 wild type, APP/PS1, and $App^{NL\text{-}G\text{-}F}$ mice were analyzed by flow cytometry. Male but not female APP/PS1 and $App^{NL\text{-}G\text{-}F}$ mice had altered spleen immune cell numbers and phenotype compared to C57BL/6 mice indicating sex-selective peripheral immune dysfunction due to the disease. To better understand the contribution of these splenic changes to the brain, male C57BL/6 wild type, APP/PS1, and $App^{NL\text{-}G\text{-}F}$ mice were divided into 3 groups: control, sham surgery, and splenectomy. Two weeks post-surgery, learning, and memory were assessed by novel object recognition and passive avoidance tests. After the behavioral testing, mice were sacrificed for blood, meninges, and brain analysis. Our data revealed a decreased systemic inflammatory condition upon splenectomy, as suggested by reduced serum proinflammatory cytokine levels such as TNF-α, IL-6, MIP-3α, and IFN-γ in APP/PS1 and $App^{NL\text{-}G\text{-}F}$ mice compared to controls. Interestingly, splenectomy improved cognitive function as suggested by an increased discrimination index and step-through latency in APP/PS1 mice. In meninges, splenectomy resulted in a decrease in the $CD4^+$ and $Ly6G^+$ (neutrophil marker) cells and an increase in $CD206^+$ (M2 macrophage marker) cells. In addition to this, splenectomy led to elevated brain CD68, CD11b, and CD16/32 but not GFAP and Iba1 immunoreactivity and increased concentrations of proinflammatory cytokines, TNF-a, IL-1b, and IL-6. Additionally, splenectomy significantly reduced Aβ levels and plaque load compared to controls. Overall, our data suggest that splenocyte phenotype directly influences cognitive function, immune phenotype, and Aβ levels in the brain.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.15/E4

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

Support: F31 NIH Grant 5F31AG079560-02
R01AG072758
1 R01 AG076448-01

Title: The effects of APOE4-R47H on disease progression and microglial function in a tauopathy model

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Abstract: Alzheimer's disease (AD) is the most common form of dementia. Inflammation prompted by microglia may precede the spread of pathogenic tau, and most AD risk genes are highly expressed in microglia, supporting a significant role for inflammation in the disease. The *R47H* variant of triggering receptor expressed on myeloid cells 2 (*TREM2*) elevates AD risk by 2–4 fold. APOE exists in three isoforms, where *APOE4* is a major AD risk gene, *APOE3* does not affect disease risk, and *APOE2* is protective against AD. APOE is a known ligand of the TREM2 receptor, and TREM2 activation robustly increases microglial *APOE* expression, as well as affecting cellular metabolism and increasing inflammation. However, little is known about the mechanistic interactions between risk variants *R47H* and *APOE4*. We investigated the effects of *R47H* on tau-triggered microglial response on either the *APOE4* or *APOE3* background in the *P301S* tauopathy mouse model. Our data shows that the combination of *APOE4-R47H* (E4-R47H) worsens tau-induced neurodegeneration compared to *APOE4* alone without significantly altering hippocampal tau load. E4-R47H exacerbates hippocampal microgliosis and increases a cluster of interferon-expressing microglia observed through single-nuclei RNA sequencing. We have also discovered increased cGAS-STING protein levels in the E4-R47H hippocampus, which may be upstream of the heightened microglial interferon response and correlates with measures of neurodegeneration. This microglial interferon cluster is unique to E4-R47H, as we do not observe it on the *APOE3-R47H* background. In addition to aggravating the cGAS-STING pathway, we have linked E4-R47H to increases in tau-induced microglial senescence. The combination of senescence and interferon-related inflammation may create a neurotoxic population of microglia which thereby exacerbate neurodegeneration. In future studies, we plan

to explore possible links between the cGAS-STING pathway and microglial senescence. Our findings provide a new understanding of how TREM2 and APOE can interact to impact microglial fitness and implicate cGAS as an important therapeutic pathway to further explore in the treatment of AD.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.16/E5

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

Support: NIH R01 AG060148
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T32 AG000096
Larry L. Hillblom postdoctoral fellowship
Edythe M. Laudati Memorial Fund

Title: Microglial deletion of C1q rescues AD cognitive decline and synaptic loss and reduces astrocytic C3 expression

Authors: *T. PETRISKO, A. GOMEZ ARBOLEDAS, S.-H. CHU, A. J. TENNER;
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Abstract: The complement system contributes to enhanced inflammation and cognitive decline in Alzheimer’s disease (AD). Previous studies have demonstrated constitutive deletion of the classical initiator protein, C1q, reduces pathology and synaptic loss in AD mouse model. As it is now known that microglia are the primary producers of C1q in the brain, the objective of this study was to determine if microglial specific deletion of C1q would attenuate cognitive impairment and synaptic loss in AD and reduce amyloid deposition and neuroinflammation. Briefly, $C1qa^{FL/FL}$CX3CR1Cre (designated $C1q^{\Delta MG}$) mice, which show deletion of microglial C1q by 2 months of age, were crossed to Arctic$C1qa^{Fl/Fl}$ mice to generate WT and Arctic (Arc) mice with and without the CX3CR1Cre transgene. At 10m, mice underwent object location memory and spatial reference Y-maze testing to assess hippocampal dependent spatial memory. Brain tissue was then collected and immunostained for pre (VGlut1) and post (PSD95) synaptic makers and superresolution Z-stacks of the hippocampal CA1 of the hippocampus obtained. The density of pre- and post-synaptic puncta and their colocalization was assessed using IMARIS. 20X confocal z-stacks were obtained and the percent volume of hippocampal coverage was quantified using IMARIS for amyloid plaque accumulation (AmyloGlo), astrocytes (GFAP), microglia (Iba1), complement protein C3, and complement receptor C5aR1 expression. Plasma

neurofilament light (NfL) levels were assessed at 10m of age.
Adult microglia deletion of C1q rescued cognitive deficits in male but not female Arc mice. Similar sex-dependent results were observed in the density of pre (Vglut1) and post-synaptic (Psd95) puncta and their colocalization in the hippocampal CA1. Male ArcC1q$^{\Delta MG}$ mice displayed increased levels of Psd95 and a trending increase in synaptic colocalization, suggesting a rescue of functional synapses in the male mouse. In contrast, female ArcC1q$^{\Delta MG}$ mice displayed slight, but significantly reduced levels of Psd95 puncta compared to female Arc mice as well as a trending reduction in colocalization of these synaptic markers. While only a trending ($p=0.08$) reduction in GFAP reactivity was observed in ArcC1q$^{\Delta MG}$ animals, a 38% decrease ($p<0.05$) in C3 expression was observed compared to Arc mice (independent of sex). Microglial deletion of C1q in Arc mice did not alter Iba1 or C5aR1 expression and failed to reduce plasma NfL levels.
Deletion of C1q in microglia prior to amyloid deposition provides protection against cognitive decline and correlates with protection of synaptic loss and reduced astrocytic C3.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.17/E6

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

Support: NIH R21 AG048498-01
NIH R01 AG056472

Title: Interactive effect of APOE and sex in modulating neuroinflammation in an Alzheimer's disease-relevant transgenic mice

Authors: *D. BALU[1], A. C. VALENCIA[1], J. M. YORK[1], F. PERI[2], F. NEUMANN[3], G. THATCHER[4], M. LADU[1], L. M. TAI[1];
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Abstract: *APOE4* and female sex synergistically increase Alzheimer's disease (AD) risk, and therefore identifying the underlying mechanisms is important for ultimately understanding and treating AD. Increasing evidence suggest that neuroinflammation may be an important pathway through which *APOE4* and female sex impact brain function. Indeed**,** both *APOE4* and female sex independently increase gliosis in humans and mice that overproduce Aβ (FAD mice). Importantly**,** microglial activation was highest in *APOE4* female AD patients. Although this data supports a correlation between neuroinflammation and AD, the extent that neuroinflammation

directly impacts cognition and AD-relevant pathology in female *APOE4* carriers is unknown. Therefore, the goal of this study was to determine whether targeting neuroinflammation is beneficial for female *APOE4* carriers *in vivo*. Initially, we determined the effect of *APOE* and sex on neuroinflammation and other AD-relevant pathology in mice that overproduce Aβ42/Aβ40 via 5xFAD mutations and express human *APOE3* (E3FAD*)* or *APOE4* (E4FAD). We found that female E4FAD mice had highest levels of gliosis, which correlated with Aβ pathology and cognitive impairment from 6 months (M) of age. These data led us to evaluate if targeting neuroinflammation may be beneficial for female E4FAD mice using a toll-like receptor 4 (TLR4) antagonist, IAXO-101. TLR4 is central for innate immunity, and we have previously shown that IAXO-101 lowers Aβ-induced cytokine production in *APOE4* mixed glia cultures. Thus, we treated E4FAD male and female mice with IAXO-101 from 4 to 6M of age. We found in female E4FAD mice that IAXO-101 treatment resulted in lower microgliosis and IL-1β levels in tandem with improvements in learning and memory, with no change in Aβ pathology. Interestingly, IAXO-101 had no effect on male E4FAD mice. These data support that neuroinflammation is an important contributing pathway to cognitive dysfunction in *APOE4* females. In addition to the interaction between sex and *APOE*, the loss of sex hormones during menopause could impact neuroinflammation with *APOE4*. In general, ovariectomy (OVX) has been shown to exacerbate AD-relevant pathology in vivo, which may be improved by Estradiol (E_2). Hence, we determined whether OVX exacerbates neuroinflammation female EFAD mice and if it can be mitigated by E_2. We found that OVX treatment increased gliosis and Aβ pathology in E4FAD mice, which was reduced by E_2. Thus, future research could focus on identifying novel ways to lower neuroinflammation in female *APOE4* carriers with low E_2. Collectively our data support that neuroinflammation is a key mechanism and therapeutic target for *APOE4* females in AD.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.18/E7

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

Support: NIH Grant NS104282-01A1
DOD Grant W81XWH2210280
DOD Grant W81XWH2210690

Title: Targeting MHC Class II-associated invariant peptide (CLIP) improves TBI-induced neurobehavioral dysfunction and neuropathology in 5xFAD mice

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Abstract: Traumatic brain injury (TBI) is a significant risk factor for Alzheimer's disease (AD). There are several pathological similarities between TBI and AD, including depression, cognitive impairment, and immune activation. Unknown, is how immune activation after TBI might facilitate AD pathogenesis. B cells play an essential role in the transition from a non-specific innate to an antigen-specific adaptive immune response. TBI can induce an adaptive immune response and evidence suggests both beneficial and pathogenic roles for B cells in nervous system disorders. Thus, specific B cell subsets may differentially influence outcomes. We previously identified expansion of B cells after TBI, including pro-inflammatory CLIP+ B cells. We developed a CLIP antagonist peptide (CAP) that competitively antagonizes CLIP binding to the antigen presenting groove of major histocompatibility complex II (MHCII) and reduces CLIP+ B cells induced by inflammatory stimuli, including TBI. We've shown that CAP is anti-inflammatory and neuroprotective after TBI, suggesting a detrimental role of CLIP+ B cells in TBI pathogenesis. We hypothesize that CLIP+ B cell expansion after TBI will be detrimental to AD pathogenesis. To test this hypothesis, we administered CAP or vehicle at 30 minutes after either sham or our fluid percussion injury (FPI) model TBI in 3-month-old male 5xFAD mice. Gait, depression, and cognition were assessed followed by pathological assessment using diffusion tensor imaging (DTI), vessel painting, and immunohistological analysis of plaques, microglia, and astrocytes. In 5xFAD mice, CAP improved post-injury gait deficits. Depression-associated behavior and cognitive impairment were identified in 5xFAD mice. CAP treatment improved some, but not all of these deficits, suggesting specific pathological domains are susceptible to CLIP+ B cells. Vessel painting revealed chronic alterations to the cerebrovasculature after FPI that were improved by CAP treatment. Chronic FPI-induced alterations to connectivity between brain regions including AD-relevant regions such as the infralimbic cortex, entorhinal cortex, and hippocampus, were selectively improved by CAP. CAP mitigated the FPI-induced alterations in amyloid plaque deposition in the hippocampus of 5xFAD mice and ameliorated the FPI-exacerbated astrocyte and microglia activation. Taken together, these findings indicate an important but selective role for CLIP+ B cells in AD-associated pathological progression following TBI and highlight a novel potential therapeutic target.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.19/E8

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

Support: NIEHS Grant ES007062

Title: Translocator Protein 18kDa (TSPO) expression in the 5XFAD animal model of Alzheimer's disease (AD): Cellular sources and association with AD pathophysiology using a life course approach

Authors: *D. A. MARTINEZ-PEREZ, J. L. MCGLOTHAN DZIEDZIC, T. R. GUILARTE; Envrn. Hlth. Sci., Florida Intl. Univ. Robert Stempel Col. of Publ. Hlth. & Social Work, Miami, FL

Abstract: Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder that affects cognition, memory, and social abilities with devastating effects on individuals and their families. The hallmark pathology of AD is the accumulation of amyloid-β (Aβ) plaques and tau neurofibrillary tangles leading to neurodegeneration and atrophy of the brain. Neuroinflammation and microglia activation play an important role in the initiation and progression of AD. TSPO is a well-validated and widely used biomarker of neuroinflammation that is expressed in glial cells, and it is markedly increased in the brain of AD subjects and AD animal models. Here, we use a life course approach to examine the trajectory of brain TSPO levels, cellular sources, and association with AD pathology. To assess cognitive performance, we used the Barnes Maze for spatial learning in male and female wild-type (WT) and 5XFAD mice at 3 months (3M), 7 months (7M) and 12 months (12M). No significant differences in cognitive performance were found at 3M. At 7M, there was a significant cognitive impairment in 5XFAD females but not in males. At 12M, we found a significant difference in spatial learning in both sexes compared to WT. To evaluate pathological endpoints, we measured Aβ plaque number, size, and load by immunohistochemistry (IHC) in different brain regions including the deep layers of the cerebral cortex, amygdala, hippocampus, and thalamus. We found an increase in Aβ-plaques as a function of age and sex, with higher levels in females compared to male 5XFAD mice. TSPO quantitative autoradiography with the TSPO-specific radioligand [^{3}H]-DPA-713 was analyzed in the cerebral cortex, amygdala, hippocampus, and thalamus. The results indicate a significant increase in TSPO levels by genotype, brain region and age, with a higher increase in TSPO levels in females than in males. TSPO levels were significantly increased in both sexes at 3M, 7M and 12M in 5XFAD mice compared to WT mice. Quadruple-label immunofluorescent confocal imaging to determine the cellular sources of the TSPO response and its association with Aβ-plaques showed that TSPO expression was highly colocalized with microglia and not with astrocytes. Furthermore, TSPO was primarily increased in microglia associated with Aβ plaques. In summary, our findings indicate that brain TSPO is an early biomarker of neuroinflammation in AD and TSPO levels increase at the same time than Aβ aggregation, but much earlier than cognitive decline, in an age- and sex-dependent manner. The increase in TSPO levels was associated with microglia surrounding or infiltrating Aβ-plaques suggesting a role in plaque formation and AD progression.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.20/E9

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

Support: NSTC Grant 111-2320-B-A49-043
Yushan Young Scholar Program

Title: Functional Study of Monocyte Derivatives in P-tau Inflicting Murine Model of Alzheimer's Disease

Authors: C.-H. YIN[1], M.-H. LIN[1], E. CHEN[1], H.-T. CHIEN HAGAR[3], M.-H. KUO[3], ***H.-R. CHEN**[1,2];
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Abstract: Phosphorylation is a normal post-translational modification of tau, but hyperphosphorylation may provoke tau aggregation into insoluble neurofibrillary tangles (NFTs), which is defining to be pathological feature of Alzheimer's diseases (AD). Recent studies suggested that pre-fibrillary, phosphorylated tau (p-tau) oligomers may already possess neurotoxicity, long before the onset of cognitive impairments. Enhancing myelin turnover reverses cognitive deficits in AD mouse models. Moreover, many studies suggested that hyperactivation of monocytes/macrophages contributes to the progression of AD and can be a promising therapeutic target in AD. Yet, several issues remain to be clarified. To investigate the role of monocyte derivatives in AD, we used bi-transgenic CCR2-CreER; R26R-GFP mice to track the monocyte derivatives in a tamoxifen-inducible manner. Our pilot study showed that intrahippocampal p-tau injection caused monocyte infiltration along with myelin sheath disruption, anxiety- like behavior and memory loss. Infiltrating monocytes in the brain parenchyma expressed high levels of Trem2, pro-inflammatory cytokines and clustered at the white matter after p-tau injection. To dissect the pathological mechanisms of p-tau inflicting AD, we performed bulk RNA-seq of 3 d and 1 m post saline/p-tau injected brains. The transcriptome analysis showed innate immune response, monocyte chemotaxis, phagocytosis and amyloid beta clearance pathway were high responder groups after exposure to p-tau in the murine brains, whereas saline injection lacks these effects. Next, pharmacological obstruction of early monocytic influx, but not late arrival monocytes, significantly attenuated anxiety-like behavior and mitochondrial metabolism defects. Together, these suggest the pathologic role of early infiltrating monocytes in p-tau inflicting murine model may promote monocytes/macrophages phagocytosis of myelin sheath and synapses leading to anxiety-like behavior and memory loss.

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Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.21/E10

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

Support: NIH R01AG059627
NIH R01AG062500

Title: Patients with Mild Cognitive Impairment (MCI) and obese prediabetics exhibit low circulating choline levels that correlate with various metabolic and brain pathologies.

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Abstract: Deficiency of dietary choline, an essential nutrient, is observed worldwide; ~90% of Americans are choline deficient. Previous work utilizing dietary questionnaires highlights a relationship between decreased choline intake and an increased risk for cognitive decline and Alzheimer's disease (AD). Examining blood circulating choline levels in Mild Cognitively Impaired (MCI) individuals, which can precede AD, would inform whether circulating choline reductions proceeds AD. Additionally, evidence in rodent models highlights that dietary choline deficiency can contribute to obesity and glucose metabolism impairments (Dave et al., 2023). This is notable as obesity and diabetes is a significant risk factor for dementia. Here, we examined the levels of circulating blood choline in two cohort of patients. One cohort of patients were diagnosed with either MCI with sparse CERAD neuritic plaque density and Braak stage between II to III (n = 10), MCI with high CERAD neuritic plaque density and Braak stage between IV to V (n = 12) or were healthy aged-matched controls (n = 10). The second cohort of patients were categorized as either lean with a body mass index (BMI) between 10 to 25 (n = 15) or obese with a BMI over 30 and prediabetic (n = 12). In the first cohort, we found that the levels of circulating choline were significantly reduced in both sparse ($p = 0.049$) and high MCI ($p = 0.0038$) cases compared to aged-matched controls. Correlation analysis revealed a significantly negative association between circulating choline levels and both CERAD neuritic plaque density ($r_{30} = -0.3548$, $p = 0.046$) and Braak stage ($r_{30} = -0.402$, $p = 0.023$), indicating that as choline levels decreased, CERAD neuritic plaque density and Braak stage increased. In the second cohort, we found that obese patients had significantly lower circulating choline levels than those in the lean BMI group ($p < 0.0001$). Subsequent correlation analysis revealed that body fat % was negatively correlated with circulating choline levels ($r_{25} = -0.6945$, $p < 0.0001$), illustrating that as choline levels went down, body fat % increased. Additionally, insulin levels ($r_{25} = -0.4578$, $p = 0.0163$) and homeostatic model assessment for insulin resistance (HOMA-IR; $r_{25} = -0.4046$, $p = 0.0363$), which approximates insulin resistance, where both significantly negatively

correlated with circulating choline levels. Collectively, these results highlight that low circulating choline levels are associated with early MCI and metabolic dysfunction, which may increase the risk of AD and other dementias, illustrating the importance of ensuring adequate dietary choline intake to offset disease.

Disclosures: W. Winslow: None. **J.M. Judd:** None. **G.E. Serrano:** None. **C. Katsanos:** None. **T.G. Beach:** None. **R. Velazquez:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.22/E11

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

Support: NIH Grant R01AG075998
NIH Grant R01AG056478
NIH Grant R01AG055865
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Title: Chlamydia pneumoniae infection and its association with NLRP3 inflammasome in retinas from Alzheimer’s disease patients

Authors: *B. GAIRE[1], Y. KORONYO[1], A. RENTSENDORJ[1], D.-T. FUCHS[1], L. SUBEDI[2,3], K. BLACK[1], M. ARDITI[2,3], T. CROTHER[2,3], M. KORONYO-HAMAOUI[1,4,5]; [1]Neurosurg., [2]Pediatrics, Cedars-Sinai Med. Ctr., Los Angeles, CA; [3]Biomed. Sciences, Infectious and Immunologic Dis. Res. Ctr., [4]Biomed. Sciences, Div. of Applied Cell Biol. and Physiol., [5]Neurol., Cedars-Sinai Med. Cneter, Los Angeles, CA

Abstract: Background: Recent advances in Alzheimer’s disease (AD) research revealed that the pathological processes associated with AD also occur in the neurosensory retina. Data have further suggested parallels between retinal and brain AD biomarkers. While the etiology of AD pathogenesis is not fully understood, an emerging theory has linked AD with microbial infection as a plausible cause for AD progression. Indeed, the presence of several microbes including *Chlamydia pneumoniae* (Cp) have been reported in the post-mortem brain of AD patients and murine models. However, the existence and role of microbial infection in AD retina has never been studied. Here, we aimed to explore the presence and distribution of Cp infection and associated inflammasome activation in the AD retina.**Method**: We employed a range of histological and biochemical techniques to detect the Cp and other associated biomarkers in postmortem retina and the brain of patients with mild cognitive impairment (MCI) or AD dementia, compared to retinas from age- and sex-matched individuals with normal cognition (NC). We assessed the presence of Cp and its potential relationship with gliosis, inflammasome activation, and pyroptosis/apoptosis.**Result**: We identified the presence of Cp inclusions in postmortem retinas of MCI and AD patients. Similar Cp inclusions were also detected in paired

brain tissues. We further correlated the extent of retinal Cp infection and brain disease severity and cognitive status. Our cross-sectional evidence revealed Cp-infected retinal cells in AD patients, with 3-fold increase in Cp immunoreactivity in retinas of AD patients versus MCI and NC subjects, possibly indicating that Cp infection mainly occur during late stages of AD progression. Retinal microglia were found to engulf Cp-infected cells. Alongside Cp infection, retinal tissues from MCI and AD patients exhibited increased NLRP3 inflammasome, gliosis, pyroptosis, and apoptosis markers. Our data suggest that Cp triggered an inflammasome activation, a detrimental process that can lead to neuronal degeneration, but that retinal inflammasome activation occurred earlier than Cp infection possibly by other triggers. We further found that retinal Cp burden was correlated with diverse AD parameters such as brain neurofibrillary tangles, Braak stage, and neuropil threads but not brain amyloidosis. **Conclusion**: Cp-triggered chronic inflammation could be a detrimental process leading to retinal degeneration in AD, suggesting potential new therapeutic avenues involving early antibiotic treatment or inflammasome attenuation.
Keywords: Alzheimer's disease, *Chlamydia pneumoniae*, retina, inflammasome, gliosis

Disclosures: B. Gaire: None. **Y. Koronyo:** None. **A. Rentsendorj:** None. **D. Fuchs:** None. **L. Subedi:** None. **K. Black:** None. **M. Arditi:** None. **T. Crother:** None. **M. Koronyo-Hamaoui:** None.

Poster

PSTR324. Alzheimer's Disease: Neuroinflammation and Immune Actions: In Vivo Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR324.23/E12

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

Support: R01AG056478
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The Haim Saban
The Tom Gordon Private Foundations

Title: Abnormal pTau accumulation within RGCs is linked with early ganglion cell loss in postmortem retinas of MCI and AD patients

Authors: *M. DAVIS[1], N. MIRZAEI[1], H. SHI[1], J. SHEYN[1], D.-T. FUCHS[1], A. RENTSENDORJ[1], C. A. MILLER[2], K. L. BLACK[1], Y. KORONYO[1], M. KORONYO[3]; [1]Neurosurg., Cedars-Sinai, Los Angeles, CA; [2]Keck Sch. of Med., USC, Los Angeles, CA; [3]Neurosurg., Cedars Sinai Med. Ctr., Los Angeles, CA

Abstract: Background: We previously identified the pathological hallmarks of Alzheimer's Disease (AD), amyloid β-protein (Aβ) plaques and neurofibrillary tangles comprised of hyperphosphorylated tau (pTau) protein, in the retina of AD patients. Retinal Aβ deposits were detected in subjects with mild cognitive impairment (MCI) and AD, with variable distribution

across cell layers and subregions. Loss of retinal ganglion cells (RGCs) was also documented in these patients. While increased pS396Tau was reported in the postmortem retina of AD patients, the topographical distribution of retinal pTau in AD and potential association with cell-type vulnerability has never been defined. **Method:** To quantify retinal pS396Tau in different subregions and cell layers and in RGCs that express the ribonucleic acid binding protein with multiple splicing (RBPMS) marker, we used quantitative immunohistochemical analyses. Manual count and immunoreactive-area of pTau-containing RGCs and RBPMS-positive RGCs were also assessed in postmortem retinae of subjects with normal cognition (NC), MCI (due to AD), or AD dementia. **Result:** We find that RBPMS cell loss was accompanied by 2-3-fold pS396Tau increases in prodromal and clinical AD retinae, especially in the superior-temporal mid-periphery region. While pS396Tau is densely observed in the inner plexiform layer (IPL) and outer plexiform layer (OPL), colocalization of pTau inside RBPMS-RGCs is also pronounced. Additionally, we find that ganglion cell layer (GCL) loss is tightly linked with pS396Tau accumulation within RGCs ($r = -0.88$, $p<0.0001$), with a substantial 44% decrease in RBPMS-expressing RGCs in retinas from both MCI and AD patients. We further defined the relationships between retinal pS396 and brain parameters, while acknowledging variability in pTau distribution amongst the patients. Our data suggest that elevated pS396Tau in remaining RBPMS-RGCs drives degeneration and displacement to deeper retinal layers in disease states. **Conclusion:** The patterns of abnormal pTau distribution in the human retina are described with increased vulnerability of RBPMS-expressing RGCs to pS396Tau accumulation. Future studies should investigate the susceptibility of other retinal cell types to tauopathy in early stages of AD.

Disclosures: M. Davis: None. **N. Mirzaei:** None. **H. Shi:** None. **J. Sheyn:** None. **D. Fuchs:** None. **A. Rentsendorj:** None. **C.A. Miller:** None. **K.L. Black:** None. **Y. Koronyo:** None. **M. Koronyo:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.01/E13

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

Support: Translational Imaging Center, USC

Title: Designing a Transgenic Zebrafish Model to Study Microglia in Alzheimer’s Disease

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Abstract: Alzheimer’s Disease (AD), the most common form of dementia, is a progressiveneurodegenerative disease most often characterized by initial memory impairment and cognitivedecline that can ultimately affect behavior, speech, visuospatial orientation and the motor system.The most common pathological characteristics of AD are the abnormal

accumulation of amyloidplaques and neurofibrillary tangles. The amyloid cascade hypothesis postulates that AD pathology is caused by the deposition of amyloid β. Microglia, the resident macrophages in thecentral nervous system, are found in the vicinity of amyloid deposits. The roles of microglia inAD development and progression are unclear as they have been reported to be both detrimentaland protective to the progression of AD. Much is still unknown about the effects of microglialinflammatory response and their effect on amyloid beta clearance;understanding the role ofmicroglia in a disease state as well as microglia stress and dysfunction allows for the design ofrelevant therapeutics for AD patients. To better understand the role of microglia in AD, we havedeveloped a zebrafish (*Danio Rerio)* AD model to study the interactions of microglia withamyloid plaques using fluorescence microscopy. We established a transgenic zebrafish line thatexpresses fluorescently tagged human amyloid protein in neurons and blue fluorescent protein inmicroglia. This transgenic model allows for the use of time-lapse microscopy to directlyvisualize microglia and amyloid plaque interactions and their contribution to neuronal health.Longitudinal studies combined with genetic perturbations enable the testing of specific pathwaysin disease progression.

Disclosures: **S. Bawarith:** None. **S. Fraser:** None. **L. Trinh:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.02/E14

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

Support: the National Key R&D Program of China(2021YFE0203000)
the Research Grants Council of Hong Kong (the Collaborative Research Fund [C6027-19GF] and the Theme-Based Research Scheme [T13-605/18W])
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Title: Il-33 stimulates microglial functional state transition in alzheimer’s disease through transcriptional regulation

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Kong Univ. of Sci. and Technol., Hong Kong SAR, China; [2]Hong Kong Ctr. for Neurodegenerative Diseases, InnoHK, Hong Kong SAR, China; [3]Guangdong Provincial Key Lab. of Brain Sci., Dis. and Drug Develop., Shenzhen–Hong Kong Inst. of Brain Science, HKUST Shenzhen Res. Inst., Shenzhen, Guangdong, China; [4]Dept. of Neurology, Hope Ctr. for Neurolog. Disorders, Knight ADRC, Washington Univ. Sch. of Med., St. Louis, MO

Abstract: Microglia surveil and maintain homeostasis in the brain by responding to and clearing damage-associated cues. In pathological conditions like Alzheimer's disease (AD), the chemotactic and phagocytic capabilities of microglia are impaired, leading to insufficient microglial clearance, resulting in accumulation of damage-associated molecular patterns. Previously, we showed that interleukin 33 (IL-33) treatment ameliorates AD pathology by enhancing chemotactic and phagocytic responses in microglia in the APP/PS1 AD transgenic mouse model. However, the underlying molecular and transcriptional regulatory mechanisms remain largely unclear. Here, we examined the transcriptomic profile of microglia in IL-33-treated APP/PS1 mice and found that the microglia undergo a functional state transition in a stepwise manner: from a homeostatic to chemotactic, then phagocytic state. This results in a beneficial functional outcome that enables the microglia to migrate towards amyloid deposition and increase the clearance of Aβ. Importantly, by comparing the gene expression levels of microglial surface proteins, we found that a surface protein expressed in migrating microglia, VCAM1, plays an important role in IL-33-induced microglial chemotaxis towards Aβ plaques. Thus, understanding the transcriptional regulatory mechanism underlying the microglial functional state transition is important for understanding the roles of microglia in AD pathogenesis.

Disclosures: W. Wu: None. **S. Lau:** None. **H. Wong:** None. **L. Ouyang:** None. **Y. Qiao:** None. **J.H.Y. Lau:** None. **J. Xu:** None. **C. Wong:** None. **Y. Jiang:** None. **D.M. Holtzman:** None. **A. Fu:** None. **N. Ip:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.03/E15

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

Support: CSIR Govt of India

Title: Icam-1 potentially improves amyloid b mediated memory and cognitive impairment by targeting microglial erk mediated inflammation

Authors: *S. GOSWAMI, SRF[1], S. C. BISWAS[2];
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Abstract: Alzheimer’s Disease (AD), the most prevalent form of neurodegeneration currently affects almost 70% of total dementia patients around the world. Although Amyloid-β (Aβ) plaque deposition marks the initiation of AD, glial neuroinflammation plays major role in neuronal loss, synaptic degeneration and ultimately cognition and memory impairment. During disease development, microglia, the major representative of brain innate immune system, fails to remove deposited Aβ plaque and gets reactivated to secrete different inflammatory proteins that modifies disease condition and severity. However, molecular targets to manipulate the transformation towards the enrichment of anti-inflammatory, actively phagocytic microglial subtype to reduce inflammation are yet to be uncovered. Here, we show that Soluble Intra Cellular Adhesion Molecule (sICAM-1) secreted from glial cells in response to Aβ, and its interaction with its microglia specific receptor Lymphocyte Function Associated Antigen 1 (LFA-1) can ameliorate pathogenesis of AD. When we incorporated ICAM-1 intraperitoneally in 5xFAD transgenic mice model of AD, it restores cognition and memory as observed by a battery of behavioral experiments, refurbishes pre and post synaptic protein expressions, reduces Aβ plaque load by increasing microglial phagocytosis and subsequent gliosis . The beneficial effects of ICAM-1 are found to be partially lost when the interaction is blocked by intranasal delivery of commercially available inhibitors such as Lifitegrast. Moreover, we could observe reduction of an early surge of pro-inflammatory protein production in primary microglia cells in response to $A\beta_{1\text{-}42}$ which correlated with their reactivated morphology, when we externally incorporated primary glial cells with rat recombinant ICAM-1. It also inhibited the $A\beta_{1\text{-}42}$ mediated microglial reactivation and reverted microglia towards anti-inflammatory isotype as observed by reduced production of pro-inflammatory proteins as well as increased AB phagocytosis. ICAM-1 is also found to inhibit the phosphorylation of ERK MAPK, the transcription factor lying upstream of these pro-inflammatory proteins. Thus, targeting ICAM-1 or its interaction with receptor LFA1 could be a good therapeutic strategy to reduce neuroinflammation and subsequent pathogenesis in AD.

Disclosures: **S. Goswami:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.04/E16

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

Support: NIH GRANT RO1AG033007
NIH GRANT R01AG073182

Title: Bri2-mediated regulation of trem2 processing in microglia and its potential implications for Alzheimer's disease and related dementias

Authors: *T. YIN, L. D'ADAMIO;
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Abstract: *ITM2B/BRI2* mutations cause familial forms of Alzheimer's disease (AD)-related dementias by disrupting BRI2's protein function and leading to the accumulation of amyloidogenic peptides. Although typically studied in neurons, our findings show that BRI2 is highly expressed in microglia, which are crucial in AD pathogenesis due to the association of variants in the microglial gene TREM2 with increased AD risk. Our single-cell RNAseq (scRNAseq) analysis revealed a microglia cluster that depends on a Trem2 activity that is inhibited by Bri2, pointing to a functional interaction between *Itm2b/Bri2* and *Trem2*. Given that the AD-related Amyloid-β Precursor protein (APP) and TREM2 undergo similar proteolytic processing, and that BRI2 inhibits APP processing, we hypothesized that BRI2 may also regulates TREM2 processing. We found that BRI2 interacts with Trem2 and inhibits its processing by α-secretase in transfected cells. In mice lacking Bri2 expression, we observed increased central nervous system (CNS) levels of Trem2-CTF and sTrem2, which are the products of α-secretase processing of Trem2, indicating increased Trem2 processing by α-secretase *in vivo*. Reducing Bri2 expression only in microglia resulted in increased sTrem2 levels, suggesting a cell-autonomous effect of Bri2 on α-secretase processing of Trem2. Our study reveals a previously unknow role of BRI2 in regulating TREM2-related neurodegenerative mechanisms. The ability of BRI2 to regulate the processing of both APP and TREM2, combined with its cell-autonomous role in neurons and microglia, makes it a promising candidate for the development of AD and AD-related dementias therapeutics

Disclosures: T. Yin: None. **L. D'Adamio:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.05/E17

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

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R35NS097273
RF1AG062171
P01NS084974-01

Title: Microglial REV-ERBα regulates inflammation and lipid droplet formation to drive tauopathy

Authors: *J. LEE[1], J. M. DIMITRY[1], J. SONG[2], M. SON[2], P. W. SHEEHAN[3], M. KING[4], T. TABOR[1], Y. GOO[2], M. A. LAZAR[5], L. PETRUCELLI[6], E. MUSIEK[1];
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Abstract: Alzheimer's disease is associated with disrupted circadian rhythms and clock gene expression. REV-ERBα (*Nr1d1*) is a circadian transcriptional repressor involved in the regulation of lipid metabolism and macrophage function. While global REV-ERBα deletion increases microglial activation and mitigates amyloid plaque formation, the cell-autonomous effects of microglial REV-ERBα on tau pathology are unexplored. Here, we show that microglial REV-ERBα deletion enhances inflammatory signaling, disrupts lipid metabolic processes, and causes lipid droplet (LD) accumulation specifically in male microglia. Inflammation and LD accumulation combine to inhibit microglial tau phagocytosis, which can be partially rescued by blockage of lipid droplet formation. Microglial REV-ERBα deletion exacerbates tau aggregation and neuroinflammation in P301S and AAV-P301L tauopathy models in male, but not female mice. Moreover, our lipidomic analysis suggests sphingomyelin is dominantly upregulated in microglia by treatment of tau brain extract while sphingomyelin metabolic process is disrupted in REV-ERBα deleted microglia. Our studies demonstrate the importance of microglial lipid droplets in tau accumulation potentially through sphingomyelin and reveal REV-ERBα as a therapeutically accessible, sex-dependent regulator of microglial inflammatory signaling, lipid metabolism, and tauopathy.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.06/E18

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

Title: Age-related pathology and microglial activation in felid brains

Authors: *V. KULIK[1], M. K. EDLER[2], M. RAGHANTI[2], A. IMAM[1,3], C. C. SHERWOOD[1];
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Abstract: Alzheimer's disease (AD) and associated pathology has been primarily identified in humans, who have relatively large brains and long lifespans. To expand what is known about aging and neurodegeneration across mammalian species, we characterized amyloid-beta (Aβ) and tau pathologies in aged felids (n=6) of five different species (cheetah, clouded leopard, African lion, serval, Siberian tiger), most of which have not previously been studied. All individuals died in the last quarter of their species' estimated maximum lifespan. We characterized Aβ and tau pathologies using immunohistochemistry for Aβ40, Aβ42, and AT8 in the gyrus sylvius and the CA1 and CA3 of the hippocampus. We also quantified Iba1-immunoreactive microglial densities and morphological types. Diffuse Aβ42 plaques, but not dense-core plaques, were present in the cerebral cortex and tended to be more common than Aβ40 plaques across species. Conversely, vascular Aβ was labeled more consistently with Aβ40 than Aβ42 for each species on average. Although all individuals showed some degree of Aβ40 and/or Aβ42 immunoreactivity (primarily in the cerebral cortex), only the cheetah and two clouded leopards had tau pathology, which was observed exclusively in the form of pretangles in the hippocampus. We also found that activated, intermediate microglia were significantly associated with pretangle load in the hippocampus of the three individuals that possessed tau pathology. This study demonstrates the co-occurrence of Aβ and tau pathology in two felid species, cheetahs and clouded leopards. Overall, these results provide an initial view of the manifestation of age-related brain pathology in these large-brained felids, which can be compared with neurodegeneration across different taxa, including domestic cats, nonhuman primates, and humans.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.07/E19

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

Title: Characterizing the Transcriptomic Landscape of Microglial Cell States in Alzheimer's Disease

Authors: S. BABU[1,2], ***H. ZHAO**[1,2,3], A. KUNISKY[1,2], K. GHAFARI[1,2], H. MATHYS[1,2]; [1]Univ. of Pittsburgh, Dept. of Neurobio., Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA; [3]Tsinghua Univ., Beijing, China

Abstract: Alzheimer's disease (AD) is a neurodegenerative disorder characterized by cognitive decline and complex pathology. Microglia, which are brain-resident macrophages, play a role in brain development, CNS homeostasis, and neurological disorders. Autopsies have shown the presence of microglia around Aβ plaques, responding to challenges. Microglia help prevent the accumulation of Aβ and clear damaged neurons. However, excessive microglia activity can

worsen AD by activating complement, leading to plaque formation, tangles, neurites, and synaptic loss. Transcriptomic studies have revealed different microglial states in AD, but more research is required. In this study, we analyzed over 80,000 prefrontal cortical immune cells from more than 400 aged post-mortem brain samples exhibiting varying levels of pathologies related to AD. Integration of immune cells revealed five major subclasses of cells, including microglia, macrophages, monocytes, and T and B Cells with microglia as the most abundant cell type comprising more than 94% of cells. Sub-clustering of the microglial cells revealed more than eight distinct transcriptomic states, including homeostatic, phagocytic, inflammatory, neuronal surveillance, antiviral, and stressed states. Using a pseudobulk-based differential gene expression analysis approach, we identified genes that were differentially expressed in high-resolution cell states across various measures of AD pathology including global AD pathology, neurofibrillary tangle burden, tangle density, overall amyloid level, neuritic plaque burden, diffuse plaque burden. We also explored the associated biological and functional ontologies, revealing that genes and gene modules related to protein phosphorylation, cell activation, innate immune response, stress response, and the TYROBP causal network are altered in AD. Additionally, using cell-type compositional analysis, we identified specific microglia cell states that are associated with multiple AD pathological variables. Furthermore, we conducted a systematic analysis of sex differences across the high-resolution cell types revealing a pronounced immune response in females. We also observed changes in the relative abundance of cell types between sex groups and their association with multiple AD pathologies. In summary, our analysis of a transcriptomic atlas encompassing immune cells from over 400 postmortem human brain samples has yielded insights into detailed cell states, pathology-associated types, and genes/pathways affected in AD. We also identified altered protein complexes and studied sexual dimorphism in the aging brain.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.08/E20

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

Support: National Health Research Institutes (NHRI-NP-PP02-014), Taiwan
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Title: Np106 promotes microglia-mediated aβ phagocytosis in an alzheimer's disease model through upregulation of atp-binding cassete transportr a7

Authors: *Y.-L. GAN[1], H.-J. TSAY[3], Y.-T. HSU[1], J.-T. HSUEH[1], J.-A. HSU[2], F.-S. SHIE[1];
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Abstract: Alzheimer's disease (AD) is a neurodegenerative disease clinically characterized by cognitive decline. The accumulation of amyloid-beta (Aβ) plaques in the brain is a hallmark of AD and contributes to disease progression. Enhancing the clearance of Aβ has emerged as a potential therapeutic approach for AD. ATP-binding cassette transporter A7 (ABCA7), a lipid transporter, plays an important role in microglial Aβ phagocytosis and has been identified as a risk gene for AD through genome-wide association studies. In this study, we investigated the effects of NP106, an anti-Aβ antibody developed in our lab, on microglial ABCA7-mediated phagocytosis and its impact on Aβ plaque in an APP/PS1 mouse model of AD. We found that NP106 administration promoted the clustering of microglia around Aβ plaques and significantly reduced cerebral Aβ levels. These plaque-associated microglia exhibited increased expression of ABCA7, which was co-localized with intracellular Aβ deposits, and displayed enhanced phagocytic activity with ramified morphology and reduced lipid droplets, indicating microglial rejuvenation. In vitro experiments confirmed that NP106 enhanced microglial Aβ phagocytosis involving up-regulation of ABCA7 and sterol-responsive/regulatory element binding protein 2 (SREBP-2). Furthermore, NP106-induced degradation of intracellular Aβ was associated with increased lysosomal activity and was not affected by ABCA7 suppression. Interestingly, NP106 and ABCA7 suppression both attenuated the gene expression of pro-inflammatory cytokines in primary microglia. In conclusion, our findings demonstrate that NP106 treatment rejuvenates microglial function, enhances Aβ clearance in an AD mouse model, and implicates the involvement of the SREBP-2/ABCA7 pathway. Our findings also suggest that the immunomodulatory effects of microglial ABCA7 may involve multiple mechanisms.

Disclosures: Y. Gan: None. **H. Tsay:** None. **Y. Hsu:** None. **J. Hsueh:** None. **J. Hsu:** None. **F. Shie:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.09/E21

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

Support: NIMH Grant R43MH122070

Title: Brain-Wide Cellular-Resolution Quantification of Microglia and Amyloid in AD Model Mice

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California Irvine, Ctr. for the Neurobio. of Learning and Memory, Irvine, CA; [3]Neurosurg., Stanford Univ., Stanford, CA; [4]Activity Signaling LLC, San Diego, CA

Abstract: Traditional histological methods have been a mainstay of neuroscience research dating back more than 100 years. Yet, despite great advances in tissue labeling and imaging technology, until very recently imaging more than a few hundred microns into a tissue has required slicing and mounting on slides. When looking for read-outs of genetic or pharmacological manipulations that affect the entire brain, this traditional focused approach is lacking, forcing researchers to limit observations to brain regions of interest. Providing access to the intricate anatomy of the whole intact brain, tissue clearing offers neuroscientists unbiased and complete views of brain anatomy and function. One area where these methods have particular utility is in the development of CNS therapeutics where they can be used to examine the regional distribution of therapeutics in the brain as well as brain-wide target engagement and phenotypic efficacy. Toward this end, we have focused on Alzheimer's Disease models developing clearing, imaging and quantification methods for brain-wide regional quantification of amyloid plaques and microglia in 5xFAD mice. Using our optimized iDISCO-based tissue clearing method and our Mesoscale Imaging System for ZEISS Lightsheet microscopes, we can image micron-scale resolution immunoreactivity across entire intact mouse brains in <20 min. Further, our machine learning-enabled Voxels software identifies individual immunostained cells and objects throughout the brain and aligns them to the Allen Reference Atlas to produce an unbiased, regionalized read-out of cellular patterns across 100's brain areas. Using antibodies targeting the microglial protein Iba1, we can label microglia across the entire brain. We have developed multiple machine learning-enabled workflows to count microglia, provide metrics of shape and measure microglial activation. We have also validated methods for labeling amyloid plaques throughout the brain and have used machine learning to differentially quantify immunoreactivity in plaques vs neuronal cell bodies in the same fluorescence channel. In 5xFAD Tg mice, rather than displaying the ramified morphology seen in WT mice, microglia become condensed and colocalize with β-amyloid plaques. Our automated methods can identify and count plaques and plaque-associated microglia (PAMs) throughout the brain. These new methods for whole-brain, next generation 3D immunohistochemistry are ideally suited to pre-clinical studies for unbiased, complete and anatomically precise mapping of the efficacy of CNS therapeutics affecting amyloid deposition and neuroinflammation.

Disclosures: **N. Guanzon:** A. Employment/Salary (full or part-time):; Translucence Biosystems Inc.. **A. Murry:** None. **E. May:** A. Employment/Salary (full or part-time):; Translucence Biosystems Inc.. **A. Wells:** None. **Y. Gallegos:** A. Employment/Salary (full or part-time):; Translucence Biosystems Inc. **C. Redd:** A. Employment/Salary (full or part-time):; Translucence Biosystems Inc. **R. Azevedo:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems Inc. F. Consulting Fees (e.g., advisory boards); Translucence Biosystems Inc. **M. Shamloo:** None. **S. Gandhi:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems Inc. F. Consulting Fees (e.g., advisory boards); Translucence Biosystems Inc. **D. Wheeler:** A. Employment/Salary (full or part-time):; Translucence Biosystems Inc. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Translucence Biosystems Inc.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.10/E22

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

Support: MODEL-AD U54AG054345
TREAT-AD U54AG065181

Title: Haplodeficiency of Inpp5d : A Modulator of Tau Pathology

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Abstract: Introduction Alzheimer's disease (AD) is characterized by the extracellular deposition of amyloid-β and the formation of intracellular neurofibrillary tangles consisting of hyperphosphorylated Tau (pTau).The intronic variant of the inositol polyphosphate-5-phosphatase-D (*INPP5D*) gene (rs35349669) has been identified as a risk factor associated with AD. INPP5D is known for its role in regulating the innate immune system within the central nervous system. In the context of AD, increased expression of the INPP5D is believed to inhibit microglial activation. Previous studies have shown a positive correlation between elevated *INPP5D* expression and amyloid burden in AD. However, the relationship between *INPP5D* and tau pathology remains unclear. **Methods** We performed fluorescence-resonance energy transfer (FRET)-based tau seeding assay on human LOAD brain samples. Furthermore, we crossed *Inpp5d*-deficient (*Inpp5d*$^{+/-}$) mice with tau mice (PS19) to assess how *Inpp5d* modulates tau pathology. **Results** We have observed a positive correlation between increased tau-seeding and the expression of *INPP5D* in subjects with late-onset Alzheimer's disease (LOAD). Additionally, in PS19 mice, there is a positive correlation between increased INPP5D expression and pTau levels at phosphorylation sites (S202/T205). Remarkably, when we reduced *Inpp5d* gene expression, we observed a concurrent decrease in pTau levels at phosphorylation sites S202/T205 and Thr231, along with a notable improvement in behavioral deficits in PS19 mice. Furthermore, our findings indicate an up-regulation of genes associated with immune-response and cell-migration in PS19.*Inpp5d*+/- mice. **Conclusion** These data demonstrate that elevated *INPP5D* expression in human LOAD subjects is recapitulated in PS19 mice, and positively correlated with tau pathology. Importantly, reduced expression of *Inpp5d* recovered behavior deficits and reduced tau pathology. Alterations in immune- and cell migration-related genes in PS19.*Inpp5d*$^{+/-}$ mice suggest that *Inpp5d* modulates the disease progression through these pathways. These discoveries shed light on the intricate relationship between *Inpp5d* expression, tau pathology, and behavioral outcomes, providing valuable insights into the underlying mechanisms of Alzheimer's disease.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.11/E23

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

Support: JSPS KAKENHI 19H01015
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JSPS KAKENHI 22K07344
AMED 18dm0207014
AMED 22dm0207073
JST JPMJMS2024

Title: Elucidating the Pathological Roles of INPP5D in Alzheimer Disease

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Abstract: Deposition and aggregation of amyloid β (Aβ) peptides in the brain are critical for the pathogenesis of Alzheimer disease (AD). In addition, recent studies have suggested the importance of genetic risk factors for AD, which are likely to modulate the amyloid cascade. Among the most important are genetic variants of *Triggering receptor expressed on myeloid cells 2* (*TREM2*), which encodes a receptor specifically expressed in microglia. Studies have demonstrated its critical role in microglial clustering around amyloid plaques, which prevents Aβ toxicity and thus restricts tau deposition around plaques. This suggests that microglia prevent AD pathogenesis by limiting Aβ-mediated exacerbation of tau pathology; however, the underlying molecular mechanism remains unclear. Here, we investigated the pathological roles of another AD risk gene, *Inositol polyphosphate-5-polyphosphatase D* (*INPP5D*), a negative regulator of phosphoinositide PI(3,4,5)P_3 signaling. Although INPP5D has been reported to play an inhibitory role in TREM2 signaling in peripheral tissues, their interaction and pathological roles in AD remain elusive. Therefore, we investigated the effects of *Inpp5d* haplodeficiency in an AD mouse model ($App^{NL\text{-}G\text{-}F}$, hereafter *NLGF* mice) as well as in a TREM2 loss-of-function AD model (Tyrobp-deficient *NLGF* mice) to ask their genetic interaction in vivo. *Inpp5d*-haplodeficient *NLGF* mice showed no effect on Aβ burden or plaque-associated changes in

microglia, astrocytes, or neurites. However, in *Tyrobp*-deficient *NLGF* mice, *Inpp5d* haplodeficiency restored microglial clustering around Aβ plaques and ameliorated TREM2 loss-of-function phenotypes such as plaque shape, astrogliosis, and phosphorylated tau deposition around plaques. Mechanistic analyses revealed that INPP5D negatively regulates cell adhesion and phagocytosis activity of Aβ fibrils in microglia. TREM2/TYROBP and INPP5D exert opposing effects on the levels of phosphorylated AKT and proteins involved in actin assembly. Our results suggest that INPP5D acts downstream of TREM2/TYROBP to regulate the microglial protective function against Aβ toxicity, thereby affecting Aβ-dependent tau deposition.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.12/E24

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

Support: F31AG063398
R01AG15819
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U01AG46152
U01AG61356
U01AG072572
R01AG063398
RF1AG057473

Title: Using iPSC-derived microglia to investigate the role of INPP5D/SHIP1 in Alzheimer’s disease

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Abstract: Genome-wide association studies have identified numerous genes linked to Alzheimer’s disease (AD), several of which are highly expressed in microglia. We investigated the role of one of these genes, INPP5D, using iPSC-derived microglia (iMGLs) and found that it plays an important role in inflammasome regulation. Transcriptomic and proteomic analyses of iMGLs treated with a selective SHIP1 inhibitor revealed changes in lysosomal protein expression and immune signaling (among other changes). To further investigate these results, we utilized iMGLs with loss-of-function (LOF) mutations in one or both INPP5D copies. INPP5D

overexpression also was induced in iMGLs using lentivirus. Proteomic and transcriptomic analyses indicated that a lower level of SHIP1 activity is associated with reduced autophagic flux and increased inflammasome activation. Moreover, our INPP5D biallelic LOF model was found to more closely resemble AD brain microglia expression profiles than the overexpression model. These findings point to INPP5D as playing an important role in inflammasome regulation in microglia, which can aid our understanding of how SHIP1 and microglia contribute to AD pathology.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.13/E25

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

Support: NIH Grant RF1AG069425
NIH Grant RF1AG069425-S1

Title: Human induced pluripotent stem cell model of Alzheimer's Disease-associated fractalkine receptor polymorphism demonstrates microglial dysfunction

Authors: *K. TUTROW[1], J. HARKIN[2], M. HERNANDEZ[3], K.-C. HUANG[5], S. PUNTAMBEKAR[3], B. T. LAMB[1], J. MEYER[4];
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Abstract: Dysfunctional microglial activity has recently been identified as a potential mechanism leading to accumulation of amyloid beta and pTau and subsequent neurodegeneration in Alzheimer’s Disease. The CX3CR1/fractalkine axis serves as a mechanism for bi-directional communication between microglia and neurons, respectively, to promote a resting, anti-inflammatory state in microglia. Previous studies have demonstrated that deficiency in CX3CR1 signaling leads microglia to a more pro-inflammatory phenotype and phagocytosis deficits and increases susceptibility of neurons to cell death. Additionally, the CX3CR1-V249I polymorphism was recently identified as a potential risk allele for Alzheimer’s Disease with worsened Braak staging in post-mortem Alzheimer’s patients. However, the role of fractalkine dysfunction in human cells and the mechanisms by which microglia with the CX3CR1-V249I SNP contribute to neurodegeneration remain unclear. Thus, to address this shortcoming, we utilized human induced pluripotent stem cells and CRISPR/Cas9 technology to elucidate the effects of the V249I polymorphism on microglia-like cells compared to an isogenic control cell

line. We demonstrate effective differentiation from both isogenic control and CX3CR1-V249I backgrounds into human microglia-like cells, which express characteristic microglial markers and are functionally phagocytic. Microglia bearing the homozygous V249I allele demonstrated decreased phagocytosis of amyloid beta in vitro compared to isogenic controls. These findings suggest that the CX3CR1-V249I polymorphism may cause a dysfunctional microglia phenotype that is further associated with a pro-inflammatory, reactive microglial state, subsequently contributing to neuronal dysfunction and death. Ongoing work will expand upon the transcriptome and secretome profile of CX3CR1-V249I microglia and elucidate how this gene variant contributes to Alzheimer's Disease-related neurodegeneration.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.14/E26

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

Title: Human-derived CD33 antibody ATLX-1088, a potent stimulator of microglial pharmacology

Authors: H. GRAVES[1], G. NOSOVITSKAYA[1], C. ZIMARINO[1], H. CAI[1], D. FINCH[1], I. PEDIADITAKIS[2], L. MITCHELL[1], A. TOBOSO-NAVASA[1], P. KOLASINSKA-ZWIERZ[1], U. GAWDA[1], J. GALSON[1], S. KESWANI[1], P. VARLEY[1], R. MINTER[1], J. OSBOURN[1], ***M. M. SIDOR**[2];
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Abstract: At Alchemab, we are harnessing the power of the immune system to find novel therapies for complex diseases. Through target-agnostic screening of immune repertoires from resilient individuals, namely people with dementia risk factors (elevated beta-amyloid, APOE4 risk allele) but no cognitive decline or Tau elevation, we have identified antibodies associated with resilience. Target deconvolution highlighted CD33 as an antigen of interest. Further repertoire mining identified a larger panel of CD33 antibodies which were characterized for their target specificity, CD33 binding affinity, cell surface CD33 binding, CD33-mediated internalization, and functional effects in iPSC-derived microglia. This screening cascade identified a human-derived lead antibody ATLX-1088, which was found to be a strong inducer of phagocytosis of beta-amyloid by microglia. In comparison to other CD33 and TREM2 antibodies, ATLX-1088 resulted in the greatest increase in phagocytosis in iPSC microglia. Interestingly, ATLX-1088 also demonstrated a greatly reduced level of CD33 depletion from the cell surface of human monocytes, which was strikingly different from other known antibodies to CD33. Additional data has been generated to characterize ATLX-1088, including pharmacokinetics, microglial transcriptomic responses, and *in vivo* target engagement. From a

development perspective, this antibody showed high levels of monomer, good thermostability, and minimal loss of binding under forced degradation conditions. Overall, these data suggest that ATLX-1088 has high potential as a therapeutic candidate for the reversal of microglial dysfunction in Alzheimer's Disease and potentially other neurodegenerative conditions.

Disclosures: H. Graves: A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **G. Nosovitskaya:** A. Employment/Salary (full or part-time):; Full Time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **C. Zimarino:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **H. Cai:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **D. Finch:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **I. Pediaditakis:** A. Employment/Salary (full or part-time):; Full Time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **L. Mitchell:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **A. Toboso-Navasa:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **P. Kolasinska-Zwierz:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **U. Gawda:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **J. Galson:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **S. Keswani:** A. Employment/Salary (full or part-time):; Part-time, Alchemab Therapeutics. **P. Varley:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **R. Minter:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **J. Osbourn:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics. **M.M. Sidor:** A. Employment/Salary (full or part-time):; Full time, Alchemab Therapeutics. E. Ownership

Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Alchemab Therapeutics.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.15/Web Only

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

Support: National Natural Science Foundation of China (#81760228, PI)
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Academician Working Station of Yunnan Province(#202305AF150139)

Title: Establishinga new model of Alzheimer’s disease to quickly test effect of IL-1β on microglia in cortex

Authors: *L. GUO[1,3], B. DONG[1], K. HE[4], Y. SHUANG[5], C. SONG[6], Y. WU[8], Q. DU[7], G. XIA[2];
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Abstract: Establishing a new model of Alzheimer’s disease to quickly test effect of IL-1β on microglia in cortex Authors * Ling Guo, Baolin Dong, Kexin He, Yingbo Shuang, Chao Song, Yuanshuang Wu, Qiong Du, Guilan Xia **Disclosures** Ling Guo, Baolin Dong, Kexin He, Yingbo Shuang, Chao Song, Yuanshuang Wu, Qiong Du, Guilan Xia : None. **Abstract Abstract Background:** Pro-inflammatory markers including Interleukin-1 beta (IL-1β), Interleukin-6 (IL-6) and other biological factors are significantly higher in elderly with Alzheimer's disease (AD). AD is assumed to be associated with different biological/genetic vulnerability. Based on the literatures of PubMed, there are no records to be found if and how cytokines like IL-1β or IL-6 can affect the microglia in the brain of TgAPP/PS1 mice through i.p. injection. The present study tried to preliminarilly establish a model to evaluate the role of IL-1β or IL-6 to AD mice or the elders. We challenged the primary microglia of rats or TgAPP/PS1 mice by using IL-1β or IL-6

in vitro or in vivo. **Method:** TgAPP/PS1 or C57BL/6 (wild type, WT) mice were treated with IL-1β or IL-6 respectively by intraperitoneal injection (i.p.). The microglial cultures were seeded into culture dishes at 2.5×10^5 cells/ml, and were treated by 10 ng/ml of IL-1β or IL-6 for 6, 9, 12, 24 or 36h, and then harvested the supernatant and the cells for the measurements including viability, nitric oxide (NO), iNOS or COX-2 proteins or mRNA, which were done via Western Blot, Griess assay or immunofluorescence staining/Confocal microscopy or RT-PCR. **Results:** The cellular viability of the microglia induced by 2 to 200 ng/ml of IL-1β showed significantly downregulation under the concentration 100 or 200 ng/ml of IL-1β. NO, iNOS and COX-2 were up-regulated in a concentration- dependent and in a time-dependent manners. Also, iNOS or COX-2 mRNA of the cells were upregulated after using IL-1β for 3h or 4 h. They were downregulated by treating with MAPK inhibitors included SB203580, SP600125 or U0126 for 24 h. **Conclusions:** IL-1β or IL-6 i.p. to TgAPP/PS1 or WT mice or incubating with the cells for 36h and displayed that iNOS were strongly long-distanced and stimulated. The working mechanisms of microglial activation induced by IL-1β or IL-6 were confirmed that MAPK signal pathway involved, and resulted in more neuroinflammation and oxidative-stress response, which further worse the inflammatory cycle. This study suggested that IL-1β or IL-6 i.p. injection model be established and it may be useful in new drug discovery.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

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

Support: KAKENHI 19H01015 (T.T.)
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AMED 22dm0207072 (T.T.)
AMED 22dm0207073 (T.T., S.T.)
JST JPMJMS2024 (T.T., S.T.)

Title: Alzheimer disease resilience factor RAB10 regulates extracellular tau uptake and inflammatory responses in microglia

Authors: *K. WANG, S. TAKATORI, T. TOMITA;
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Abstract: The two pathological hallmarks of Alzheimer disease (AD), amyloid β (Aβ) and tau, play crucial roles in AD pathogenesis. Intriguingly, some individuals with these pathologies maintain normal cognitive function, a phenomenon called as resilience. Although recent studies emphasize the importance of genetic factors in resilience, the molecular mechanisms underlying this process remain elusive. In this study, we focus on RAB10, a genetic resilience factor encoding a small GTPase implicated in the macropinocytosis of phagocytes. Emerging evidence suggests that microglia, the brain resident phagocytes, internalize extracellular tau aggregates, contributing to their metabolism and initiating neuroinflammatory responses against them. We therefore investigated whether RAB10 influences the uptake of, and the response to, extracellular tau by microglia. We developed a flow-cytometry-based assay to quantify the internalization of fluorescent tau fibrils by cultured cells and investigated the effects of Rab10 knockdown and knockout in microglial cells. Microglia were treated with tau fibrils to analyze the effect on tau clearance, and the amount of tau remaining in the conditioned medium was determined using ELISA. To examine the effect of RAB10 on tau-mediated inflammatory responses, we measured mRNA levels of inflammatory cytokines in tau fibril-treated microglia using real-time PCR. Tau fibril uptake was reduced by Rab10 knockdown in primary microglia and in Rab10-deficient microglial MG6 cell lines, with the latter being rescued by overexpression of human RAB10. Rab10 deficiency led to impaired macropinocytosis, and treatment with a macropinocytosis inhibitor reduced tau uptake. Treatment of microglial cells with tau fibrils resulted in the upregulation of TNFα and IFNβ mRNA expression, whereas Rab10 deficiency specifically inhibited the tau-mediated increase in IFNβ expression. Our findings suggest that tau uptake in microglia depends on macropinocytosis, facilitated by RAB10. Interestingly, Rab10 deficiency specifically suppressed tau-induced Type I IFN expression. Further investigations are needed to elucidate the molecular mechanisms underlying RAB10 function in tau-mediated microglial responses. Understanding the role of RAB10 in microglia could provide a potential therapeutic target for enhancing resilience against cognitive decline in AD.

Disclosures: K. Wang: None. **S. Takatori:** None. **T. Tomita:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.17/E28

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

Support: NIH Grant AG077610
NIH Grant AG079141
NIH Grant AG056061
BrightFocus Fellowship

Title: Alzheimer’S risk factor bin1-regulated microglial intrinsic and extrinsic mechanisms affect tau pathology in ps19 mice

Authors: *A. SUDWARTS[1], M. HANSEN[1], S. WANG[2], S. SMIRNOU[1], V. SKOROBOVENKO[1], M. DELOZIER[1], G. THINAKARAN[3];
[1]USF Byrd Alzheimer's Inst., Tampa, FL; [2]Neurobio. department, university of south florida, Tampa, FL; [3]USF Morsani Col. of Med., Univ. of South Florida Neurosci. Program, Tampa, FL

Abstract: Microglia play significant roles in Alzheimer's disease (AD) pathophysiology. There is evidence microglia may function in both protective and degenerative capacities, which has received little clarity from transcriptionally-characterised phenotypes uncovered from transgenic pathologies alone. Here we used a reverse-genetic approach to conditionally delete *Bin1* - the second-most significant risk gene for late-onset AD (LOAD) - in microglia of PS19 mice. Our data demonstrate that microglial BIN1 facilitates levels of pathologically phosphorylated tau (pTau), specifically in female PS19 mice. RNAseq analysis identified cell autonomous and non-autonomous effects for microglial BIN1 during tau pathogenesis in the PS19 model. Weighted gene co-expression analyses revealed complex networks of genes regulated by microglial gene expression during tau pathogenesis. We also identified gene networks correlated with levels of formic acid-soluble pTau, irrespective of microglial Bin1 gene manipulation. These gene networks offer novel insight into microglial responses to tau pathology. Future studies will shed crucial light on how these networks impact pathological outcomes.
Funded by NIH grants AG077610, AG079141, and AG056061; and BrightFocus Foundation fellowship to A.S.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.18/Web Only

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

Support: EU Grant 764860

Title: Cortical Spreading Depolarization (CSD) is largely unaltered in a mouse model of Alzheimer's disease despite distinctive histopathology

Authors: F. GIMENO-FERRER, A. EITNER, N. NOORA, R. BAUER, C. SCHMIDT-HIEBER, H.-G. SCHAIBLE, ***F. RICHTER**;
Univ. Hosp. Jena, Jena, Germany

Abstract: To assess the impact of Alzheimer's disease (AD) on cortical homeostasis the TASTPM mouse model of AD was used. TASTPM mice were bred from sperm provided by Glaxo Smith Kline Co (U.K.). To assess cortical excitability and histopathology, we recorded ongoing brain activity and induced cortical spreading depolarization (CSD) both in male and female TASTPM and in wildtype (WT) mice at ages of 3, 6, and 12 months, measured the size of

the extracellular space (ECS) fraction, and stained brain slices for Amyloid-β (Aβ) deposits, microglia activation, and astrogliosis. In spontaneously breathing anesthetized WT and TASTPM mice (sodium thiopentone, 100 mg/kg, i.p.) the direct current (DC-) electrocorticogram was recorded with arrays of glass microelectrodes in two cortical areas at a depth of 200-250 µm. The size of the ECS volume was assessed with the real time-tetramethylammonium (TMA) method. CSD were induced by microinjection of 1 M KCl. CSD-related DC potential shifts, and changes in extracellular potassium concentration were monitored over a period of 4 h. After euthanasia, the fixed brains were stained for Aβ deposits, phosphorylated Tau (P-Tau) tangles, astroglia, and activated microglia. A proliferation of Aβ deposits in cortex was observed with ageing between 3 and 12 months after birth, females had more deposits than males. P-Tau tangles were also detected in some cortical neurons at the age of 12 months. With proliferation of the Aβ-plaques microglial reaction (CD68, CD39 and Galectin-3) and astrogliosis (GFAP) in cerebral cortex progressively developed. Aβ accumulates were also found in brain blood vessels. The TMA method indicated a shrinkage of the cortical ECS with development of AD from 0.22±0.02 to 0.14±0.01 (females) and from 0.23±0.02 to 0.17±0.01 (males). CSD both in TASTPM and in WT mice expressed a large heterogeneity, but no typical impact of AD. At the age of 3 months, CSD in TASTPM mice could be easier elicited than in older ages. The observed changes in brain histology could not explain CSD-heterogeneity. However, a typical hyperexcitability in AD animals was manifested as convulsive behavior after thiopentone anesthesia. Thus, in the TASTPM model of neurodegeneration, replicable pathohistological changes have no well-defined correlation with functional parameters of homeostasis. This deserves future investigations.

Disclosures: F. Gimeno-Ferrer: None. **A. Eitner:** None. **N. Noora:** None. **R. Bauer:** None. **C. Schmidt-Hieber:** None. **H. Schaible:** None. **F. Richter:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.19/E29

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

Support: NIA 5R01AG062762-02
NIA 1R01AG062762-01A1
NIA 1R56AG062762-01

Title: Assessing the role of neuroinflammation in a mouse model of hyperglycemia with relevance to Alzheimer’s disease

Authors: *K. S. HERNANDEZ, A. A. ORTIZ, A. M. L. OSSE, A. R. PLATT, L. E. ROMERO, J. W. KINNEY;
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Abstract: Alzheimer's disease (AD) is the most common form of dementia that leads to progressive neurodegeneration. Key symptoms of AD include cognitive and memory impairments in middle or old-aged adults. In 2023, AD and other dementias will cost our nation $345 billion; by 2050, this number is projected to increase to over $1.1 trillion. AD is marked by three core pathological features including a build-up of beta-amyloid plaques, neurofibrillary tangles, and sustained immune response in the brain. Age is the largest known risk factor for AD, however, AD is not a normal part of aging. Diabetes is a risk factor for AD and can increase the risk of developing the disease by up to 4-fold. Diabetes is characterized by chronic hyperglycemia - a sustained period of high blood glucose levels. Several animal and human studies have shown that a sustained state of hyperglycemia can initiate neuroinflammation and exacerbate AD pathology; however, the mechanism by which this is occurring has yet to be elucidated. We investigated the effects of hyperglycemia by examining glial cells involved in neuroinflammation to determine whether the increased AD-like pathology diabetes confers, is a result of a hyperglycemic state itself, or if it’s the result of hyperglycemic-induced neuroinflammation. We hypothesize that if we treat a hyperglycemic state with an antidiabetic agent, AD-like pathology will continue to persist.We used 18-month-old male wild-type mice, and administered two different treatments: streptozocin (STZ), a drug that causes increased glucose levels by destroying cells in the pancreas that produce insulin; and phloridzin (PZ), an antidiabetic agent that lowers blood glucose levels, to rescue the hyperglycemic state. Blood glucose levels were taken weekly. We utilized immunohistochemistry to examine the hippocampus, a region that is involved in learning and memory and first impacted in AD, in mice. More specifically, we measured activated microglia and astrocytes in different regions within the hippocampus. In AD, activation of microglia and astrocytes can promote and worsen AD pathology. Briefly, we observed changes of microglial and astrocytic activity in STZ and PZ-treated mice. This data further emphasizes the importance of the brain's immune response and its role in AD progression. Given the rapid increased trajectory of diabetes and AD diagnosis, it is imperative to understand the mechanistic role by which chronic hyperglycemia influences neuroinflammation and AD, to better ameliorate treatments targeting AD.

Disclosures: K.S. Hernandez: None. **A.A. Ortiz:** None. **A.M.L. Osse:** None. **A.R. Platt:** None. **L.E. Romero:** None. **J.W. Kinney:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.20/E30

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

Support: NIA Grant R56 AG049870
NIA Grant R01 AG059028
NIA Grant P01 AG005138
NIA Grant P30 AG066514
NIA Grant 1R01AG078755-01

Title: Glial and vascular changes proximal to neurodegenerative lesions in areas of the human neocortex vulnerable to Alzheimer's disease

Authors: A. K. MCKENDELL[1], C. M. FREIRE-COBO[1], A. M. BADINA[2,3], E. SELMANOVIC[1], T. SETHI[1], E. NAING[1], A. PELLAGRINI[1], M. D. LAZARCZYK[1], S. MAGALHAES[1], J. SLOBIN[1], B. WICINSKI[1], E. MCDONOUGH[4], L. LOWERY[4], M. MEDALLA[5], B. B. TOURNIER[2,3], J. I. LUEBKE[5], D. E. MEYER[4], P. R. HOF[1,6], *M. VARGHESE[1,6];
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Abstract: Brain region-specific vulnerability to Alzheimer's disease (AD)-related neurodegeneration is well known. We sought to uncover the underlying cellular factors contributing to this selective vulnerability. We performed highly multiplexed immunofluorescence (MxIF) staining 26 markers and comparing postmortem human samples from an AD-susceptible brain area, the prefrontal cortex (PFC, Brodmann's area 9, 10 or 46) against an AD-resilient brain area, the primary visual cortex (V1, area 17). Subjects included AD (n = 3, clinical dementia rating CDR 3, Thal stages 3-4, Braak stages V-VI, age = 83-95 years), mild cognitive impairment (n = 4, CDR 0.5, Thal stages 1-3, Braak stages I-V, age = 61-89 years), and age-matched healthy controls (n = 5, CDR 0, Thal stages 0-1, Braak stages I-II, age = 74-97 years), representing both females and males. Antibodies for MxIF were selected to include markers for various cell types, cell features, cell states, tissue architecture, and AD neuropathology. The MxIF images were preprocessed to flatten the illumination field, stitch fields-of-view into panels, register across staining rounds using a nuclear stain as the fiduciary, and subtract the autofluorescence background. The images were analyzed using QuPath and a custom-made plugin for the Fiji distribution of NIH ImageJ. We combined manual and machine-learning tools available in these programs to automate segmentation and classification by marker expression and localization. Additional analysis was done in R to determine the average proximity of detected cells or features based on their centroids. Densities of astrocytes, microglia, oligodendrocytes, and blood vessels were comparable across CDRs in both brain regions, whereas that of neurons decreased in CDR 3 compared to CDR 0 specifically in the PFC. This decrease was driven by neurons located in layers 3 and 4 of the PFC. Interestingly, in PFC layer 3, we observed a trend towards more microglia expressing major histocompatibility complex protein II in CDR 3 compared to CDR 0.5. Moreover, vasculature in the PFC of CDR 3 subjects also showed an upward trend for proximity of reactive astrocytes expressing glial fibrillary acidic protein. Reactive astrocytes showed a trend to be closer to amyloid-beta plaques in CDR 3 subjects. These preliminary results point towards a regional inflammatory response in glia proximal to vasculature and pathological protein deposits in areas with selective loss of neurons. Further investigation into markers of cell state in their spatial context will provide information on *in situ* cellular interactions and protein changes contributing to neuronal vulnerability in AD.

Disclosures: **A.K. McKendell:** None. **C.M. Freire-Cobo:** None. **A.M. Badina:** None. **E. Selmanovic:** None. **T. Sethi:** None. **E. Naing:** None. **A. Pellagrini:** None. **M.D. Lazarczyk:** None. **S. Magalhaes:** None. **J. Slobin:** None. **B. Wicinski:** None. **E. McDonough:** A.

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Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.21/E31

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AG077002

Title: Effects of chronic corticosterone and 4-MU treatment on PNN components in primary astrocyte and microglia cultures

Authors: *P. M. M. WONNENBERG[1], S. VICINI[2], K. CONANT[3];
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Abstract: Alzheimer’s disease (AD) is a progressive condition characterized by cognitive impairment, amyloid beta aggregates, and tau protein deposits. Risk factors in human and/or animal models include the apolipoprotein E ε4 allele (ApoE4), corticosterone (CORT) treatment, and major depressive disorder (MDD), conditions in which changes in extracellular matrix (ECM) effectors have been observed. Excess ECM can restrict neuroplasticity and excitatory neuronal function, and it may also sequester amyloid and tau. Perineuronal nets (PNNs), a specialized form of ECM, are present in various brain regions and can increase the firing of parvalbumin-expressing (PV+) GABAergic interneurons to in turn inhibit pyramidal cells. We have shown that corticosterone is linked to increased deposition of PNN proteins in a rodent model of depression, and hypothesize that PNN changes might also increase AD risk. In this study, we examine the effects of long-term exposure to CORT on PNN effectors in primary astrocyte and microglia cultures. We observe that chronic CORT treatment induces significant changes in PNN components and effectors, including TIMP-1, Pro-MMP-9, CCL5, versican, and neurocan. We are evaluating 4-MU, an inhibitor of HA synthesis, for its ability to attenuate PNNs and enhance plasticity during long-term CORT. Parallel studies are determining the effect of chronic CORT treatment on the firing patterns of PV+ interneurons and pyramidal (PYR) excitatory neurons with single cell recordings from PV-Cre/Td-tomato C57BL/6 hippocampal

neurons. We hypothesize that extended exposure to CORT will cause increased excitation of PV cells and reduced excitation of pyramidal cells and that treatment with 4-MU will normalize these effects. Finding from these studies could support the use of 4-MU for disorders with excess ECM deposition.

Disclosures: P.M.M. Wonnenberg: None. **S. Vicini:** None. **K. Conant:** None.

Poster

PSTR325. Microglial Roles in Alzheimer's Disease

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR325.22/E32

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

Support: UPMC Endowed Professorship, D.S.
University of Pittsburgh Brackenridge Fellowship Fredrick Honors College, M.M.

Title: Na^+/h^+ exchanger isoform 1 (nhe1) upregulation in reactive astrocytes in Alzheimer's disease neuropathology and neurological function

Authors: *J. M. COLLIER[1,3], S. METWALLY[1], M. MCFARLAND[1], V. FIESLER[1], S. KRISHNA[4], M. STAUFFER[2], G. BEGUM[1], J. KOFLER[2], D. SUN[1];
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Abstract: Reactive astrogliosis has been indicated as an early biomarker in Alzheimer's Disease (AD) pathology. Reactive astrocytes undergo morphological, transcriptional, and functional changes in response to acute and chronic neurological disease. Previous findings in our lab have shown that Na+/H+ exchanger isoform 1 (NHE1) expression and activity in reactive astrocytes contribute to neuroinflammation and cognitive function deficits in murine models of ischemic stroke and vascular stenosis. In this study, using immunostaining assays in control and AD with comorbidity patient post-mortem brain tissues, we detected significant increase in NHE1 protein expression localized in the GFAP+ reactive astrocytes in cerebral cortical regions of AD brains compared to controls ($p=0.007$). ~60% of GFAP+ reactive astrocytes expressed NHE1 protein in cortical regions of the AD brains, while control tissues displayed less than 20% of NHE1+/GFAP+ reactive astrocyte. 3D reconstruction of these astrocytes revealed significantly more process branch points in reactive astrocytes of the cortical regions from AD patients ($p=0.004$) along with larger soma volume and process diameter than in controls. In the well-characterized APP/PS1dE9 AD mouse model, we detected reactive astrocytes in hippocampal dentate gyrus regions at 4-6 months old age and in CA3 regions between 6-8 months of age. Interestingly, elevation of NHE1 protein expression occurred in hippocampal GFAP+ reactive astrocytes between 6-8 months of age. Cognitively, APP/PS1dE9 mice did not display any preference in the novel arm of the Novel Spatial Recognition Test ($p>0.999$) at 4 months of age,

which is in contrast of the performance of wild-type control mice (p=0.0475). Moreover, in the Light-Dark assay to test anxiety-like behavior, APP/PS1dE9 mice spent significantly more time in the dark zone (p=0.022) than wild-type mice at 4 months of age. We speculate that development of reactive astrogliosis and loss of homeostatic astrocyte function (including intracellular pH homeostasis) may contribute to neurological function impairment and AD pathology in APP/PS1dE9 mice. We are currently investigating the impact of upregulation of NHE1 protein in reactive astrocyte function and potential as a therapeutic target for AD.

Disclosures: J.M. Collier: None. **S. Metwally:** None. **M. McFarland:** None. **V. Fiesler:** None. **S. Krishna:** None. **M. Stauffer:** None. **G. Begum:** None. **J. Kofler:** None. **D. Sun:** None.

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.01/E33

Topic:

Support: NIH / National Institute on Aging funding P01AG026572 to RDB
NIH / National Institute on Aging funding T32AG061897 to RDB
Center for Innovation in Brain Science to RDB

Title: Physiological and Behavioral profiles of precision midlife combinatorial therapy in hAPP hAPOE risk model of AD

Authors: *G. TORRANDELL[1,2], H. VAN ROSSUM[1,2], A. KALINA PARKER[1], Y. SHANG[3], R. D. BRINTON[2];
[2]Ctr. for Innov in Brain Sci., [1]Univ. of Arizona, Tucson, AZ; [3]Ctr. for Innov in Brain Sci., Univ. of Arizona, TUCSON, AZ

Abstract: The preclinical phase of Alzheimer's Disease can begin decades prior to the onset of clinical symptoms. During this stage, physiopathological changes of AD that drive beta amyloid and tau hallmark pathologies. These changes include neural and peripheral inflammation and dysregulated metabolism. Targeting these systems of biology through FDA-approved therapeutics could be an effective strategy to address AD risk factors thereby reducing the risk of AD. Our previous analyses indicated that lipid-lowering, glucose metabolic regulators, and anti-inflammatory therapeutic resulted in statistically lower risk of AD. To address the impact of combinatorial therapeutics on AD pathology, we utilized an AD risk humanized (h) hAPP hAPOE3/3 and hAPP hAPOE4/4 mouse models. Female and male hAPP hAPOE3/3 and hAPP hAPOE4/4 mice were exposed at midlife to combination therapy intervention starting at 15 months old for a period of 1 month or 3 months. Combination drug formulations were administered daily via chow. Mice underwent open field, novel object recognition (NOR), and EchoMRI body composition assessment before and after treatment. Additionally, mice

performed weekly nesting test and weekly body weight monitoring. Results of these analyses indicated sex and APOE genotype differences in body weight and body composition. Behavioral differences between groups were assessed by NOR, open field, and weekly nesting to evaluate cognitive function, anxiety level, and general welfare. Mice were longitudinally monitored for body weight and calorie intake measures, which were used to calculate daily dose for drug dosing accuracy. Additionally, mice were screened weekly for dermatitis, barbering, or any possible adverse event associated with the given therapeutics.
Observational studies support the use of FDA-approved risk factor therapeutics to reduce the risk of AD, with a greater benefit when used in combination. This study evaluated the therapeutic potential of combination therapy targeting drivers of AD pathology and supports a precision medicine strategy for AD based on sex and APOE genotype.

Disclosures: G. Torrandell: None. **H. Van Rossum:** None. **A. Kalina Parker:** None. **Y. Shang:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RDB is President of NeuTherapeutics, LLC..

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.02/E34

Topic:

Support: NIH/NIA Grant T32AG061897
NIH/NIA Grant R01AG05793
The University of Arizona Center for Innovation in Brain Science

Title: Translational potential of novel LOAD risk mouse model hAPP+hAPOE: Biomarkers and pathologies across chronological aging

Authors: *N. DELATORRE[1,2,3], H. VAN ROSSUM[3,4], J.-P. WIEGAND[3], M. MASTRIANI[3], J. W. MCLEAN[1,4], R. D. BRINTON[3,2,4];
[2]Dept. of Pharmacol., [3]Ctr. for Innovation in Brain Sci., [4]Grad. Interdisciplinary Program Neurosci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Late-onset Alzheimer's disease (LOAD) is a progressive neurodegenerative disease with a decades-long prodromal phase. There are four well-documented risk factors for LOAD: age, APOE4 genotype, female chromosomal sex, and maternal history of AD. Each risk factor impacts multiple systems, adding to the complexity of LOAD. Previous findings demonstrated sex-driven inflammatory profiles and metabolic shifts in the female humanized-APOE mice compared to aged-matched males. Herein, we report outcomes from a novel AD risk factor model that includes both humanized APOE (hAPOE) and APP (hAPP). The mouse model was generated by breeding transgenic hAPOE (APOE3/3 & APOE4/4) KI mice (JAX) with

homozygous B6(SJL)-App tm1.1Aduci/J mice (JAX).
To investigate impact of LOAD risk factors on AD relevant outcomes, we conducted metabolomic, proteomic, behavioral and β-amyloid assessments across aging at 6, 12, and 15-month-old (human equivalent ~30, 40, and 50-year-old respectively) hAPP+hAPOE3/3 & hAPP+hAPOE4/4 in both female and male mice.
Metabolic analyses indicated sex-dependent differences in the plasma profiles of 6m mice whereas 12m mice exhibited a genotype-dependent metabolic profile that was further amplified at 15m. Specifically, female hAPP+hAPOE4/4 mice exhibited lower total cholesterol and HDL plasma levels compared to their respective hAPP+hAPOE3/3 same-sex counterparts. Conversely, at the same time point, both sexes hAPP+hAPOE4/4 mice had higher triglyceride levels with the male hAPP+hAPOE4/4 exhibiting the highest levels. Proteomic analyses indicated that β-amyloid was detectable in plasma at 12m & 15m in both sexes. hAPP+hAPOE4/4 females exhibited an accelerated aging phenotype at 12m & 15m, indicated by increased proportion of irregular and acyclic cycling mice, compared to hAPP+hAPOE3/3 animals.
Collectively, the data indicate an interaction across AD risk factors which is consistent with an ongoing genotype-dependent metabolic shift that is amplified by age and sex. Metabolic dysregulation has been closely linked with AD development and progression. Low HDL cholesterol and high triglycerides levels are determinants for other disease that are risk factors for AD, such as stroke and heart disease. Together, these findings establish a foundation for the development of precision therapeutics targeting the prodromal phase of LOAD. This critical stage offers the greatest potential for reversing, preventing, and delaying the progression of LOAD. Future studies include metabolic and proteomic assessments at 18m and further determination of AD pathology in the brain.

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Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.03/E35

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

Support: NIH/National Institute on Aging: P01AG026572 (Perimenopause in Brain Aging and Alzheimer’s Disease)
NIH/National Institute on Aging: R01AG057931 (Sex Difference in the Molecular Determinants of Alzheimer’s Disease Risk: Prodromal Endophenotype)
Center for Innovation in Brain Science to RDB

Title: Hapoe increases weight and survival probability in happ transgenic mouse models

Authors: *J.-P. L. WIEGAND, T. STANLEY, A. DALTON, L. CAMPBELL, R. D. BRINTON;
Ctr. for Innovation in Brain Sci., Univ. of Arizona, Tucson, AZ

Abstract: Background: The 4th isoform of apolipoprotein E is the most prevalent genetic risk factor for Alzheimer's Disease (AD) and transgenic hAPOE animal models are widely used. However, many studies investigate humanized APOE in isolation from key interactors, such as humanized amyloid precursor protein (hAPP).Method: To address knowledge gaps regarding APOE and how it interacts with hAPP, transgenic mice carrying hAPOE alleles (JAX#27894, APOE3/3 KI, https://www.jax.org/strain/027894 and JAX#29018, APOE4/4 KI, https://www.jax.org/strain/029018) were obtained and bred. APOE KI mice were subsequently bred to homozygous B6(SJL)-App tm1.1Aduci/J animals (JAX#030898, hAbeta-loxP-KI). These animals were longitudinally tracked for identification and analysis of susceptible and resilient subpopulations through population-level analyses including monthly weights, clinical observations, and survival rates.Result: Colony-wide analyses indicated multiple key differences: 1) hAPP*hAPOE mice exhibit higher body weight as compared to hAPOE and hAPP mice, across both males and females. hAPP*hAPOE mice also display clear genotypic differences (with hAPP*hAPOE4 exhibiting a failure to thrive) where hAPOE do not show any colony weight differences. No difference was observed in longitudinal barbering. 2) hAPP*hAPOE mice had the highest survival probability, followed by hAPOE, and then hAPP mice. 3) hAPP*hAPOE show a reduced prevalence towards dermatitis, as compared to hAPOE mice. Conclusion: The addition of hAPP to the well-characterized hAPOE gene vastly changes even basic results such as weight, survivability, and the commonly observed dermatitis. It is clear from these results that humanized transgenes are necessary to produce human effects. Further work should explore how necessary other downstream neurological mechanisms are to create more translatable models. Acknowledgements: Research was supported by the NIH/National Institute of Aging P01AG026572 and R01AG057931 to RDB and the Center for Innovation in Brain Science to RDB.

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Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.04/E36

Topic:

Support: NIH/NIA P01-AG026572 to RDB
NIH/NIA R01-AG057931 to RDB

Woman's Alzheimer's Movement to RDB
Center for Innovation in Brain Science to RDB

Title: First-line therapies for neuropsychiatric disorders modify risk for development of Alzheimer's disease

Authors: *H. CORTES-FLORES, G. TORRANDELL, R. D. BRINTON;
Univ. of Arizona, Tucson, AZ

Abstract: Neuropsychiatric disorders including depression, insomnia, epilepsy, schizophrenia and attention-deficit and hyperactivity disorder (ADHD) have been associated with a neurodegenerative process and linked to increased risk for Alzheimer's Disease (AD). Because biologic mechanisms of AD and neuropsychiatric disorders, we hypothesized that pharmacologic treatment for neuropsychiatric disorders could impact risk for AD. CNS drugs that are first-line therapies for neuropsychiatric disorders (including antidepressants, sedatives, anticonvulsants, antipsychotics and stimulants) were investigated for impact on AD incidence.
To address this hypothesis, we conducted a retrospective medical informatics analysis of insurance claims of patients aged 60 years and older, with and without exposure to CNS drugs. To reduce health status and demographic bias, we utilized propensity score matching to adjust for age, gender, Charlson comorbidity index (CCI), and comorbidities. The propensity score matched population was surveyed for AD diagnosis following at least 1 year of exposure to CNS drugs. Additionally, responder vs non-responder phenotypes were identified based on comorbidity and co-treatment profiles using sensitivity analysis.
Exposure to CNS drugs was associated with a decreased risk for AD (RR [95%CI]: 0.50 [0.47-0.53]; *P*<.0001), and women (RR [95%CI]: 0.46 [0.42-0.50]; *P*<.0001) exhibited a slightly greater risk reduction compared to men (RR [95%CI]: 0.55 [0.50-0.61]; *P*<.0001). Drug stratification indicated that antidepressant, sedative, anticonvulsant, and stimulant treatment were associated with reduced AD risk, while antipsychotics were associated with increased risk for AD. Responders displayed fewer comorbidities although they had a higher incidence of obesity. Further, responders exhibited a greater use of anti-inflammatories and menopause hormonal treatment.
Collectively, these results provide evidence indicating that antidepressants, sedatives, anticonvulsants, and stimulants act as risk modifiers for AD by restoring dysregulated neurotransmission function, while antipsychotics appear to increase the vulnerability of the brain for AD development. These findings have the potential to contribute to advancing neuropsychiatric treatment that could impact risk of Alzheimer's disease.

Disclosures: H. Cortes-Flores: None. **G. Torrandell:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); President of NeuTherapeutics, LLC..

Poster

PSTR326. ApoE and Associated Pathways II

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Program #/Poster #: PSTR326.05/E37

Topic:

Support: NIH/NIA P01AG026572
Women's Alzheimer's Movement
Center for Innovation in Brain Science

Title: Age, Sex and APOE as modifiers of Alzheimer's Disease-On-Ramp risk factors profiles: a UK Biobank retrospective study using extended Cox models

Authors: *S. MERLINI[1,2,3], F. VITALI[3,4,5], R. D. BRINTON[3,6,5];
[2]Biomed. Engin., [3]Ctr. for Innov in Brain Sci., [4]Neurol., [5]Ctr. for Biomed. Informatics and Biostatistics, [6]Pharmacol., [1]Univ. of Arizona, Tucson, AZ

Abstract: Obesity, diabetes (T2D), hypertension (HTN) and hyperlipidemia (HLP) are relevant Alzheimer's Disease (AD)-on-ramp modifiable risk factors (RFs). However, it remains unclear whether the risk conferred by these conditions is modified in time by other unmodifiable AD risk factors, such as chromosomal sex and APOE genotype. In this study, we conducted a retrospective analysis using the UK Biobank to evaluate age-specific effect of AD-on-ramp RFs, in combination with sex and APOEε4 carrier status. Inclusion criteria were age older than 55 years, no prior history of neurodegenerative disease, neurosurgery, or cancer, and enrollment with at least 3 years of follow-up. Propensity score matching was performed based on age at recruitment, educational level, center, and Charlson comorbidity index. Stratified Cox proportional hazard models (CPHMs) with counting process formulation were used to examine the association between each time-depending modifiable RFs and AD onset, adjusted for sex and *APOE* genotype. Age stratification was evaluated when violation of hazard ratio (HR) proportionality assumption occurred during aging. Finally, we performed extended stratified CPHM for recurrent event to estimate AD onset based on the development of multiple RFs in time. Preliminary findings revealed RF-specific differences in AD on-set based on the age of RF diagnosis. The risk of developing AD was significantly greater than APOEε4 effect if HTN was diagnosed before 63yo, while if diagnosed after 72yo, APOEε4 was the major contributor of increased AD risk. Similarly, HLP was associated with higher risk of AD if diagnosed before 65yo or in the 65-72 age group, while T2D in the &lt64 or 64-72 age groups, and obesity in the &lt70 age group. Again, the age-stratum-specific decline of RF impact on AD diagnosis were modulated by a substantial increased effect of APOEε4 carrier status within the age-strata. Additionally, when considering a CPHM with all the RFs combined, higher risk of developing AD was consistently associated with earlier RF diagnosis (&lt62), while APOEε4 carriers exhibited higher risk of AD onset in the older age. This study identified critical tipping points indicating a decline in the hazard ratio of modifiable RFs with aging and a stronger association of APOEε4 with the late-onset AD. Age stratification within CPHMs provided valuable insights into age-specific hazard ratios and identified age-dependent RFs. Furthermore, these results highlight interactions between age, sex, and APOE genotype which could inform a precision medicine approach for AD prevention.

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Poster

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Program #/Poster #: PSTR326.06/E38

Topic:

Support: NIA Grant T32AG061897
NIA Grant R01AG057931
Center for Innovation in Brain Science

Title: Behavioral and metabolic profiles in a humanized APP/APOE mouse model of Alzheimer's Disease risk

Authors: *J. W. MCLEAN[1,2], A. BHATTRAI[1,3], R. D. BRINTON[1,3,4];
[1]Ctr. for Innovation in Brain Sci., [2]Grad. Interdisciplinary Program in Neurosci., [3]Dept. of Pharmacol., [4]Dept. of Neurol., Univ. of Arizona, Tucson, AZ

Abstract: Research into mechanisms driving Alzheimer's Disease (AD) pathology has frequently utilized animal models with dominant mutations; however, the vast majority (>95%) of AD cases are idiopathic. Animal models with AD risk factors instead of dominant mutations represent an approach with greater translational validity. The predominant genetic risk factor for AD is the Apolipoprotein E *ε4 (APOE4)* polymorphism, with *APOE4* homozygosity conferring ~15-fold higher risk relative to the normative *APOE3/3* genotype. Additionally, women are nearly twice as likely to develop AD. To address the translational validity of a risk factor model approach for AD research, we investigated behavioral and metabolic differences in a novel mouse model with homozygous expression of humanized (h) amyloid precursor protein (h*APP*), encoding the precursor of the major protein in amyloid plaques, and homozygous replacement of murine *Apoe* with humanized h*APOE3* or h*APOE4*. Aged (22-24 months) h*APP*/*APOE3* and h*APP*/*APOE4* mice underwent open field (OF), novel object recognition (NOR), EchoMRI body composition analysis, fasting blood glucose (FBG) and ketone body (FKB) determinations. Data were analyzed by 2-way ANOVA for effects of biological sex and *APOE* genotype followed by post-hoc *t*-tests and considered significant at $p<0.05$. Female mice, regardless of h*APOE* genotype, traveled significantly further and for a greater percentage of time during both OF and NOR, and interacted more with both familiar and novel objects during NOR. There were no group differences in either thigmotaxis or novelty recognition. EchoMRI body composition analysis revealed significant weight reduction in both male and female h*APP*/*APOE4* mice. While weight loss included decline in both lean and adipose mass, greater loss of adipose tissue in h*APP/APOE4* mice resulted in lower body fat percentage and was the major contributor to weight loss. Metabolically, female mice had lower FBG and higher FKB relative to males, regardless of genotype. The sex difference was greater in h*APP/APOE4* females, which had the lowest FBG and highest FKB. The h*APOE4*-driven reduction in body weight, especially in adipose tissue, further indicates a metabolic consequence in mice expressing the AD risk gene. Metabolomic and brain imaging analyses are underway. Collectively, this program of research contributes to determination of the translational validity of the humanized *APOE* and *APP* mouse model for preclinical target identification and therapeutic development for AD.

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Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

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Program #/Poster #: PSTR326.07/E39

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

Support: P01AG026572 to RDB
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Women's Alzheimer's Movement to RDB
Center for Innovation in Brain Science

Title: Network-based identification of precision therapeutics for Alzheimer's disease specific to endocrine and APOE genotype status

Authors: *F. VITALI[1], J. ZHANG[2], T. WANG[3], R. D. BRINTON[2];
[2]Ctr. for Innov in Brain Sci., [1]Univ. of Arizona, Tucson, AZ; [3]Ctr. for Innov in Brain Sci., Univ. Of Arizona, Tucson, AZ

Abstract: Alzheimer's disease (AD) affects an estimated 6.5 million people in the US, two-thirds of which are women, yet highly efficacious, disease-modifying treatments for AD are currently lacking. The greatest risk factors for AD are age, chromosomal female sex and APOEε4 genotype. A key driver of chronological and endocrinological aging is the activation of innate and adaptive immune systems occurring in midlife female brain. The development of computational methods screening for personalized therapeutics accounting for sex, APOE genotype, endocrine age is crucial to prevent and treat AD efficiently.We analyzed RNAseq data of 15-month acyclic vs 9-month regular APOEε3/ε3 (E3) and APOEε4/ε4 (E4) female mouse models to identify APOE-specific Differentially Expressed Genes (DEGs, pvalue<.01). STRING Protein-Protein Interaction (PPI) database was used to extract protein interactors for the two APOE3 and APOE4 DEG lists and construct two APOE-specific AD PPI networks. Target Proteins (TPs) candidates in each network were identified by using DrugBank database and selecting only proteins targeted by at least one FDA-approved drug. A score of Drug Synergy was developed to rank FDA-approved drugs considering the number of target proteins in- vs out-the APOE-AD specific network and their distance to DEG nodes.Comparing 15 months acyclic and 9 months regular RNAseq data of mouse brain, we identified 265 DEGs for APOE3 and 452 DEGs for APOE4. The resulting APOE-AD-specific PPI resulted in 1,711 nodes (proteins) and 21,445 edges (PPIs) for APOE3 network and 2,841 nodes (proteins) and 35,493 edges (PPIs) for APOE4 network. The use of DrugBank combined with topological network properties enabled the identification of 342 and 568 target proteins in the APOE3 and APOE4 networks that were ranked according to TP_{score}. Finally, the application of the drug synergy score, allowed the

ranking of 99 therapeutics for APOE3 network and 102 drugs for APOE4 network. Of these only 19 drugs are in common to both networks.Our computational analyses provide a network-based approach for the identification of personalized therapeutics based on topological properties of PPI specific of APOE genotype and endocrine transition changes. Our findings suggest significantly greater transcriptomic dysregulation in female APOE4 vs APOE3 brains (452 vs 265 DEGs). The network-based approach method identified 99 and 102 potential therapeutics specific to female, endocrine transition, and APOE3 and APOE4 genotype. Future studies will involve the computational identification of combination of the identified therapeutics to effectively advance precision medicine for prevention and treatment of AD.

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Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.08/E40

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

Support: NIH/NIA P01AG026572 to RDB
Center for Innovation in Brain Science to RDB

Title: Endocrinological aging drives alterations in white matter architecture: Evidence from a humanized APOE risk model of Alzheimer’s disease

Authors: *A. RAIKES, A. BHATTRAI, T. WANG, R. D. BRINTON;
Univ. of Arizona, Tucson, AZ

Abstract: Alzheimer’s affects women 2:1 compared to men, suggesting sex-specific factors driving risk. Menopause, a female-specific phenomenon, induces a multi-system response across endocrine, metabolic, and immune-inflammatory systems. Despite known effects on these systems, the impact on the brain and AD risk remains incompletely understood, limiting preventative options. Here, we examined menopause effects on white matter properties in a late-onset AD mouse model. 6-, 9- and 15-month humanized APOEε3/ε3 (JAX #29018), ε4/ε4 (JAX #27894), and ε3/ε4 (bred in-house) female mice were stratified into 3 endocrine aging groups based on vaginal cytology profiles: regular cyclers (consistent 4-5 day cycles), irregular cyclers (6-9 day cycles), and acyclic (no cycling >9 days). Animals were euthanized on estrous day and transcardially perfused with cold PBS. Ex-vivo MRI included diffusion-weighted imaging. Fixel-based analyses were used to analyze white matter fiber density (FD), cross-section (FC) and their product (FDC), in addition to anisotropy (FA) and diffusivity (MD). Data were analyzed using generalized additive models with main effects of endocrine aging (ref: 9-month regular), hAPOE

genotype, and total brain volume (all metrics except FD). Findings are reported at FDR corrected $p < 0.05$. Compared to 9-month regularly cycling mice, both 6-month regular and 15-month acyclic mice had lower FC. Differences were primarily in the bilateral fimbria, stria terminalis, corpus callosum as well as gray matter regions including bilateral hippocampal subfields and cingulate cortex (15 month acyclic mice only). 15-month acyclic mice had lower FD/FDC primarily in the left cingulate, piriform, and bilateral entorhinal cortices and greater FD/FDC in the bilateral habenular commissure and dorsal tenia tecta. FA and MD were significantly lower in the 15-month acyclic mice in most white matter regions. Additionally compared to 15-month irregularly cycling mice, 15-month acyclic mice exhibited lower FA in white matter tracts and striatal projection fibers. In these mice, menopause, not genotype, altered white matter bundle properties, shifting toward smaller bundles of varying compactness and reduced bundle coherence that cannot be solely explained by age. The most pronounced differences were between 9-month pre-menopausal mice and 15-month post-menopausal mice. Differences between peri- and post-menopausal mice at 15 months were only observed in FA. Collectively these findings implicate endocrine aging as a driving factor in white matter changes that may precede AD presentation. On-going work is focused on conducting translational analyses in the human brain.

Disclosures: **A. Raikes:** None. **A. Bhattrai:** None. **T. Wang:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuTherapeutics.

Poster

PSTR326. ApoE and Associated Pathways II

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Program #/Poster #: PSTR326.09/E41

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

Support: NIA Grant R37AG053589
Center for Innovation in Brain Science

Title: Key role of ERβ/ESR2 in estrogenic regulation of neuronal mitochondrial transcription and function

Authors: *T. WANG[1], Z. MAO[2], S. CHEN[2], Y. SHANG[2], J. B. STANTON[2], G. QI[2], F. YIN[2], R. D. BRINTON[2,3,4];
[2]Ctr. for Innovation in Brain Sci., [3]Dept. of Pharmacol., [4]Dept. of Neurol., [1]Univ. of Arizona, Tucson, AZ

Abstract: Estrogen is a master regulator of the bioenergetic system in the female brain, exerting broad control over a variety of metabolic processes from glucose transportation to glycolysis, mitochondrial respiration and ATP generation. While both ERα and ERβ have been reported to mediate E2 regulation on brain bioenergetic function, their cell-type specific contribution to

bioenergetic homeostasis has yet to be elucidated. Herein, we investigated the role of ERα and ERβ in E2 regulation of neuronal bioenergetics. We developed novel conditional estrogen receptor α and β knock-down rat models by inserting two loxP sites upstream and downstream of exon 3 of *Esr1 or Esr2* allele using Crispr/Cas9-mediated genome engineering technique. Our novel ERαlox and ERβlox rat models enable inducible and brain cell-type and region specific ERα and ERβ knockdown without disrupting normal development of estrogen dependent organs and systems. Results of our analyses indicated that ERβ was primarily involved in E2 regulation of neuronal mitochondrial OxPhos *in vivo.* Neuronal ERβ knockdown resulted in selective decrease in *Esr2* gene expression, accompanied by significant decreases in hippocampal mitochondrial respiration, impaired complex activities and reduced NAD+ levels. Whereas neuronal ERα knockdown did not impact hippocampal mitochondrial respiration nor mitochondrial complex activities. Further mechanistic investigation using primary embryonic neurons isolated from ERαlox and ERβlox rats confirmed ERβ dependent transcriptomic regulation of TCA and oxidative phosphorylation genes. Importantly, ERβ knockdown resulted in increased UCP2 expression and decreased AKT and ERK signaling, leading to increased proton leak and decreased mitochondrial respiration. Collectively, these data demonstrate that ERβ plays a pivotal role in maintaining neuronal mitochondria function and bioenergetic homeostasis that requires a mitochondrial transcription-dependent mechanism.

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Poster

PSTR326. ApoE and Associated Pathways II

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Program #/Poster #: PSTR326.10/F1

Topic:

Support: NIA Grant P01AG026572
NIA Grant R01AG057931
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Center for Innovation in Brain Science

Title: Impact of humanized APOE on brain volumetrics and white matter microstructure within a mouse model for Alzheimer's disease

Authors: *A. BHATTRAI[1,2,3], A. RAIKES[1,3], J. MCLEAN[1,3], J.-P. WIEGAND[1,3], R. D. BRINTON[3,2,1];
[2]Dept. of Pharmacol., [3]Ctr. for Innovation in Brain Sci., [1]Univ. of Arizona, Tucson, AZ

Abstract: Frequently utilized Alzheimer's disease (AD) preclinical models, while exhibiting strong disease etiology, rely on risk factors expressed in familial AD, which accounts for <1% of the clinical AD population. Apolipoprotein (APOE) ε4 is the strongest genetic risk factor for the development of the more prevalent late-onset Alzheimer's disease (LOAD). MRI studies demonstrate a link between APOE-ε4 and reduced gray matter volume as well as lower fractional anisotropy (FA) in AD patients. However, brain volumetric and white matter integrity (WMI) data in preclinical models is lacking. Herein, we report the effect of sex and genotype on local brain volume and WMI in an aged humanized APOE (hAPOE) LOAD mouse model. Aged h*APOE* ε3/3, ε3/4, and ε4/4 mice (N=43, mean age=24.1 months, Female=20) underwent high-resolution ex-vivo structural (T2-weighted RARE; 75 microns isotropic) and diffusion-weighted ($b = 1000\ mm/s^2$; 200 microns isotropic) MRI imaging. Deformation based morphometry was used to identify volumetric differences and WMI was quantified using FA. Mice were classified as ε4 carriers (at least one allele) or non-carriers (ε3/ε3). Two-way ANOVAs were used to identify main effects of ε4 genotype and sex. Results are reported as FDR corrected. $p \leq 0.05$ was considered statistically significant. hAPOE-ε3/ε3 mice had larger olfactory bulb and parieto-temporal lobes than ε4 carriers and greater mean FA across the major white matter tracts. Independent of genotype, females had larger cortical areas in parts of bilateral temporal and parietal lobes, as well as bilateral thalami compared to males. Males had greater volume in the bilateral olfactory bulb, amygdala, hypothalamus, and hippocampi. No sex differences were observed in FA. At 24 months of age (~70 human years), h*APOE* ε4 mice have decreased WMI compared to ε3s. Decreased WMI in ε4 carriers is consistent with recent work linking APOE with reduced myelin in APOE ε4 human brain. Further, sex differences in local volume point to unique sex-specific patterns of aging in this model. Outcomes of these analyses indicate both sex and APOE genotype differences on both gray matter volumes and white matter integrity. On-going research includes comprehensive systems biology modeling of AD presentation to identify therapeutic targets as well as translating these findings to human brain imaging analyses.

Disclosures: A. Bhattrai: None. **A. Raikes:** None. **J. McLean:** None. **J. Wiegand:** None. **R.D. Brinton:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); RDB is Founder and President of NeuTherapeutics, LLC..

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.11/F2

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

Support: National Institute on Aging, Grant Number:R01 AG054672

Title: Microglia-specific targeting of Apoe restores cognitive function and induces Apoc1 in aged $APP^{NL\text{-}G\text{-}F}$ knock-in mouse model

Authors: S. HERRON[1], Z. YIN[2], ***Y. CHEN**[3], S. IKEZU[3,1], O. BUTOVSKY[2,4], T. IKEZU[3,1]; [1]Dept. of Pharmacol. and Exptl. Therapeut., Boston Univ. Sch. of Med., Boston, MA; [2]Dept. of Neurol., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA; [3]Dept. of Neurosci., Mayo Clin., Jacksonville, FL; [4]Evergrande Ctr. for Immunologic Dis., Brigham and Women's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Polymorphisms in the apolipoprotein E (*APOE*) gene are the most significant genetic risk factor for the development of Alzheimer's Disease (AD), with the e4 allele carrying the highest risk. Previous studies have indicated the essential role of APOE in microglia transitioning from a homeostatic phenotype to Microglia Neurodegenerative Phenotype (MGnD) or Disease-Associated Microglia (DAM) in neurodegenerative disease conditions. However, the role of microglial APOE in AD pathology has not been fully elucidated. To this end, we conditionally deleted *Apoe* in microglia in the $APP^{NL\text{-}G\text{-}F}$ knock-in mouse model ($APP^{NL\text{-}G\text{-}F}$:$Apoe^{cKO}$) and comprehensively characterized microglial transcriptome, brain tissue proteome, and characterized AD pathology development. Interestingly, abrogation of microglial *Apoe* preserved cognitive function at 10 months of age in $APP^{NL\text{-}G\text{-}F}$: $Apoe^{cKO}$ mice as compared to $APP^{NL\text{-}G\text{-}F}$ mice. While the microglial bulk RNA seq showed minimal changes in microglial transcriptome other than expected suppressed expression of *Apoe,* we observed significant upregulation in the level of Apolipoprotein C-1 (*Apoc1*) in $APP^{NL\text{-}G\text{-}F}$: $Apoe^{cKO}$ mice. Apoc1 is a lipoprotein located at a neighboring genetic locus to *Apoe,* in $APP^{NL\text{-}G\text{-}F}$: $Apoe^{cKO}$ mice. This result was further validated using RNA scope, which showed significantly high expression of *Apoc1* and low expression *Apoe* in plaque-associated regions of $APP^{NL\text{-}G\text{-}F}$:$Apoe^{cKO}$ mice. These findings indicate a reciprocal relationship between Apoe and Apoc1. In addition to a reduced sphericity of amyloid plaques determined by immunofluorescence paired with 3D structural analysis, we found significantly reduced plaque-induced neuritic dystrophy and the reduced synaptic load of Aβ in $APP^{NL\text{-}G\text{-}F}$:$Apoe^{cKO}$ mice. These findings further support the result of recovered cognitive function suggesting a possible counteracting effect of Apoc1 on Apoe. Our study will provide insights into the role of microglial Apoe in cognitive function, plaque-associated pathology and the mechanistic link between Apoe and Apoc1 gene expression.

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Poster

PSTR326. ApoE and Associated Pathways II

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Program #/Poster #: PSTR326.12/F3

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

Support: NIH Grant P30 AG072973
KUMC Biomedical Research Training Program

Title: Primary mitochondrial dysfunction promotes APOE upregulation in neuronal models

Authors: *A. P. GABRIELLI[1], R. H. SWERDLOW[2];
[1]Cell Biol. and Physiol., Univ. of Kansas Med. Ctr., Kansas City, KS; [2]Univ. Kansas Sch. Med., Leawood, KS

Abstract: Objective: Identify the relationship between primary mitochondrial (mito) dysfunction and APOE upregulation across neuronal models and establish potential mechanisms.
Rationale: Genetic and post-mortem studies implicate both mito dysfunction and APOE in Alzheimer's disease (AD) pathogenesis. Evidence suggests that APOE, particularly APOE ε4, can form neurotoxic fragments that disrupt mito function. Comparatively little has been known about the role of mito function in APOE biology. Understanding this relationship could yield therapies that attenuate neurotoxic stress due to feedback between mito dysfunction and APOE.
Methods: SY5Y human neuroblastoma cells were subjected to either a partial or complete mtDNA depletion with ethidium bromide incubation to create a primary mito dysfunction. Mito dysfunction was also achieved in SY5Y cells through FCCP-induced manipulations of the mito membrane potential, oligomycin-induced ATP synthase inhibition, glucose deprivation, and mito ribosomal inhibition with chloramphenicol. APOE expression levels were assessed under each treatment condition through a combination of qPCR, western blotting, ELISA, and ICC. ATP synthase inhibition was also performed in human neural progenitor cells (NPCs) derived from iPSCs. A minimum of 6 biological replicates were assessed under each condition.
Results: Each condition producing a primary mito dysfunction resulted in significantly increased expression of APOE. Complete, chronic mtDNA depleted SY5Y (ρ0) cells exhibited a 65-fold increase in *APOE* mRNA, an 8-fold increase in secreted apoE protein, and a significant increase in intracellular apoE protein. Partial (95%) mtDNA-depletion caused a 4-fold increase in *APOE* mRNA. FCCP and oligomycin both produced a 2-fold increase in *APOE* mRNA. Glucose deprivation increased mRNA levels 5-fold, while ribosomal disruption with chloramphenicol contributed to a 3.5-fold increase. Human NPCs treated with oligomycin to inhibit ATP-synthase activity, exhibited a 10-fold increase in *APOE* mRNA.
Conclusions: Primary mito dysfunction, achieved through various mechanisms, was universally associated with a significant, and robust increase in APOE expression. This phenomenon was observed not only in human neuroblastoma cells, but also in noncancerous human neural progenitor cells. Understanding the mechanisms by which mito dysfunction upregulates APOE expression, particularly in carriers of the neurotoxic APOE ε4 isoform, could yield potential therapeutic targets that focus on the interplay between mitochondrial dysfunction and apoE biology in AD.

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Poster

PSTR326. ApoE and Associated Pathways II

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

Support: Greenberg Research Grant
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Dean's Research and Teaching Funds

Title: α-Synuclein treatment of iPSC-derived mature astrocytes homozygous for apolipoprotein E ε4

Authors: *A. KOOB, A. BRAHIMI, I. J. JENKINS, S. ATASHPANJEH;
Univ. of Hartford, West Hartford, CT

Abstract: Increasing evidence indicates that the apolipoprotein E ε4 allele (apoE4) is a risk factor for synucleinopathies such as dementia with Lewy bodies. Synucleinopathy is also a common pathological hallmark in Alzheimer's diagnoses, of which inheritance of apoE4 allele has long been associated with increased disease prevalence. Astrocytes remove and degrade neuronally derived α-synuclein (αS) via autophagy, and apoE4 has recently been shown to result in impaired autophagy. In order to further investigate the association between αS, apoE and autophagy, two types of astrocytes were challenged with αS: iPSC-derived mature astrocytes from a skin fibroblast of a patient homozygous for apolipoprotein E ε4 allele (astro-apoE4), and controls homozygous for apolipoprotein E ε3 allele (astro-apoE3). Astrocytes were exposed to monomeric native αS for 24 hours at 100 nM, 250 nM and 500 nM. αS conjugated to HyLite 488 confirmed αS uptake by astrocytes at all levels. However, autophagic flux, as evidenced by increased LC3B and decreased p62 expression, did not occur at 100 or 250 nM in astro-apoE4 cells. Also, analysis of LDH release indicated significant cytotoxicity in astro-apoE4 cells at all concentrations. Lamp1 and cd63 labeling to distinguish early and late autophagy confirmed disruptions to autophagy when comparing astro-apoE4 and astro-apoE3 cells. Therefore, further studies examining the mechanisms of apoE4 and autophagy in astrocytes may provide insight into the etiology of synucleinopathies.

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Poster

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

Support: NIA NIH Grant P01AG066591
NIA NIH RO1 Grant AG061879

Title: Apoe2 enhanced resilience to aging is mediated by eif4a1

Authors: *T. L. M. MCHUGH, N. MARKOV, J. BONS, L. MCFARLIN, S. MAK, K.-T. TSHILENGE, K. WILSON, B. SCHILLING, T. E. TRACY, L. M. ELLERBY;
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Abstract: The *E2* allele of Apolipoprotein E is associated with substantially increased odds for extreme longevity and confers protection against aging and Alzheimer's disease (AD). Meanwhile, the strongest genetic risk factor for late-onset sporadic AD is *APOE4*. While the detrimental effects of APOE4 have been well studied, little is known about how *APOE2* contributes to neuronal protection from general aging and AD. To address this knowledge gap, our lab generated a unique series of isogenic iPSCs engineered with CRISPR/Cas9 to express the three isoforms (i.e., APOE2, -3 and -4). Differentiation of this series into glutamatergic neurons and subsequent proteomic analysis by mass spectrometry identified EIF4A1, a eukaryotic initiation factor, as the key protein driving the separate clustering of the proteomic signature of the three isoforms. We verified by western blot that EIF4A1 is significantly increased in APOE2 neurons compared to APOE4. Additionally, by immuno-cytochemistry (ICC) we identified an increased intensity of EIF4A1 in second-order dendrites of APOE2 neurons compared to APOE4. Given EIF4A1's role in mRNA translation, we hypothesize its increased expression in APOE2 dendrites allows for enhanced regulation of local translation at the synapse. This is significant as local translation plays a critical role in synaptic plasticity, protein synthesis, and overall synaptic function, and dysregulation of this process is implicated in the pathology of various neurodegenerative and neurodevelopmental disorders, contributing to synaptic dysfunction. APOE4 is known to alter synapse function decades before the onset of AD and may promote increased synapse density and hyperactivity. We have identified an increased number of synapses in 7-week old APOE4 compared to APOE2 and APOE3 neurons by ICC and colocalization of the post-synaptic marker PSD95 and the presynaptic marker Synapsin. Supporting this data, we measured by whole-cell voltage-clamp electrophysiology and APOE4 neurons have an increased frequency of miniature excitatory post-synaptic currents (mEPSCs) compared to APOE2 and APOE3 neurons. Collectively, our data suggest that increased expression of EIF4A1 is responsible for APOE2's protection against increased neuronal synapse density and hyperactivity. By modulating the levels of EIF4A1 in APOE human neurons, we will determine if EIF4A1 is responsible for APOE2's protective effect against neuronal dysfunction, thus revealing the cellular and molecular mechanisms that underly APOE2's enhanced resilience to aging and AD.

Disclosures: T.L.M. McHugh: None. **N. Markov:** None. **J. Bons:** None. **L. McFarlin:** None. **S. Mak:** None. **K. Tshilenge:** None. **K. Wilson:** None. **B. Schilling:** None. **T.E. Tracy:** None. **L.M. Ellerby:** None.

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.15/F6

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

Support: NIH R01 AG067258

Title: The chemotherapeutic agent doxorubicin induces brain senescence, with modulation by APOE genotype

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Abstract: Many cancer patients experience serious cognitive problems related to their treatment, which can limit their ability to maintain treatment regimens. The molecular mechanisms of this cancer chemotherapy-induced cognitive impairment (CICI) are unknown, thus slowing the development of preventative approaches. We hypothesized that cancer chemotherapies could induce cellular senescence in the brain, creating a pro-inflammatory environment and damaging normal brain communication. We tested this hypothesis using the common chemotherapeutic agent doxorubicin in two independent mouse models. In the first model, we used mice that express tdTomato under the pdkn2a (p16) promoter; p16 is a regulator of cellular senescence, and its upregulation is demoted by the presence of fluorescently tagged cells. Two weeks after exposure to three doses of 5 mg/kg doxorubicin, the number of tdTomato positive cells were increased nearly three-fold in both the cerebral cortex and the hippocampus ($p<0.05$). This co-localization occurred in both neurons and microglia, but not astrocytes or oligodendrocyte precursor cells. In the second model, we used APOE knock-in mice; the APOE4 allele is a risk factor for CICI in humans and mouse models. We isolated RNA from the cerebral cortex of APOE3 and APOE4 mice from one to 21 days after a single dose of 10 mg/kg doxorubicin. Using NanoString analysis of over 700 genes related to neuroinflammation and RT-qPCR analysis of cerebral cortex transcripts, we found two-fold induction of four senescence-related genes at three weeks in the APOE4 mice: p21(cdkn1a), p16, Gadd45a, and Egr1. We conclude that doxorubicin promotes cellular senescence pathways in the brain, supporting the hypothesis that drugs to eliminate senescent cells could be useful in preventing CICI.

Disclosures: T. Demby: None. **P. Gross:** None. **L. Aitakli:** None. **J. Mandelblatt:** None. **J. Huang:** None. **G. Rebeck:** None.

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.16/F7

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

Support: Bright Focus foundation A2021025S
Cure Alzheimer’s Fund
Glaucoma Research Foundation
NIA/Mayo Clinic Alzheimer’s Disease Research Center P30 AG062677

Title: Regulation of meningeal lymphatic function by APOE

Authors: *N. DELIVANOGLOU[1], K. TODD[2], M. J. BARBER[1], S. PEREIRA DAS NEVES[1], R. SCHRADER[1], G. SANCHEZ[1], S. REGO[1], Y. A. MARTENS[3], G. BU[3], J. D. FRYER[2], S. DA MESQUITA[1];
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Abstract: The lymphatic vasculature that is present in the meningeal dura and drains cerebrospinal fluid (CSF) becomes dysregulated during aging and in models of neurodegenerative disorders such as Alzheimer's disease. Apolipoprotein E (apoE), in particular the isoform apoE4, increases the risk for late-onset sporadic AD. However, little is known about the sex-specific effects of *APOE4* expression on meningeal lymphatic morphology and drainage. In the present study, we are investigating the effects of human apoE3 or apoE4 on meningeal lymphatic function and immunity in female and male mice. We have evaluated the morphology of lymphatic vessels in dural whole mounts of *APOE3* and *APOE4* mice at different ages. This assessment was performed in both males and females. The drainage function of meningeal lymphatics has been assessed in the same groups of mice by *in vivo* imaging of CSF-derived fluorescent tracers into the deep cervical lymph nodes. We have also performed RNAscope and protein measurements using murine meningeal tissue to investigate the main cellular source(s) of apoE, and single-cell RNA sequencing to explore the cell-specific transcriptomic signatures linked to the changes in meningeal lymphatic function. We observed alterations in meningeal lymphatic vessel morphology that were apoE isoform and sex specific. *APOE4* expression also induced marked transcriptional changes in meningeal immune cells that accompany the altered lymphatic function. Altogether, our data suggests that *APOE4* expression induces a sex-specific deleterious immune response that affects meningeal lymphatic function and that rebalancing meningeal immunity and lymphatic drainage in *APOE4* expressing mice might have beneficial implications for brain function and cognitive behavior.

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Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.17/F8

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

Title: Targeting apoE-dyslipidation as a therapeutic for Alzheimer's Disease

Authors: *A. VALENCIA[1], D. BALU[1], C. T. LEWANDOWSKI[2], A. MOORE[1], S. BELLUR[1], J. M. YORK[1], M. LADU[1], G. THATCHER[3], L. M. TAI[1];
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Abstract: *APOE* is the greatest genetic risk factor for Alzheimer's disease (AD), with *APOE4* increasing AD risk up to 12-fold compared to *APOE3*. Therefore, it is important to identify therapeutic approaches that target mechanisms through which *APOE4* regulates AD-relevant pathology. One approach is to focus on the fundamental structural differences between apoE3 and apoE4. In the brain interstitial fluid, most, if not all apoE is found on lipoproteins. Importantly, data from our group and others suggest that apoE4-lipoproteins are poorly lipidated which may contribute to higher Aβ pathology in AD. Hence, the goal of this study was to determine if apoE4 lipidation can be restored pharmacologically to impact Aβ pathology and synaptic viability. Research on the complex and dynamic apoE-lipoprotein modeling processes is continually evolving. Currently, apoE is thought to be post-translational lipidated inside the cell and by ABCA1/G1 at the plasma membrane. We therefore tested two approaches to increase apoE lipidation in E4FAD mice that express *APOE4* and overproduce Aβ ($5xFAD^{+/-}$/$APOE4^{+/+}$). First, we focused on increasing ABCA1 at the transcriptional level using nuclear receptor (NR) agonists. We found that retinoid X receptor (RXR) agonists increased ABCA1/ABCG1 levels and apoE4 lipidation, reduced Aβ and increased synaptic viability. However, RXR agonists induced severe lipogenic hepatomegaly, limiting its use as AD treatment. We therefore developed a novel non-lipogenic LXRβ partial agonist and PPAR/RXR antagonist, CL3-3, which increased apoE4 lipidation, reduced Aβ levels and increased synaptic viability without inducing hepatomegaly in E4FAD mice. Our second approach was to increase free cholesterol availability for apoE lipidation by inhibiting Acyl-CoA:cholesterol acyltransferase (ACAT). ACAT inhibition reduced Aβ pathology and increased synaptic viability, but surprisingly did not increase apoE4 lipidation. Instead, ACAT inhibition reduced production of Aβ from the amyloid precursor protein (APP). Thus, ACAT inhibition may address a general lipid processing deficit associated with *APOE4* and Aβ, independent of apoE lipidation. Overall, our data support targeting lipid processing deficits including apoE4-dyslipidation as a key therapeutic approach for AD.

Disclosures: A. Valencia: None. **D. Balu:** None. **C.T. Lewandowski:** None. **A. Moore:** None. **S. Bellur:** None. **J.M. York:** None. **M. LaDu:** Other; posthumous submission. **G. Thatcher:** Other; Inventor on patents owned by the University of Arizona. **L.M. Tai:** None.

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.18/G1

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

Support: AARF-22-973952

Title: Apoe variant alters hyperexcitability associated with Alzheimer's disease in dentate granule neurons

Authors: *M. R. BERCHULSKI[1], S. SINGH[1], S. L. A. MARTIN[1], Z. BRIDGES[1], K. M. S. O'CONNELL[1], C. C. KACZOROWSKI[2];
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Abstract: Cognitive decline in Alzheimer's disease (AD) differs based on background genetics even when faced with similar pathology. Using genetic linkage analysis, we previously identified a coding variant in the receptor binding domain of ApoE that leads to changes in cognitive performance across a genetically diverse mouse population harboring familial (FAD) mutations known as the AD-BXDs (Neuner et al., 2019). Using CRISPR-based gene editing, we developed a C57BL/6J (B6) mouse carrying the ApoE allele from the DBA/2J (D2) strain ($ApoE^{E163D}$) and determined that $ApoE^{E163D}$ mice with 5XFAD transgene ($ApoE^{E163D}$-5XFAD) are more susceptible to cognitive decline compared to B6-5XFAD mice (Kaczorowski, et al., 2022). Analysis of hippocampal RNA sequencing profiles from the hippocampus indicates robust transcriptomic changes in dentate gyrus (DG) granule cells associated with cognitive resilience to FAD mutations in the presence of the B6 ApoE genotype. Here we aim to evaluate the functional implications. We adapted the Patch-Seq method (Cadwell et al., 2017) to investigate transcriptional, electrophysiological, and morphological changes within the DG associated with both FAD mutations and ApoE genotype. Whole-cell patch clamp was used to evaluate intrinsic excitability of granule cells from 14 month old B6 mice carrying FAD mutations and/or $ApoE^{E163D}$, compared to age matched controls immediately following contextual fear conditioning to evaluate cognition. The cells were filled with biocytin during the recording for staining and downstream analysis of morphology. The cellular contents, including the nucleus, were collected after completion of recording to characterize the transcriptome. We observed a significant interaction of the 5XFAD and ApoE genotypes on DG granule cell excitability. Specifically, $ApoE^{E163D}$-5XFAD DG granule cells required less current stimulus to elicit a single action potential, and to fire in response to a current ramp (rheobase). Although input resistance was comparable across all 4 groups, the sag ratio of $ApoE^{E163D}$-5XFAD was also reduced. Enhancement of DG excitability of $ApoE^{E163D}$-5XFAD mice may underlie increased seizure susceptibility that has been reported previously in FAD animal models on D2 genetic background and associated with increased risk of seizures in AD patients. Ongoing work will integrate DG excitability with single-cell RNAseq, morphology, and behavior to dissect mechanisms underlying the modifier effect of ApoE genotype on susceptibility and resilience to cognitive deficits in FAD mutation carriers.

Disclosures: **M.R. Berchulski:** None. **S. Singh:** None. **S.L.A. Martin:** None. **Z. Bridges:** None. **K.M.S. O'Connell:** None. **C.C. Kaczorowski:** None.

Poster

PSTR326. ApoE and Associated Pathways II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR326.19/G2

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

Support: NIH Grant R01AG53242

Title: Neuronal IDOL regulates LDLR-APOE pathway and affects amyloid pathology

Authors: *H. KARAHAN, M. M. AL-AMIN, S. WIJERATNE, S. K. JOHN, B. MCCORD, D. C. SMITH, H. M. RONDON CORDERO, J. MANTOR, D. J. ACRI, B. KIM, J. KIM; Indiana Univ., Indianapolis, IN

Abstract: In our earlier studies, we reported the critical roles of the low-density lipoprotein receptor (LDLR) in regulating ApoE level and amyloid-beta (Aβ) clearance in the brain. Overexpression of LDLR in the brain inhibits Aβ aggregation by decreasing ApoE levels and increasing Aβ clearance (1). Furthermore, we demonstrated that global deletion of the Inducible Degrader of LDLR (IDOL) gene markedly increases LDLR levels and decreases ApoE levels in the brain (2). Importantly, the loss of *Idol* expression significantly reduces amyloid plaque burden and ameliorates neuroinflammation in an APP/PS1 mouse model (2). To determine the cellular mechanisms by which IDOL affects the levels of ApoE and Aβ, we generated neuronal and microglial *Idol* conditional knock-out (cKO) mouse models by crossing *Idol*-floxed mice with the Camk2a-Cre and Cx3cr1-CreER mouse lines, respectively. To investigate the effect of cell type-specific deletion of *Idol* on Alzheimer's disease (AD) pathology, we crossed these *Idol* cKO mouse lines with the 5XFAD mouse model. We assessed the pathological changes in these mice by using biochemical and histological methods. While deletion of microglial *Idol* in 5XFAD mice did not cause a significant change in Aβ levels, neuronal *Idol* deletion significantly decreased Aβ accumulation in female mice. Furthermore, we found a significant increase in LDLR and a decrease in ApoE levels in female neuronal *Idol* cKO;5XFAD mice. Overall, these findings suggest that deletion of neuronal *Idol* can have a protective effect against Aβ accumulation in females which warrants further investigation. Since there was an increase in LDLR and a decrease in ApoE levels in female neuronal *Idol* cKO mice, these findings strengthen the critical role of the LDLR-ApoE axis in Aβ accumulation.
References
1) Kim et al., Neuron (2009) 64, 632-644. 2) Choi et al., Sci Transl Med (2015) 7(314):314ra184.2.

Disclosures: H. Karahan: None. **M.M. Al-Amin:** None. **S. Wijeratne:** None. **S.K. John:** None. **B. McCord:** None. **D.C. Smith:** None. **H.M. Rondon Cordero:** None. **J. Mantor:** None. **D.J. Acri:** None. **B. Kim:** None. **J. Kim:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.01/Web Only

Topic: C.03. Parkinson's Disease

Support: R01NS107513

Title: White Matter Microstructural Abnormalities in Parkinson's Disease Revealed Using Multi-Site Bundle Analytics: A Pilot Study

Authors: *C. OWENS-WALTON[1], B. Q. CHANDIO[2], C. MCMILLAN[3], P. OPRIESSNIG[4], K. POSTON[5], P. SCHWINGENSCHUH[4], M. SHAHID[5], D. TOSUN[6], S. I. THOMOPOULOS[2], Y. VAN DER WERF[7], N. JAHANSHAD[2], P. M. THOMPSON[2];
[1]Keck Sch. of Med., USC, San Francisco, CA; [2]Keck Sch. of Med., USC, Los Angeles, CA; [3]Neurol., Univ. of Pennsylvania, Philadelphia, PA; [4]Neurol., Med. Univ. of Graz, Graz, Austria; [5]Neurol. and Neurolog. Sci., Stanford Univ., Stanford, CA; [6]Ctr. for Imaging of Neurodegenerative Dis., San Francisco VA Med. Ctr., San Francisco, CA; [7]VU Univ. medical center, Amsterdam, Netherlands

Abstract: Diffusion MRI (dMRI) tractography studies have reported microstructural white matter (WM) deficits in Parkinson's disease (PD) across multiple brain networks. However, these studies have been performed in small samples, with low statistical power. In this pilot study, we investigate fine-scale WM microstructural differences along specific bundles in PD compared to controls (CN), using a multi-site BUndle ANalytics (BUAN) tractography approach. We analyzed 3D dMRI data from 492 PD participants (HY stage: 2.0±0.7; 64.5y±9.4; 34% female) and 124 CN (63.1±9.5y; 44% female) from 6 cohorts. Images were preprocessed using the ENIGMA-DTI pipeline. Constrained spherical deconvolution reconstruction and the deterministic EuDX tracking method were used to generate whole-brain tractograms. These were then registered to a bundle atlas template to extract bundles. Tract profiles were generated consisting of 100 segments/tract in each subject. PD and CN group comparisons were conducted using linear mixed models to localize differences in diffusion tensor imaging metrics. Group, age, and sex were modeled as fixed effects, and participant and site as random effects, adjusting for multiple comparisons using a false discovery rate. PD participants showed *lower* AD and RD in tracts in the brainstem (medial longitudinal fasciculus); *lower* RD and MD in tempo-parietal association tracts (middle longitudinal fasciculus); *lower* MD in fronto-temporal tracts (uncinate fasciculi); *higher* FA in ascending somatosensory tracts (spinothalamic tract); and *lower* RD and MD in ascending visual tracts (optic radiations; Fig1). Alterations to WM bundles in PD are extensive, impacting brain regions implicated in a range of clinical functions. Here we demonstrate that multi-site group-level tractometry analyses are possible, yielding significant power to better uncover microstructural WM abnormalities associated with PD. Future work will track how these effects depend on PD staging.

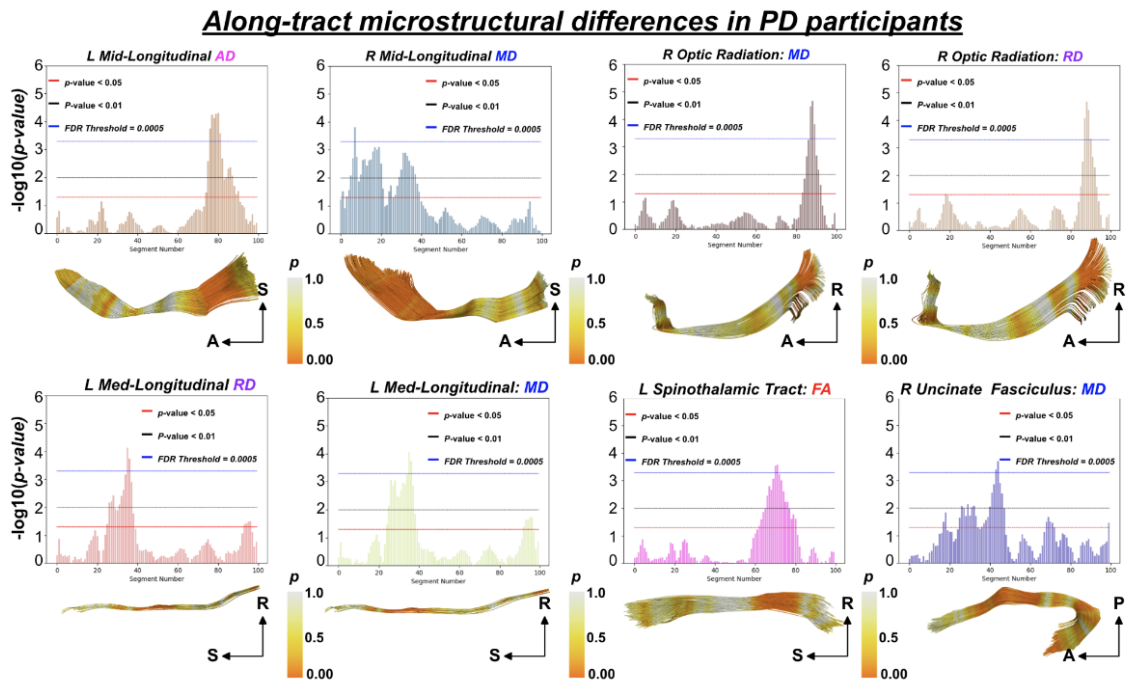

Fig 1: Localized along-tract microstructure alterations in Parkinson's disease (PD). Compared to healthy controls, the PD group exhibited microstructural differences in 8 tracts. **Abbreviations**: AD, axial diffusivity; MD, mean diffusivity; RD, radial diffusivity; med-longitudinal, medial longitudinal tract; mid-longitudinal, middle longitudinal fasciculus; p, p-value; A, anterior; P, posterior; S, superior; R, right.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.02/G3

Topic: C.03. Parkinson's Disease

Title: The Roles and Interplay of Reinforcement-Based and Error-Based Processes on Exploratory Behaviour in Neurologically Intact and Parkinson's Disease

Authors: *A. ROTH[1], J. H. BUGGELN[1], J. A. CALALO[2], R. LOKESH[2], S. R. SULLIVAN[1], T. T. NGO[1], M. J. CARTER[3], J. J. JEKA[2], J. G. A. CASHABACK[1];
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Abstract: Exploration is important when attempting to relearn motor skills following a neurological disorder. Reinforcement-based adaptation has been closely linked to the basal ganglia, which is compromised in Parkinson's disease. Error-based motor adaptation has been attributed to corrective processes in the cerebellum. Converging neuroanatomical evidence shows bidirectional connections between reinforcement-based (basal ganglia) and error-based (cerebellum) neural circuitries, suggesting an interaction between the two. Yet we know little on how reinforcement-based and error-based processes interact to influence behaviour. Here we designed two experiments and a computational model to investigate the unique and interacting roles of reinforcement and error feedback on motor exploration. Participants grasped the handle of a robotic manipulandum and made reaching movements to a large target that promoted exploratory behaviour, without vision of their hand. Participants received either reinforcement feedback (pleasant sound, monetary gain for a success) and/or error feedback (small cursor showing hand position) at the end of their reach. We computed trial-by-trial statistical random walks (lag-1 autocorrelations) to quantify exploration. In Experiment 1, both model predictions and empirical results show that neurologically intact individuals display significantly greater exploration with reinforcement feedback compared to error feedback ($p < 0.001$). Participants displayed moderate levels of exploration when receiving both forms of feedback, which was greater than isolated error feedback ($p = 0.03$) and less than isolated reinforcement feedback ($p < 0.001$). These results show that reinforcement-based processes and error-based processes lead to dissociable behaviour and interact to influence exploration. In Experiment 2 we considered those with Parkinson's disease, who have compromised reinforcement-based neural circuits. Parkinson's participants showed less exploration with reinforcement feedback compared to healthy age-matched controls ($p < 0.001$). Parkinson's participants displayed no difference in exploration compared to age-matched controls when receiving error feedback ($p = 0.431$) or both forms of feedback ($p = 0.273$). These results support the idea that reinforcement-based processes play a mechanistic role in boosting exploratory behaviour. Taken together, our results and model suggest that reinforcement-based and error-based processes respectively boost and suppress exploration, while in concert these processes oppose one another to result in moderate exploratory behaviour.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.03/G4

Topic: C.03. Parkinson's Disease

Support: Parkinson's Foundation Postdoctoral Fellowship for Basic Scientists Award PF-PRF-839073
NIH Grant R01NS9580

Title: Restoration of reduced M4-mediated cholinergic transmission in parkinsonian mice improves motor symptoms and alleviates levodopa-induced dyskinesia

Authors: *B. E. NIELSEN, C. FORD;
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Abstract: A proper balance between dopamine (DA) and acetylcholine (ACh) in the dorsal striatum is required for normal motor function, while imbalances are associated with movement disorders, such as Parkinson's disease (PD). Despite the classical assumption about their opposite roles, dopaminergic and cholinergic systems interactions are dynamic and coordinated, taking place at multiple levels, including direct modulation of striatal output cells through G protein-coupled receptors. Among ACh receptors, muscarinic M4 receptor has the highest expression level in the striatum, particularly in direct medium spiny neurons (dMSNs), and is the primary subtype involved in DA signaling modulation and related motor behaviors. However, how M4-mediated cholinergic transmission occurs at the synaptic level across striatum regions still remains unclear, as well as its alterations following DA depletion. Combining brain slice electrophysiology and two-photon imaging, we examined M4 transmission in dMSNs across dorsomedial (DMS) and dorsolateral (DLS) striatum in presence of normal DA levels and after DA loss in a 6-hydroxydopamine mouse model of PD. Although PD has been assumed as a hypercholinergic state, we found a significant reduction in the strength of M4-mediated cholinergic transmission in the DA-depleted striatum, being DLS more sensitive than DMS. Surprisingly, a decrease in postsynaptic M4 function and not a change in presynaptic ACh release was accountable for the observed impairment. We developed two strategies to rescue M4-cholinergic transmission selectively in dMSNs: overexpression of M4 receptors and ablation of regulator of G-protein signaling 4 (RGS4) to prolong intracellular signaling. Both strategies were effective in restoring transmission to normal levels and in improving balance and coordination motor deficits. While DA-depleted mice typically develop levodopa-induced dyskinesia after chronic levodopa treatment, rescuing M4-mediated cholinergic transmission also ameliorated the characteristic abnormal involuntary movements observed with dopamine-replacement therapy. Taken together, our findings reveal a progressive reduction in M4-mediated cholinergic transmission following DA loss with implications for motor symptoms in parkinsonian and levodopa-induced dyskinetic states, positioning cholinergic system, and in particular M4-signaling, as a promising therapeutic target for restoring DA-ACh balance.

Disclosures: B.E. Nielsen: None. **C. Ford:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.04/G5

Topic: C.03. Parkinson's Disease

Support: NFRF Exploration Grant

Title: The Virtual Brain for Parkinson Disease: Capturing pathological oscillations with dopamine-mediated circuits

Authors: H. SHEHEITLI[1], **D. DEPANNEMAECKER**[1], K. GUDIBANDA[1], A. MCINTOSH[2], P. SORRENTINO[1], V. JIRSA[1];
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Abstract: The Virtual Brain (TVB) is a computational framework with specialized data processing workflows that aims to simulate and model the dynamics of the human brain. Personalized brain network modelling in epilepsy has demonstrated its predictive power [1] and is being systematically evaluated in the prospective large scale clinical trial EPINOV for epilepsy surgery. Here we aim to adopt and translate the approach for studying Parkinson's disease. To accomplish this, it is essential to incorporate specific features relevant to Parkinson's disease within the TVB simulation framework. Notably, Parkinson's disease is characterized by an excessive occurrence of beta-burst activity in the basal ganglia. To capture these dynamics we modify a neural mass model based on a mean-field approach [2]. The novel neural mass model that now incorporates diverse types of synapses. We demonstrate how the model captures the transition from a resting state to beta oscillations and characterize its dynamical properties. Finally, we have integrated a basal ganglia model into TVB, based on this model, and we observed how dopamine-mediated modifications in the dorsal striatum can lead to beta oscillations within the basal ganglia network.
References:
[1] Jirsa V, Wang H, Triebkorn P, Hashemi M, Jha J, Gonzalez-Martinez J,et al. Personalised virtual brain models in epilepsy. The Lancet Neurol-ogy. 2023 May;22(5):443-54. Available from: https://doi.org/10.1016/s1474-4422(23)00008-x.
[2] Chen L, Campbell SA.Exact mean-field models for spiking neu-ral networks with adaptation. Journal of Computational Neuroscience.2022 Jul;50(4):445-69.Available from: https://doi.org/10.1007/s10827-022-00825-9.1

Disclosures: H. Sheheitli: None. **D. Depannemaecker:** None. **K. Gudibanda:** None. **A. McIntosh:** None. **P. Sorrentino:** None. **V. Jirsa:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.05/G7

Topic: C.03. Parkinson's Disease

Support: Parkinson Society Canada
NSERC Discovery to JFXD

Title: Learning dance modifies fMRI signals in regions associated with movement, mood, and reward in Parkinson's disease.

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Abstract: Research has identified multiple benefits of dance for people with Parkinson's disease (PwP). These include improvements in gait, balance, and posture, as well as a range of non-motor benefits including improvements in cognition, mood, and quality of life. However, the neural structures and mechanisms involved in these outcomes of dance for PwP remain unknown. The present analysis investigated behavioural and neural measures in 10 PwP (9 males; age 52-76 years; disease duration 0-17 years) attending dance classes over a period of 8 months. Functional MRI was conducted up to 4 times across the 8-month period. Participants imagined dancing in the scanner while listening to music associated with a learned choreography. A blocked design consisted of one-minute blocks of music and imagery alternating with 30-seconds of no music, repeated for 5 cycles. A general linear model was used to functionally map brain areas related to imagining the dance. Regions were activated using the GLM of music and imagery versus no music were auditory cortex, supplementary motor area (SMA), premotor area, parietal, insula, and cerebellum. To identify signals associated with mood circuitry, an anatomical seed was placed at the subcallosal cingulate gyrus (SCG) and BOLD signals were correlated with scores on the Geriatric Depression Scale (GDS) completed before and after class. There was a significant positive correlation between change in blood-oxygen-level-dependent (BOLD) signal and average pre-post change in GDS scores ($r(5)=0.83$, $t=-3.31$, $p<0.05$). To explore reward circuitry, an anatomical seed was placed in the ventral tegmental area, and the BOLD signal was modulated by learning the dance over time ($p<0.05$). Our results show modulations of BOLD signals across the 8-months in preSMA, SMA, SCG, ventral tegmental area and cerebellum, which suggests a putative mechanism of neuroplastic changes in motor and nonmotor regions as a function of learning dance in PwP.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.06/G8

Topic: C.03. Parkinson's Disease

Support: NIH NINDS 1R01NS129517-01

Title: Impairments in visual perception and cortical circuit function in a model of Parkinson's disease dementia

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder. In addition to motor deficits such as bradykinesia, tremor and rigidity, many patients experience non-motor symptoms, such as cognitive impairment. One of the most affected cognitive domains in PD patients is visuospatial/perceptual function and it has been hypothesized that these deficits are attributable to the dysfunction of specific cortical circuits. However, the mechanisms underlying cortical neuron dysfunction are not fully understood. Hence, we aim to understand how α-synuclein (α-syn), a pathological feature of PD, contributes to cortical circuit impairment in PD. We hypothesize that α-syn pathology directly impairs neuronal activity in cortical circuits, leading to visuoperceptive impairments. To study PD-associated visuoperceptive impairments, we have developed a model to monitor the progression of α-syn pathology and visuoperceptive function in relation to neuronal dysfunction in primary visual cortex (V1) of mice injected with pre-formed fibrils (PFF) into V1. Injection of PFFs directly into V1 leads to time-dependent formation of Lewy-like inclusions in cortical neurons. Using longitudinal in vivo two-photon calcium imaging in the visual system, we record evoked activity of pyramidal cells in layer 2/3 of V1 while mice passively view a series of sinusoidal gratings drifting in 8 directions and random dot kinematograms (RDKs). We then quantify the proportion of visually-responsive pyramidal cells and the distribution of orientation selectivity indices of cortical neurons as measures of visual cortical circuit function. In addition, we measure visuoperceptive function using a novel head-fixed coherent motion discrimination "go/no-go" task. After training, mice are able to perform significantly above chance when discriminating RDKs displaying motion in horizontal or vertical directions at 90% coherence. Introducing "test" trials with lower coherence values, we then quantify coherent motion discrimination thresholds as a measure of visuoperceptive function. Experiments are ongoing, but we expect that there will be fewer responsive cells and neurons may be more broadly tuned in PFF-injected mice compared to saline-injected controls. We also expect the average motion discrimination threshold to be higher, indicating impaired visuoperceptive function, in PFF-injected mice. Together, our results will shed light on the mechanisms by which α-syn pathology drives cortical circuit dysfunction and cognitive impairment in PD.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Topic: C.03. Parkinson's Disease

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Title: Parkinson by acute dopamine receptors blockade, or 6-OHDA lesion in Substantia Nigra pars compacta, produces changes in cortical oscillations and network dynamics

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Abstract: The primary motor cortex (M1) is a crucial brain region responsible for processing and executing motor commands by integrating inputs from the premotor cortex and activating glutamatergic pyramidal neurons. These neurons produce motor outputs to the basal ganglia (BG) and spinal cord. Functionality associated with BG activity is regulated by dopamine (DA). In patients with Parkinson's disease (PD) and in *in vivo* Parkinsonian mouse models, Beta oscillations (13 - 40Hz) have been detected in BG local field potential (LFP) recordings. In this research, a hemiparkinsonian mouse model with a unilateral lesion caused by 6-OHDA in the SNc was employed. This lesion depletes DA from the BG while leaving the ventrotegmental area (VTA) intact to maintain cortical DA modulation. The main question was whether DA depletion in the BG alone could cause increased beta rhythm in cortical slices *in vitro*, despite the fact that most BG-thalamo-cortical connections are severed in this model. The combined activity of numerous neurons was evaluated in conjunction with the local field potential using microelectrode arrays (MEAs) *in vitro*. The findings reveal that abnormal synchronization of the beta band in the motor cortex is observed in brain slices following DA depletion exclusively in the BG. This abnormal synchronization was eliminated after levodopa administration. This scenario was compared with the situation of acute administration of DA receptor antagonists. Abnormal beta oscillations were also observed in the motor cortex, but with differing sequential network states and reconfiguration of functional connections. The observation of cortical beta oscillations *in vitro* due to the absence of DA in the BG suggests that these oscillations may originate outside the BG-thalamo-cortical loop.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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UCSB Institute for Collaborative Biotechnologies under Cooperative Agreement W911NF-19-2-0026 with the Army Research Office

Title: Resting-state functional mapping of open loop circuit motor network connectivity with ultra-high field MRI in humans

Authors: *E. RIZOR, N. DUNDON, J. WANG, J. STASIAK, R. LAPATE, S. GRAFTON; Univ. of California, Santa Barbara, Goleta, CA

Abstract: Individuals suffering from Parkinson’s Disease (PD) may experience paradoxical kinesia (PK), a phenomenon characterized by the ability to temporarily overcome bradykinesia (slowness of movement) and display relatively normal motor functioning. This phenomenon occurs despite the known detrimental impact of PD on the closed loop circuit (CLC) motor network through the dorsal putamen and basal ganglia. Here, we hypothesize that there is preservation of function in a separable open loop circuit (OLC), wherein signals originating from the amygdala are posited to travel to motor cortex (M1) via the ventral putamen and cholinergic neurons of the nucleus basalis of Meynert (NB). While this putative pathway may be relatively preserved in PD and underlie PK, precise identification of maximally connected voxels between OLC nodes has not been characterized using measures of functional connectivity (FC) derived from high-resolution fMRI. Recent advancements in multi-echo imaging have allowed for greater separation of BOLD from the non-BOLD signals that significantly reduce the quality of single-echo FC investigations. Therefore, we acquired high-resolution multi-echo resting-state fMRI scans (voxel size=1.5x2x2mm^3) with a Siemens 7T Terra ultra-high field scanner from 6 young, healthy individuals (average age = 27.0 years, 4M/2F). EPI images underwent ME-ICA denoising, followed by mapping of the OLC using the amygdala, putamen, NB, and M1 as seed regions. Exploratory FC analyses showed that the amygdala was positively functionally connected to bilateral M1, the putamen (peak voxels in ventral putamen), and NB ($t>4$, $p<0.05$). NB was positively connected to all other OLC nodes, notably in the left ventral putamen, bilateral central nucleus (CN) of the amygdala, and bilateral basolateral nucleus (BLN) of the amygdala ($t>4$, $p<0.05$). The putamen was positively connected with bilateral dorsolateral M1 and NB ($t>4$), with peak voxels in right dorsolateral M1 and left NB ($t>9.34$, $p<0.05$). Finally, M1 was positively connected to bilateral putamen (peak voxel along the ventrolateral putamen/insula border, $t=28$), bilateral NB, and the left BLN of the amygdala ($t>4$, $p<0.05$). These results suggest that the hypothesized nodes in this putative pathway are functionally connected to one another, and that the ventral/lateral putamen, the CN and BLN of the amygdala, and bilateral NB may be an important sites of OLC activation. The precise mapping of OLC nodes in healthy adults can serve as a guidepost for PD researchers, paving the way for future targeting of the OLC as a potential treatment for the onset and symptomatology of PD.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.09/H1

Topic: C.03. Parkinson's Disease

Support: NIH P20NS123151

Title: Human subthalamic nucleus activity during interval timing tasks in Parkinson's disease

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Abstract: Cognitive and executive function deficits in Parkinson's disease (PD) have a huge impact on quality of life and independence. In PD, the perception of time and interval timing is one well-documented aspect of impairment, with PD patients demonstrating high performance variability and low accuracy. Research using fMRI and electrophysiological data has linked these timing deficits to the thalamo-cortico-striatal circuit, implicating basal ganglia structures and the subthalamic nucleus (STN) in timing. Stimulation of the STN can improve performance on timing tasks in individuals with PD, but the mechanisms for this are unclear. Eight individuals with Parkinson's disease (5 M, 3 F) between 55 and 75 years of age participated in a simple interval timing task requiring the participant to respond after they perceived that intervals of either one or three seconds had elapsed from an auditory cue. The task was completed intraoperatively during surgical implantation of Deep Brain Stimulator (DBS) electrodes in the STN, which allowed for electrophysiological recordings directly from STN during task performance. Simultaneous single- and multi-unit spikes were recorded from the STN using microelectrodes. Spikes were sorted and epoched offline, resulting in 49 identifiable single- and multi- units. Modulation indices were calculated and used to compare firing patterns before, during, and after both cues and responses. Firing patterns within the STN were heterogenous, with clear modulation of a subset of neurons further supporting the role of the STN in timing tasks. The participants demonstrated high variability in response time, consistent with previous evidence of deficits in timing in PD. These preliminary results support the role of the STN in timing tasks, and further research is needed to determine functional connectivity between the STN and frontal cortices to better elucidate the neurophysiological mechanisms of timing deficits in individuals with Parkinson's disease.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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Program #/Poster #: PSTR327.10/H2

Topic: C.03. Parkinson's Disease

Support: Boston Scientific Postdoctoral fellowship

Title: Topography and waveform morphology of neurons within the subthalamic nucleus of patients suffering from Parkinson's disease

Authors: *S. VASNIK[1], H. H. SUBRAMANIAN[2], H. S. BOKIL[3], P. DOSHI[1];
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Abstract: Background: Microelectrode recordings (MER) are routinely undertaken during deep brain stimulation (DBS) surgery to determine the optimal positioning of the DBS lead. We studied the topology and waveform morphology of the subthalamic nucleus (STN) to understand the cellular pathophysiology of Parkinson's disease. **Methods:** Intraoperative MERs were acquired from 7 subjects undergoing DBS surgery. Analysis was carried out using custom-built scripts on Matlab R2021b. The spike time, amplitude, rate, duration, and morphology of clusters were annotated, and the anatomical location was estimated using Lead-DBS toolbox ver.2.5.2. **Results**: We identified 182 clusters (17 MER tracks) distributed within the STN. Five types of firing patterns were observed: irregular (I) (53), tonic (T) (40), irregular burst (IB) (37), periodic burst (PB) (27), and group discharge (GD) (25). Based on waveform morphology, spikes were classified as positive dominant (23), negative dominant (81), biphasic negative (39), biphasic positive (24), and triphasic (15). For reporting the spatial location, we divided the STN in the anteroposterior axis into anterior, central, and posterior compartments, which were further subdivided in the mediolateral and dorsoventral axes (12 segments). The anterolateral dorsal region showed T and I firing patterns, and the ventral region exhibited T, I, and IB. No track was found in the anteromedial region. The dorsal centromedial region showed T, I, PB, and IB, and the ventral part had T, I, and GD. The centrolateral dorsal and ventral region had I, IR, and PB discharge. The posteromedial STN is a rarely spiking region. Finally, the posterolateral dorsal STN demonstrated T, I, and axonal spikes, and the posterolateral ventral region had I, IB, and PB. **Conclusions**: The anterolateral STN contains tonically firing fast-spiking interneurons. The centromedial region predominantly has IB neurons, in contrast to the centrolateral STN, which contains neurons that burst phasically. The posteromedial region is a non-spiking area and is supported by small RMS values suggestive of low neuronal density. The posterolateral STN has a high density of cells that exhibit irregular firing with a few axonal spikes. The understanding of STN cellular physiology is critical in optimizing DBS therapy for PD. Our study elucidates salient neuronal subtypes in PD patients. **Declaration:** This study was wholly undertaken at Jaslok hospital & Research Center under the institutional ethics approval. **Patients undergoing DBS surgery were implanted with Boston Scientific Leads for treatment of Parkinson's disease.**

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.11/H3

Topic: C.03. Parkinson’s Disease

Support: ASAP-020572

Title: Elucidating cortical dysfunction and impaired motor dexterity in parkinsonism

Authors: *H. CHEHADE, D. BEREZHNOI, H.-Y. CHU;
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Abstract: Background: Impaired motor dexterity often occurs in people with Parkinson’s disease (PD), a devastating progressive neurodegenerative disorder. However, the underlying pathophysiology of the dexterity deficits remains elusive. Recent research reports that proper control of skilled motor activity largely depends on an intact motor cortical function. Thus, the goal of the present study is to elucidate the role of motor cortical circuits in the development of impaired dexterity in parkinsonian state. **Methods:** We first evaluate acquisition and performance of forelimb reach-to-grasp task in a mouse model of progressive parkinsonism (MitoPark). Second, we correlate the forepaw kinematics with cortical neuronal activity using *in vivo* GCaMP6f imaging. **Preliminary results:** Our results show that, prior to 10 weeks of age, there is no difference in the learning curve of MitoParks compared to controls. However, kinematics analysis suggests an increased variability of endpoint of reaches in 10 weeks old MitoParks compared to littermate controls. We are currently working on Ca^{2+} signal analysis as well as their correlation with different stages of forelimb movements. Studies from the other age groups are undergoing.

Disclosures: H. Chehade: None. **D. Berezhnoi:** None. **H. Chu:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR327.12/H4

Topic: C.03. Parkinson’s Disease

Support: The Michael J. Fox Foundation for Parkinson's Research 18691

Title: A dual-hit mouse model of Parkinson's disease with anxiety and depression symptoms that precede the onset of motor impairment

Authors: *A. VAZQUEZ, E. YAO, R. VAN DER MERWE, C. DURKEE, B. D. MARGOLIN, S. CHATTERJEE, A. KREITZER, M. WOOD, K. R. THOMPSON;
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Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by tremor, rigidity, bradykinesia, and balance problems. Although PD is a movement disorder, there is high comorbidity with depression and anxiety, with up to 50% of all PD patients experiencing these psychiatric symptoms over the course of their disease. Dopamine replacement therapies do little to improve psychiatric symptoms, which often appear several years prior to the onset of motor symptoms. To investigate the basis of anxiety and depression symptoms related to neurodegeneration in PD, we focused on the GBA1 D409V KI x mThy1-hSNCA double transgenic mouse line (GBA x aSyn), recently developed by the Michael J. Fox Foundation (Polinski et al., 2022). This dual-hit model combined the strongest genetic risk factor for PD (GBA1 gene mutation) with the most well-validated driver of PD pathology (overexpression of alpha-synuclein). We performed a brain-wide histological analysis in 24-month-old mice to identify signs of pathology. Brain slices from GBA x aSyn and age-matched C57BL/6NJ control mice were stained for phosphorylated alpha-synuclein (pSer129), tyrosine hydroxylase, choline acetyltransferase, and serotonin. In male GBA x aSyn brains, pSer129 was significantly elevated in brain regions involved in the regulation of movement and mood. Slices from female GBA x aSyn mice showed less evidence of pathology. To understand whether there was an impact on behavior, male and female GBA x aSyn mice and controls were run through a behavioral test battery at 6, 12, 18, and 24 months of age to define measures of locomotion and thigmotaxis (open field test), exploratory anxiety (light-dark test), anhedonia (sucrose preference), sociability (3-chamber interaction), and behavioral despair (tail suspension test). At the 6-month time point, male GBA x aSyn mice displayed hyperlocomotion, light-dark anxiety, and tail suspension immobility. At the 12-, 18-, and 24-month time points GBA x aSyn mice continued to display anxiety and immobility, as well as deficits in locomotor activity. In line with histological results, female GBA x aSyn mice displayed a milder behavioral phenotype profile compared to males, with consistent defects observed only on locomotion and tail suspension immobility. Additional local field potential electrophysiology and pharmacological experiments were conducted in separate cohorts of male GBA x aSyn mice to define the neural circuit dynamics that contribute to mood and movement dysregulation. Results point to the preclinical utility of the GBA x aSyn line as a model of prodromal PD that displays multiple psychiatric symptoms and a later onset motor impairment.

Disclosures: **A. Vazquez:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **E. Yao:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **R. Van der Merwe:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **C. Durkee:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **B.D. Margolin:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **S. Chatterjee:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **A. Kreitzer:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **M. Wood:** A. Employment/Salary (full or part-time):; MapLight Therapeutics. **K.R. Thompson:** A. Employment/Salary (full or part-time):; MapLight Therapeutics.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

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KTH Digital Futures

Title: Detailed modelling and topological analysis of the striatal microcircuitry in health and Parkinson's disease

Authors: *I. CARANNANTE, J. HJORTH, A. KOZLOV, M. SCOLAMIERO, L. GUO, A. KUMAR, W. CHACHÓLSKI, J. HELLGREN KOTALESKI;
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Abstract: Simulating large-scale networks of neurons is an important approach both when interpreting and synthesising experimental data from healthy and diseased brains. Moreover, simulations are a powerful tool to investigate the relationship between the structure and network dynamics within a network and can provide insights into both healthy and disease states.
Here we focus on the striatum, the main input stage and the largest nucleus of the basal ganglia. The basal ganglia are involved in motor learning, action-selection and reinforcement learning. Dysfunction in these areas lead to a variety of brain disorders such as Parkinson's disease (PD).
We built a full-scale data driven model of the mouse striatal microcircuitry using available data on cellular morphology, electrophysiological properties, and cell density. Synaptic connectivity is predicted based on touch detection in combination with pruning rules (Hjorth et al. 2020, 2021). In addition we model the progression of Parkinson's disease. The morphologies are iteratively degenerated and the changes in connectivity are estimated. In particular, neurodegeneration is modelled as a progressive loss (PD0 control, PD1, PD2, PD3) of the most distal fragments of the dendritic arbours of the striatal projection neurons (SPN). The compensatory growth of fast spiking (FS) interneuron axons that takes place during early stages of PD has also been included. These processes lead to substantial reduction of the number of distal synapses on SPNs, and the addition of some GABAergic FS synapses, mimicking the disease progression.
Single-cell models, for both healthy and PD states, are optimised using BluePyOpt. Each model is fitted to single cell somatic voltage recordings and is able to describe both the subthreshold and suprathreshold behaviours.
In order to compare and quantify the connectivity and structure of the healthy and Parkinsonian network, we applied topological methods.
In particular directed cliques analysis was used, quantifying groups of neurons sharing at least one presynaptic (source) and one postsynaptic neuron (sink). The distribution of the sizes of such local connectivity motif (simplices) were counted and their composition with regard to cell types were investigated. We have shown that progressive dendritic degeneration not only alters the global connection probabilities but also dramatically affects statistics of simplices. We found that

interneurons, despite being the minority, can have a surprisingly large effect on the distribution of simplices. These results suggest that interneurons may play a crucial role in shaping the striatal network structure during PD progression.

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Poster

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Title: Frequency probe of DBS local evoked potentials in the subthalamic nucleus suggests no resonance in therapeutic range

Authors: *J. A. DALE[1], S. L. SCHMIDT[1], J. J. PETERS[1], D. A. TURNER[1,3], W. M. GRILL[1,2,3];
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Abstract: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective treatment for the motor symptoms of Parkinson’s disease (PD). Despite its success, DBS settings are difficult to optimize due to the large parameter space. Moreover, PD symptoms vary throughout the day based on medication state and internal state (e.g., sleeping vs. awake, sitting vs. walking). DBS local evoked potentials (DLEPs) have recently received attention as a potential biomarker to accelerate DBS programming or to serve as a feedback signal for adaptive DBS. DLEP amplitude correlates with clinician-chosen stimulating contacts for DBS (Xu et al. 2022), but it is unknown how changes in DBS parameters such as frequency affect the DLEP amplitude and this lack of information complicates analysis and interpretation of DLEP characteristics. For example, the presence of DLEP resonance would suggest greater symptom relief at lower stimulation amplitudes if an appropriate stimulation frequency is selected.
We measured DLEPs in response to a range of DBS frequencies in participants with PD receiving STN lead implants. All procedures were approved by Duke Health IRB and the participants gave informed consent. Each participant received ten second trials of DBS at varying frequencies with ten second periods of no DBS between trials. We also simulated DBS across the same frequencies in a biophysical model of DLEP generation (Schmidt et al. 2020). The average DLEP amplitude decreased with increasing DBS frequency (Mann-Kendall trend test, p=0.0003,

N=4). In line with our experimental data, the model simulations also predicted decreasing DLEP amplitude with increasing DBS frequency. These results indicate that frequency must be considered and controlled when evaluating and comparing DLEP amplitudes. Furthermore, this work gives greater insight into the mechanisms of DLEP generation and the properties of the network that produce this phenomenon. This frequency probe experiment suggests DLEPs are not resonant in the therapeutic range of DBS frequencies.

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Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.15/H7

Topic: C.03. Parkinson’s Disease

Title: Mid-frontal theta and beta oscillations can classify Parkinson’s disease with freezing gait

Authors: S. ROY, K. C. SANTOSH, ***A. SINGH**;
Univ. of South Dakota, Vermillion, SD

Abstract: People with Parkinson's disease (PD) who experience Freezing of Gait (FOG) are at risk of falling and have a reduced quality of life. Abnormal mid-frontal cortical oscillations have been linked to PDFOG+ but have not been extensively studied to distinguish between PDFOG+ and PDFOG- using machine learning models. In this study, we used resting-state mid-frontal cortical oscillatory features and employed shallow machine learning models to classify PDFOG+ and PDFOG-. We collected scalp electroencephalography (EEG) recordings from 82 PD patients (41 PDFOG+ and 41 PDFOG-) and 41 healthy age-matched controls. We segmented EEG signals into 3-second segments and analyzed the absolute power values from different frequency bands for classification models. But our focus was on mid-frontal theta (4-7 Hz) and beta (13-30

Hz) frequency bands, based on our previous reports. We implemented six machine learning algorithms and used a k-fold cross-validation approach to classify PDFOG+ from PDFOG- and healthy controls. Our results showed that the Deep Neural Network model had the highest accuracy, precision, recall, and F1-score in differentiating PDFOG+ from PDFOG- and healthy controls using a) mid-frontal theta power, b) mid-frontal beta power, and c) a combination of mid-frontal theta and beta power values. Deep Neural Network model performed the best in classifying PDFOG+ based on combined mid-frontal theta and beta power values, with an accuracy of 78%, compared to other models and cortical regions. Our machine learning classifying methods demonstrate the effectiveness of mid-frontal theta and beta power values in identifying PDFOG+. This study enhances our understanding of the cortical characteristics of PDFOG+ during the resting-state condition and may contribute to improving the objective classification of PDFOG+.

Disclosures: S. Roy: None. **K.C. Santosh:** None. **A. Singh:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.16/H8

Topic: C.03. Parkinson’s Disease

Support: NIH Grant K99NS131447

Title: Mapping Neural Responses to Deep Brain Stimulation: Comparing Subject Specific and Normative Approaches

Authors: *D. H. LENCH, G. J. REVUELTA;
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Abstract: Background: Deep brain stimulation (DBS) of the subthalamic nucleus (STN) is an effective treatment to improve motor fluctuations and medication induced dyskinesias in patients with Parkinson’s Disease. Despite its success, STN DBS inconsistently improves gait, and can cause unwanted side effects such as changes in cognition. Understanding how DBS modulates brain networks has the potential to improve targeting and parameter optimization to address these challenges. To date most studies have investigated DBS effects on networks using normative connectomes derived from large samples of healthy controls, however, subject specific approaches may more accurately capture heterogeneity in neural response to DBS. In this study, we assessed the feasibility of subject specific DBS-fMRI and compared findings to a normative approach. **Methods:** Four participants with STN DBS implants were recruited to undergo an fMRI scan on a 1.5T Siemens Sola MRI scanner. Prior to scanning stimulation was set to a bipolar mode and cycled on and off every 30 seconds. Blocks of stimulation were modeled in SPM12 to identify brain regions with a BOLD response to the stimulation. Lead trajectories were reconstructed in LEAD-DBS. Volumes of tissue activated (VTAs) were

modeled and used as seeds in normative connectome analyses derived from 1000 healthy adult brains from the Brain Genomics Superstruct Project (GSP). Brain regions engaged by DBS in the subject specific and normative approaches were compared. **Results:** Brain regions activated in the subject specific DBS-fMRI included primary motor cortex, supplemental motor area, pallidum, and parietal cortex (voxel threshold $p<0.01$, cluster $k>50$). While three participants showed increased motor cortex (M1) BOLD responses, one participant showed a reduction in BOLD signal within M1 in response to stimulation. Artifact associated with the lead and lead extensions primarily effected the posterior temporo-parietal junction and the STN. Across all participants normative functional data demonstrated VTA connectivity (Fisher $z > 0.1$) to the putamen, pallidum, thalamus, medial cerebellum, and anterior cingulate cortex, and anti-correlated connectivity (Fisher $z < -0.1$) with sensorimotor cortex. **Conclusions:** Combined DBS fMRI is feasible in participants with MRI compatible devices and results in heterogenous brain activation patterns. Relative to normative datasets, subject specific DBS-fMRI may provide complimentary yet unique information about DBS effects on brain networks. Future studies in larger samples will examine the relationship of DBS-fMRI activation patterns to behavioral outcomes, and stimulation settings.

Disclosures: D.H. Lench: None. **G.J. Revuelta:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.17/H9

Topic: C.03. Parkinson’s Disease

Title: Segregated cortico-subthalamic nucleus connectivity underlies cardinal features of Parkinson´s disease

Authors: *R. RODRIGUEZ-ROJAS, J. MÁÑEZ-MIRÓ, J. PINEDA-PARDO, M. DEL ÁLAMO, R. MARTINEZ-FERNANDEZ, J. OBESO;
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Abstract: Imaging-guided focused ultrasound targeting the subthalamic nucleus (FUS-STN) improve all Parkinson´s disease (PD) motor signs. Variable benefit in cardinal features is recognized, and tremor and rigidity are known to improve more than bradykinesia after treatment. However, the underlying pathophysiological basis for such differences remain unclear. This study aimed to define correlations between the topography of FUS-STN lesions and the improvement of PD cardinal signs. We analyzed volume and location of FUS-STN lesions in the T1/T2 MRIs at 24-hours post-procedure in 39 PD patients treated unilaterally. Lesion topography was correlated to changes in the motor UPDRSm from baseline to last follow-up in the “off” medication state. Sub-items for bradykinesia, rigidity and tremor from body side contralateral to subthalamotomy were scored and analyzed independently. Hierarchical multiple regression model analyses were used to examine the relationship of lesion location to

clinical outcome. Antiparkinsonian effect in the off state on the treated side was 55.9% and specifically change from baseline was 54.4, 46.6 and 74.8 % for rigidity, bradykinesia and tremor respectively. Benefit on bradykinesia was associated with impacting the rostral motor subthalamic nucleus subregion connected to the supplementary motor area (***r***=-0.34; P=0.041). Conversely, anti-tremor effect was explained by more lateral and caudal lesions thus within the STN projecting to the primary motor cortex (***r***=0.48; P=0.006). Rigidity was related to the impact between bradykinesia and tremor spots, being significantly correlated with the volume of the STN ablation (***r***=0.492; P=0.002). These findings suggest separated circuits underlying each cardinal feature and thus a pathophysiological segregation of the STN motor region.

Disclosures: R. Rodriguez-Rojas: None. **J. Máñez-Miró:** None. **J. Pineda-Pardo:** None. **M. del Álamo:** None. **R. Martinez-Fernandez:** None. **J. Obeso:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.18/H10

Topic: C.03. Parkinson’s Disease

Support: ASAP grant

Title: M1 cortical neuron contributions to Parkinson’s disease pathology: computer models

Authors: *D. W. DOHERTY[1], L. CHEN[2], H.-Y. CHU[3], W. LYTTON[4];
[1]SUNY Downstate Hlth. Sci. Univ., Pittsburgh, PA; [2]Neurodegenerative disorder department, Van Andel Res. Inst., Grand Rapids, MI; [3]Van Andel Inst., Grand Rapids, MI; [4]DHSU, Brooklyn, NY

Abstract: The focus in studies of Parkinson’s disease (PD) pathology has long been on substantia nigra (SN), where major anatomical pathology occurs, and on striatum, which receives major projections from SN. Recent evidence shows decreased excitability of primary motor cortex (M1) pyramidal-tract type (PT) neurons of layer 5B in mouse models of PD. PT dysfunction may be particularly important in producing motor symptoms because these are the cells that project downward to brainstem and spinal cord, leading to the direct motor expression of PD pathology. PD pathophysiology has been associated with timing anomalies. Using NEURON/NetPyNE simulators, we implemented detailed computer simulations of PT neurons alone and in the network context, generating pathophysiological models from healthy vs 6-OHDA-treated mouse slice data, matching current-frequency curves. Pathophysiological PT parameter alterations included an increase in NaT and especially BK currents. The simulated control and 6-OHDA PT neurons were next run in a simulated M1 slice. Self-organized and self-sustained activity was initiated in the M1 model using a brief stimulating current (100 ms; 57 nA) to seven layer 5B intratelencephalic pyramidal neurons. Stimulation in simulated control M1 slices resulted in sustained PT spiking activity. In contrast, simulated M1 slices using 6-OHDA

PT neuron simulations produced bursts of activity in the delta range. Our simulations will be examined further to indicate how relatively small changes in PT neuron excitability alters activity throughout the circuit in a way that might disrupt motor activity in Parkinson's disease.

Disclosures: D.W. Doherty: None. **L. Chen:** None. **H. Chu:** None. **W. Lytton:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.19/I1

Topic: C.03. Parkinson's Disease

Support: Grant, Ministerio de Industria, Economía y Competitividad SAF2017-86246-R
Fellowship, Comunidad de Madrid 2017-T2/BMD-5231

Title: Dopamine-independent cortical disinhibition in manifested Parkinson's disease

Authors: *C. AMMANN[1,2], M. DILEONE[1], C. PAGGE[1,3], D. MATA-MARÍN[1], M. MATARRAZZO[1], M. H. MONJE[4], Á. SÁNCHEZ-FERRO[1], B. FERNÁNDEZ-RODRÍGUEZ[1], C. GASCA-SALAS[1], J. MÁÑEZ-MIRÓ[1], R. MARTÍNEZ-FERNÁNDEZ[1], L. VELA-DESOJO[1], F. ALONSO-FRECH[1], A. OLIVIERO[5], J. OBESO[1,6], G. FOFFANI[1,6,5];
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Abstract: Primary motor cortex alterations observed in Parkinson's disease (PD) are classically attributed to the secondary effects of dopaminergic degeneration in the nigrostriatal pathway and altered basal ganglia output. Conversely, our recent cross-sectional study, involving a relatively large sample size, revealed that cortical disinhibition in PD seems to be independent on the stage of the disease (Ammann et al., 2020), as determined through short-interval intracortical inhibition (SICI) using transcranial magnetic stimulation (TMS). This finding indicates that cortical disinhibition may not be influenced by the extent of nigrostriatal degeneration and altered basal ganglia output throughout the evolution disease after diagnosis (i.e. in manifested PD). Thus, the objective of the present study was to formally validate this hypothesis.We employed paired-pulse SICI protocols on both hemispheres in a relatively large sample of PD patients tested off medication. Additionally, SICI was evaluated in one subset of patients after administration of 100-150% of the morning dose of levodopa medication. In a second subset of recently diagnosed patients, SICI was reassessed after a period of 1-5 years from the initial evaluation. All statistical analyses were performed with Bayesian methods.Our findings demonstrated comparable levels of cortical disinhibition (i.e. reduced SICI compared to healthy

controls) in both hemispheres when comparing the clinically more affected and less affected side of PD patients. Also, cortical disinhibition did not show recovery following an acute and clinically effective dose of levodopa. Furthermore, cortical disinhibition did not worsen after 1-5 years of clinically evident disease progression.These results provide evidence that cortical disinhibition is predominantly dopamine-independent in manifested PD. Cortical disinhibition may thus either arise as an early compensatory mechanism during the prodromal phase of PD in response to initial dopamine depletion or act as a life-long risk factor for the development of the disease. *Ammann C; Dileone M; Pagge C; Catanzaro V; Mata-Marín D; Hernández-Fernández F; Monje MHG; Sánchez-Ferro A; Martínez-Fernández B; Vela-Desojo L; Alonso-Frech F; Oliviero A; Obeso JA; Foffani G. 2020. Cortical disinhibition in Parkinson's disease. Brain. 143-11, pp.3408-3421.*

Disclosures: C. Ammann: None. **M. Dileone:** None. **C. Pagge:** None. **D. Mata-Marín:** None. **M. Matarrazzo:** None. **M.H. Monje:** None. **Á. Sánchez-Ferro:** None. **B. Fernández-Rodríguez:** None. **C. Gasca-Salas:** None. **J. Máñez-Miró:** None. **R. Martínez-Fernández:** None. **L. Vela-Desojo:** None. **F. Alonso-Frech:** None. **A. Oliviero:** None. **J. Obeso:** None. **G. Foffani:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.20/I2

Topic: C.03. Parkinson’s Disease

Support: BRFSG-2022-06

Title: Chloride dysregulation in the substanitia nigra pars reticulata of 6-OHDA parkinsonian rats

Authors: *A. R. UPRETY, J. WOO, A. OSTROUMOV;
Pharmacol. and Physiol., Georgetown Univ., Washington, DC

Abstract: Parkinson’s Disease (PD) is a neurogenerative disorder hallmarked by progressive loss of dopaminergic neurons of the substantia nigra pars compacta and basal ganglia (BG) dysfunction. PD has been found to result in increased firing, irregular, synchronous and oscillatory activity within the BG. While these electrophysiological changes have been linked to behavior their mechanisms are not fully understood. To resolve if alterations of gamma-aminobutyric acid (GABA) signaling explain the aberrant electrophysiological properties observed in PD, we performed slice electrophysiology within the substantia nigra pars reticulata (SNr) using a unilateral 6-hydroxydopamine induced dopamine depletion rat model. We found an increased chloride reversal potential and an impaired chloride extrusion capability in the dopamine depleted hemisphere of SNR GABA neurons. Further, we found that intra-nigral inhibition is altered in the PD state. Lastly, we found that found that stimulation of GABAergic

terminals arising from the globus pallidus externus (GPe) resulted in an increased firing rate of SNr GABA neurons. Our previous studies demonstrate that depolarized chloride reversal potential and impaired extrusion was due to a change in chloride potassium symporter 2 (KCC2) activity. Indeed, we found that altered intra-nigral inhibition was reversed following application of KCC2 agonist CLP290. Hence, we propose that dopamine depletion disrupts KCC2 function causing the reduced synaptic inhibition and even paradoxical GABAergic excitation of SNr neurons. Further, as oscillatory activity within the SNr has been previously shown to be in part driven by GPe input, we believe that this shift in GABAergic signaling may underlie the increased irregular, synchronous, and oscillatory activity of the BG.

Disclosures: A.R. Uprety: None. **J. Woo:** None. **A. Ostroumov:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR327.21/Web Only

Topic: C.03. Parkinson’s Disease

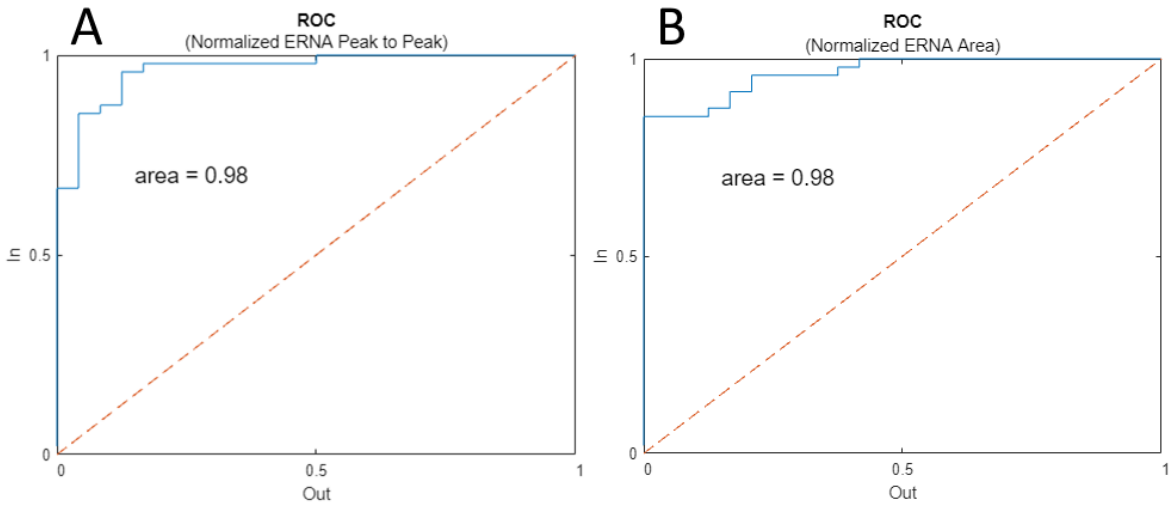
Support: NINDS Grant R01NS124650

Title: Evoked resonant neural activity for localizing Subthalamic Nucleus of patients with Parkinson’s Disease: preliminary results

Authors: *L. R. F. BRANCO[1], H. HEYDARI[1], C. SWAMY[1], A. TARAKAD[2], N. VANEGAS-ARROYAVE[2], A. VISWANATHAN[3], N. F. INCE[1];
[1]Biomed. Engin., Univ. of Houston, Houston, TX; [2]Neurol., [3]Neurosurg., Baylor Col. of Med., Houston, TX

Abstract: Introduction Evoked resonant neural activity (ERNA) is a promising biomarker for identifying the subthalamic nucleus (STN) during deep brain stimulation (DBS) surgery. Key advantages of ERNA include the ability to record ERNA in the anesthetized patient and the large signal amplitude. **Objectives** Determine whether ERNA can be used as a quantitative biomarker for determining if a DBS contact is located within the STN. **Methods** Awake neurophysiological recordings were obtained from 9 STN (6 patients) during DBS surgery after securing the directional leads. Monopolar simulation (2mA, 130 Hz, 60microsec) was delivered (Ripple, Summit) separately to the top and bottom contacts for 25 seconds. The stimulation train incorporated a 50ms gap at a rate of 2 gaps per second to obtain a total of fifty ERNA recordings per lead. Peak to peak amplitude and area under detrended, average ERNA waveforms were computed from each DBS contact and normalized across each hemisphere. LeadDBS was used to quantify each DBS contact as located inside versus outside the STN. **Results** Contacts located within the STN (48 out of ***n***=72) had a significantly higher ERNA peak to peak amplitude compared with those outside the STN (425.7 ± 287.5 versus 99.5 ± 58.5 μV, p-value = 7.81e-12, two-sample t-test from normalized data). Similarly, the area under the curve was significantly

higher for contacts located within the STN compared with those outside STN (10.296 ± 5.794 versus 3.151 ± 1.652 mWb, p-value = 2.82e-11, two-sample t-test from normalized data). Receiver operating characteristic (ROC) curves demonstrate high discriminative power comparing IN versus OUT of STN from both peak to peak (AUC=0.98, Fig. 1A) and area under the ERNA waveform (AUC=0.98, Fig. 1B). **Conclusions** ERNA recorded from contacts within the STN have higher amplitude and resonance. Hence, ERNA may be an effective biomarker to confirm STN DBS lead implantation intraoperatively.



Disclosures: L.R.F. Branco: None. **H. Heydari:** None. **C. Swamy:** None. **A. Tarakad:** None. **N. Vanegas-Arroyave:** None. **A. Viswanathan:** None. **N.F. Ince:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.22/I3

Topic: C.03. Parkinson’s Disease

Support: UH3- NS100553
UG3-NS130202
R01-NS119520

Title: Paired deep brain stimuli elicit short-term facilitation in globus pallidus interna and subthalamic nucleus

Authors: *S. BRINKERHOFF, J. OLSON, M. AWAD, C. GORDON, C. GONZALEZ, A. NAKHMANI, N. BENTLEY, M. HOLLAND, B. GUTHRIE, H. WALKER;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: High frequency deep brain stimulation (DBS) is a highly effective neurosurgical treatment for Parkinson's disease. Despite its efficacy, knowledge about how DBS interacts with local brain circuitry is limited. Paired DBS pulses elicit local event-related electrical potentials near the stimulation site in the subthalamic nucleus (STN), the globus pallidus interna (GPi), and the ventral intermediate thalamus (VIM). These event-related potentials occur across all targets at <0.5 milliseconds latency after stimulus onset and presumably represent direct depolarization of proximal neural elements by the stimulus pulse. Subsequent oscillatory event-related potentials, termed evoked resonant neural activity (ERNA), occur at longer latencies (~4 ms) in STN and GPi but not in the VIM thalamus. Greater knowledge on the fast dynamics of ERNA could shed light on mechanism of action, disease pathophysiology, and novel biomarkers to guide DBS therapy. Here, we contrast ERNA features in the STN versus GPi, the canonical functional targets for Parkinson's disease. We hypothesized that ERNA amplitude, temporal dynamics, and number of peaks differ in STN (n=14) versus GPi (n=12). We delivered pairs of DBS pulses across a range of interstimulus intervals and amplitudes during surgery and recorded local evoked potentials from non-stimulating bipolar configurations on the implanted lead. Following artifact removal, we contrasted ERNA amplitude, frequency, onset latency, offset latency, wave period, and number of peaks by interstimulus interval and brain target. STN and GPi DBS both elicit ERNA, but ERNA amplitude was considerably larger in STN than in GPi (p=0.001). Otherwise, ERNA displayed similar onset latencies, offset latencies, peak-to-peak frequencies, wave periods, and number of peaks across targets. In conclusion, pairs of DBS pulses elicit larger amplitude ERNA responses in STN than in GPi. The presence of ERNA in these targets (but not the VIM) suggests a role in the pathophysiology of a tremulous motor features of Parkinson's disease and dystonia. These and other evoked local field potential responses could eventually guide open-loop DBS programming or serve as control signals for adaptive therapy.

Disclosures: S. Brinkerhoff: None. **J. Olson:** None. **M. Awad:** None. **C. Gordon:** None. **C. Gonzalez:** None. **A. Nakhmani:** None. **N. Bentley:** None. **M. Holland:** None. **B. Guthrie:** None. **H. Walker:** F. Consulting Fees (e.g., advisory boards); Varian.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.23/I4

Topic: C.03. Parkinson's Disease

Title: Spatiotemporal characterization of the motor cortex and the striatum in Parkinson`s Disease mice models with Levodopa- induced Dyskinesia (LID).

Authors: *M. KHATEB[1], S. ACHVAT[2], F. AEED[2], S. RON[2], J. SCHILLER[2], Y. SCHILLER[1,2];
[1]Neurol., Rambam Hlth. Care Campus, Haifa, Israel; [2]Neurosci., Ruth and Bruce Rappaport Fac. of Medicine, Technion- Israel Inst. of Technol., Haifa, Israel

Abstract: Background: L-dopa-induced dyskinesia (LID) is a common and very difficult-to-treat complication in Parkinson's disease (PD) patients. The pathomechanism of LID has not been fully elucidated. At the network level, LID may be the result of hyperactivation of the primary motor cortex (M1) because of hypoactivation of the basal ganglia (BG) output nuclei. Nevertheless, electrophysiological evidence is sparse mainly due to technological limitations related to the poor ability of data acquisition from a large number of neurons from different involved regions simultaneously and without depth limitation. The Neuropixels technology overcomes these obstacles. Methods: We investigated the spatiotemporal dynamics of M1 and the striatum in PD-LID mice using Neuropixels. The recordings were acquired from awake head-restrained PD mice having LID. First, the mice underwent a surgical procedure in which a headpost was installed. Next, recording at the wild-type state was obtained as a control. Mice were then injected with the neurotoxin 6OHDA at the right motor striatum creating hemiparkinsonism and LID was induced by serial intraperitoneal injections of Ldopa and Benserazide. Neural activity was recorded multiple times from M1 and the beneath striatum simultaneously in Parkinsonian and dyskinetic states. During electrophysiological recording, the mice were monitored using fast cameras. Results: 1. LID is associated with a hyperkinetic state, manifested in exaggerated contralateral rotations. Compatible with previous reports, parkinsonian mice are bradykinetic and tend to rotate ipsilaterally in relation to the lesioned side of the brain. After inducing LID the rotations significantly shifted contralaterally.
2. LID was associated with changes in firing rates in M1 and the striatum.
Neural firing rate in M1 was decreased in PD compared to WT state. In LID, firing rate was increased, yet not reaching the baseline values at WT (n= 7 mice, 6971 neural clusters). An opposite pattern of firing rate changes was observed at the striatum. Conclusion: Our big-data, single-cell recordings partially support the classical hypothesis as overactivation of M1 was found in LID only compared to PD. The data sheds light on a possible limitation of the classical theory because neural activity at M1 in LID did not normalize or exceed the baseline activity level of WT thus failing to properly explain the hyperactivity in LID by changes in the firing rates at M1. Next, we are addressing the highly interesting issue of LID-associated changes in cortico-BG synchrony and the intrinsic properties of networks within M1 and striatum and between them taking advantage of the simultaneous recording.

Disclosures: **M. Khateb:** None. **S. Achvat:** None. **F. Aeed:** None. **S. Ron:** None. **J. Schiller:** None. **Y. Schiller:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.24/I5

Topic: C.03. Parkinson's Disease

Support: The Grainger Foundation

Title: Optimization of Spike-Timing Dependent Plasticity Deep Brain Stimulation for the Treatment of Parkinson's Disease

Authors: *L. PRAMANIK[1], S. HILLAN[1], S.-Y. CHANG[2,3], L. LUJAN[2,3];
[1]Mayo Clin. Grad. Sch. Biomed. Engin. and Physiol., Rochester, MN; [2]Neurologic Surgery, [3]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Techniques modulating neural plasticity for treatment of neurologic and psychiatric conditions have recently emerged. However, plasticity-inducing parameters that lead to therapeutic outcomes remain to be characterized, especially in the context of movement disorders such as Parkinson's disease (PD). Deep Brain Stimulation (DBS) is commonly used to treat refractory PD. Thus, there is a need for and unique opportunity to develop stimulation protocols that modulate neural plasticity for the treatment of PD and other movement and psychiatric disorders. Here, we propose a machine learning-based approach to optimize a Spike-Timing Dependent Plasticity (STDP) approach to DBS in the motor cortex. We will first characterize stimulation parameters using patch clamp in M1 brain slices during theta-burst, low-frequency (1 Hz) STDP stimulation. We will measure baseline evoked and spontaneous excitatory post-synaptic currents (EPSCs) for 30 minutes at rest prior to implementation of plasticity-induction approaches. Theta-burst and low-frequency electrical stimulation are applied through a pair of electrodes located close to the patched neurons. For STDP, local electrical stimulation depolarizes pre-synaptic components and depolarization of M1 neurons activates post-synaptic components. For each trial, stimulation of these two targets will be paired 100 times, and this procedure will be repeated while varying the pulse width, stimulation amplitude, and inter-target stimulation lag. We measure the amplitudes of evoked EPSCs along with the frequency and amplitude of spontaneous EPSCs. Our primary outcome is the duration of the evoked EPSCs until return to baseline levels, as this should indicate maximal modulation of plasticity.
We expect machine learning approaches such as Ridge or LASSO regression will allow modeling of the relationship between the parameters modulated and the duration of the evoked EPCSs. This will allow identification and prediction of the parameters that produce the longest-lasting evoked potentials. Future work will include evaluating the efficacy of these parameters for plasticity-implemented DBS to rescue motor deficits through behavioral testing in awake animals.

Disclosures: L. Pramanik: None. **S. Hillan:** None. **S. Chang:** None. **L. Lujan:** None.

Poster

PSTR327. Parkinson's Disease: Circuit Mechanisms and Deep Brain Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR327.25/I6

Topic: C.03. Parkinson's Disease

Support: NIH R01 NS107336
The Grainger Foundation

Title: Neural activity in the medial prefrontal cortex, medial amygdala, and ventral pallidum in response to STN DBS and its effect on anxiety

Authors: *S. HILLAN[1], W. LUJAN[2], J. SILVERNAIL[2], S.-Y. CHANG[2], J. LUJAN[2]; [1]Mayo Clin. Grad. Sch. of Biomed. Sci., Rochester, MN; [2]Neurosurg., Mayo Clin., Rochester, MN

Abstract: While effective for the treatment of motor symptoms of Parkinson's disease (PD), deep brain stimulation (DBS) of the subthalamic nucleus (STN) may cause psychiatric side effects, such as anxiety. The cause of these psychiatric effects is believed to be the result of stimulation spread from the motor to the limbic STN, however the precise mechanisms remain elusive. Given that psychiatric disorders are also an exclusion criterion for STN DBS there is a need to improve understanding of how STN stimulation affects neuronal circuitry associated with psychiatric effects such as anxiety. The medial prefrontal cortex (mPFC) and medial amygdala (mAmyg) are areas of the brain that have been associated with anxiety, while the ventral pallidum (VP) is the primary limbic output of the STN. This study investigates changes in neural activity in in these three brain regions in a hemi-parkinsonian 6-hydroxydopamine (6-OHDA)-lesioned model of PD. A unilateral injection of 6-OHDA was delivered into the striatum. Following three weeks to allow for the lesion to take full effect, the animal was anesthetized with urethane, and microwire recording electrodes were implanted bilaterally in the medial prefrontal cortex, medial amygdala, and ventral pallidum. Stimulating electrodes were implanted bilaterally in the STN while cortical evoked potentials were recorded from the motor cortex. A reference was placed over the cerebellum. Stimulation was delivered unilaterally at frequencies varying from 1 Hz to 130 Hz with local field potentials recorded bilaterally from the mPFC, mAmyg, and VP before, during, and after stimulation. Additionally, evoked potentials were recorded from these same regions before and after stimulation. Experiments are still ongoing, and our expected results are that there will be an increase in theta band power in these regions. Next steps include performing these electrophysiological analyses on awake animals during risk avoidant behavioral assays.

Disclosures: S. Hillan: None. **W. Lujan:** None. **J. Silvernail:** None. **S. Chang:** None. **J. Lujan:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.01/I7

Topic: C.03. Parkinson's Disease

Support: NINDS R15 NS115032-01A1

Title: Pallidothalamic Circuit-Specific Manipulation ameliorates motor symptoms in a rat model of parkinsonian

Authors: *J. JACKSON, H. LOUGHLIN, O. SIDDIQUA PROVA, C. LOOMAN, C. YU; Biomed. Engin., Michigan Technological Univ., Houghton, MI

Abstract: Deep brain stimulation (DBS) has been widely used to alleviate motor symptoms in patients with advanced Parkinson's disease (PD). Among the various anatomical targets for DBS, the globus pallidus interna (GPi) has shown clinical effectiveness. However, the precise mechanisms underlying the therapeutic effects of GPi DBS remain unclear. This study aimed to investigate the effects of optogenetic stimulation in the entopeduncular nucleus (EP), the rat homologue of GPi. Experiments were conducted in a unilateral 6-hydroxydopamine (6-OHDA) lesioned rat model of PD to evaluate the behavioral and neural circuit changes induced by optogenetic EP DBS. Motor deficits were assessed through the quantification of circling behavior and the adjusting steps test in response to optogenetic EP DBS at different frequencies. Single-unit recordings were performed in the EP and ventral lateral motor thalamus (VL) to analyze neural circuit activity during 130Hz optogenetic EP DBS in PD rats. The results demonstrated that optogenetic stimulation of EP local neurons exhibited frequency-dependent effects. High-frequency DBS at 130Hz ameliorated pathological ipsilateral turning and corrected abnormal forelimb stepping, whereas low-frequency DBS at 20Hz was ineffective. Furthermore, optogenetic activation of EP at 130Hz led to both increased and decreased neural activity in the EP, while neurons in the VL showed mixed changes in firing rate during 130Hz optogenetic EP DBS. Spectral analysis of single-unit firing times demonstrated that a majority of GPi neurons exhibited decreased beta-band oscillatory activity during 130Hz optogenetic EP DBS, along with a reduced oscillatory peak in the beta band in the majority of VL neurons. These findings suggest that the modulation of neural firing rates within the EP and VL circuits alone cannot fully explain the frequency-dependent behavioral effects of EP DBS. Instead, high-frequency optogenetic EP DBS may ameliorate parkinsonian motor symptoms by reducing pathological oscillatory activity in the EP-linked neural circuit. Our study highlights the effectiveness of circuit-specific manipulation in the pallidothalamic pathway through optogenetic EP DBS for improving motor deficits in a rat model of PD. These findings provide valuable insights into the underlying mechanisms of EP DBS and may contribute to the selection and optimization of clinical GPi DBS targets.

Disclosures: J. Jackson: None. **H. Loughlin:** None. **O. Siddiqua Prova:** None. **C. Looman:** None. **C. Yu:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.02/I8

Topic: C.03. Parkinson’s Disease

Support: NIH Grant F31NS127483-01

Title: Characterization of behavioral impairments and effects of DBS in a 3K tremor mouse model

Authors: S. NANIVADEKAR, A. GITTIS;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Parkinson's disease (PD) is a progressive, neurodegenerative movement disorder characterized by tremor, bradykinesia, and postural instability. Pathologically, there is a loss of dopaminergic neurons in the midbrain that produces excessive inhibition. Aggregates of alpha-synuclein (a-Syn) in midbrain and cortical neurons is thought to underly the impairments seen in PD. Recently, a new genetic model of PD showed that destabilizing tetramers of a-Syn using a missense mutation produces tremor and gait instability correlated with the level of a-Syn pathology (Nuber et al., 2018). In this study we aim to characterize the behavioral deficits in this mouse model (3KPD) at various stages of the disease, identify interventions that might correct the phenotypic features, and eventually probe the neural correlates with electrophysiology and lesion studies. For the characterization of motor impairments, we compare the behavior of the 3K mice vs. control mice at 4, 7, 10, and 13 months. We analyze movement in an open-field arena task by looking at % time the mice are immobile, the velocity during movement bouts, and the % time spent in a tremulous state. Fine motor deficits are quantified by using a grip strength assay. Balance and coordination are assessed using a tapered beam walk task. To study non-motor deficits, we perform the tail-suspension task for depression, and the hole-board open field task for exploratory behavior. Deep brain stimulation (DBS) is an invasive therapy used to treat patients with PD. In a mouse at the most severe stage of this model (~ 13 months) we delivered DBS to the EPN (rodent homolog of the GPi). Preliminary findings in this mouse showed an acute reduction in immobility. The 6-OHDA model of PD that causes degradation of dopaminergic projections in the brain, produces pathological firing activity in the substantia nigra pars reticulata (SNr) which is the output nucleus of the basal ganglia. We performed electrophysiology recordings in the SNr of a 3K mouse to study whether a similar pathophysiology is observed in this model.

Disclosures: S. Nanivadekar: None. **A. Gittis:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.03/J1

Topic: C.03. Parkinson's Disease

Support: NIDDK RO1 DK124098 to Thyagarajan Subramanian
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DOD NETP GRANT13204752 to Thyagarajan Subramanian

Additional funding from Anne M. and Phillip H. Glatfelter, III Family Foundation and Ron and Pratima Gatehouse Foundation

Title: S129-alpha synuclein phosphorylation in paraquat and lectin model of parkinson's disease

Authors: *C. SWAIN[1], K. LE[1], J. A. KALBUS[1], K. SAUTER[4], K. VENKITESWARAN[2], T. SUBRAMANIAN[3];
[1]Neurol., [2]Neurol. and Neurosci., [3]Neurology, Neuroscience, and Bioengineering, Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH; [4]Col. of Med., Penn State Univ., Red Lion, PA

Abstract: Epidemiologic studies have shown that a commonly used pesticide, paraquat, has been linked to the development of Parkinson's Disease (PD). Lectins are ubiquitous in the human diet and when uncooked, have the ability to enhance the toxicity of pesticides such as paraquat. A rat model of PD using oral administration of paraquat and lectin (P+L) has shown that the pathology is mediated via the monosynaptic dopaminergic nigrovagal pathway, which connects the gut to the SN via the vagus nerve. In the disease state, α-synuclein that is phosphorylated at the Ser129 site travels retrogradely via the vagus nerve to reach the SN via the nigrovagal pathway. Previously, I presented data in the Society for Neuroscience 2022 conference to show that the prokinetic squalamine lactate (S) prevents motor symptoms of PD in the P+L rat model (Swain, et al., 2022). In this experiment, subthreshold doses of P+L were given to Sprague Dawley rats (n=33, 250-400g) via oral gavage for 7 days. An experimental group (n=17) were given squalamine in their drinking water for 30 days post-P+L treatment. A control group (n=16) received no squalamine post-P+L treatment. Motor symptoms were assessed with the vibrissae-evoked forelimb placement test (VEFPT) and stepping test. P+L animals showed significantly lower VEFPT and stepping test scores (#$p<0.05$) at 4 weeks compared to P+L+S group. Here, I present additional data demonstrating the neuronal loss in SNpc depicted by Tyrosine Hydroxylase (TH) immunohistochemistry and Cresyl Violet (CV) staining and phosphorylated S129-αSyn aggregates depicted by S129-αSyn immunohistochemistry staining. Stereological quantification of TH+ neurons, CV+ neurons, and S129-αSyn aggregates in the SNpc of P+L and P+L+S animals are ongoing. Our study indicates that squalamine may play a protective role in preventing α-synuclein misfolding and aggregation, and therefore may represent a translational opportunity for treatment of early PD.

Disclosures: C. Swain: None. **K. Le:** None. **J.A. Kalbus:** None. **K. Sauter:** None. **K. Venkiteswaran:** None. **T. Subramanian:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Research funding from National Institutes of Health and from the Department of Defense, UCB pharma, Bukwang and BlueRock. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Squalamine is supplied by Enterin. D. Fees for Non-CME Services Received Directly from Commercial Interest or their Agents (e.g., speakers' bureaus); Honoraria for serving on scientific advisory board for Teva, Neurocrine and Supernus; Honoraria for study section service from the National Institutes of Health.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.04/J2

Topic: C.03. Parkinson's Disease

Title: Pna5, a glycosylated angiotensin (1-7) peptide improves cogntion in a chronic, progressive mouse model of parkinson's disease

Authors: *K. BERNARD[1], J. A. MOTA[1], M. J. CORENBLUM[1], M. HAY[4], L. MADHAVAN[2], T. FALK[3];
[2]Neurol., [3]Dept. Of Neurol., [1]Univ. of Arizona, Tucson, AZ; [4]Dept. of Physiol., The Univ. of Arziona, Tucson, AZ

Abstract: Parkinson's Disease (PD) is a chronic age-related neurodegenerative disease. While clinical diagnosis is based upon the presence of the hallmark motor symptoms, non-motor symptoms such as cognitive decline are a prominent feature of disease. Currently, there are no treatments that can intervene in disease progression, nor satisfactorily address PD associated cognitive decline. Angiotensin (1-7) (Ang(1-7)) is an endogenous peptide classically known for its role in the systemic renin-angiotensin system, however it is also well established that Ang1-7 signaling through endogenous Mas receptors modulates inflammation, oxidative stress, and protein clearance pathways. However, its poor stability and low blood-brain barrier (BBB) permeability limit its therapeutic potential. To address this problem, we designed and synthesized a glycosylated Ang(1-7) analog (PNA5) that shows increased **peptide stability (and therefore half-life) and enhanced CNS penetration.** Other groups have demonstrated that PNA5 is able to improve cognition in heart-failure driven models of cognitive decline. To examine the *in vivo* effects of PNA5 in relation to PD, male mice overexpressing human wild-type alpha-synuclein (Thy1-Syn, line 61) were treated with 1 mg/kg PNA5 (SC, daily) or saline (n = 16-18 /group) starting at 4 months of age. After two months of treatment, mice were assessed behaviorally through specific motor and cognitive tasks, and subsequently sacrificed for immunohistochemical or immunoblot assays to assess Syn pathology, inflammation, and synaptic alterations. Evaluation via a novel object recognition task (NOR) revealed that Syn mice spent less time interacting with the novel object compared to wild-type, suggesting impaired recognition memory (58.43% of time compared to 37.96%, p = 0.0031), and treatment with PNA5 reverses this deficit (52.53% of time compared to 37.69%, p = 0.0412). Similarly, Syn mice show reduced working memory in a Y-maze as described by percentage of alterations (65.61 % compared to 56.69%, p = 0.0292), and PNA5 restores this deficit (66.27% compared to 56.69%, p = 0.0292). These data indicate that PNA5 improves cognitive function in the human Syn expressing animals. Interestingly, there were no changes to burden of phosphorylated Syn (psyn) in the frontal cortex (immunohistochemistry and western blot), however a non-significant (20%) reduction of psyn was seen in the hippocampus. We are currently evaluating inflammation and autophagy markers, as well as changes to synapse number in these regions to identify pathological correlates of the improved cognition.

Disclosures: K. Bernard: None. **J.A. Mota:** None. **M.J. Corenblum:** None. **M. Hay:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent

holder, excluding diversified mutual funds); Dr. Meredith Hay is the founder and President of a biopharmaceutical company, ProNeurogen, that has licensed the technology described in this abstract. **L. Madhavan:** None. **T. Falk:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.05/J3

Topic: C.03. Parkinson's Disease

Title: Pre- and post-synaptic actions of the multimodal serotonin compound vortioxetine in reducing dyskinesia in hemiparkinsonian rats

Authors: M. COYLE, ***E. R. SARINICK**, C. BUDROW, C. R. BISHOP;
Binghamton Univ., Binghamton, NY

Abstract: Parkinson's disease (PD) is a movement disorder characterized by bradykinesia, muscle stiffness, tremors, and postural instability caused by loss of nigrostriatal dopamine neurons. The most widely used treatment for patients with PD is levodopa (L-DOPA), the precursor for dopamine. However, chronic L-DOPA use results in abnormal involuntary movements known as L-DOPA-induced dyskinesia (LID). Previous studies have demonstrated that vortioxetine, a multimodal selective serotonin reuptake inhibitor and serotonin 1A (5-HT1A) receptor agonist, can reduce LID while maintaining the benefits of L-DOPA treatment, however its mechanisms of action remain to be determined. Given prior work demonstrating that 5-HT compounds may act at pre- and post-synaptic sites within and outside the basal ganglia, we sought to better characterize vortioxetine's influence. To do so, male and female adult Sprague-Dawley rats were first rendered hemiparkinsonian through a unilateral injection of 6-hydroxydopamine into the medial forebrain bundle and rendered dyskinetic through chronic L-DOPA treatment (6 mg/kg; s.c.). In Experiment 1, rats received striatal microdialysis cannulae at the time of lesion to measure the pre-synaptic effects of vortioxetine (0, 3, 10 mg/kg; s.c.) on dopamine release while concurrently measuring LID. In Experiment 2, dyskinetic rats were administered vortioxetine (0, 3, 10 mg/kg) just prior to the dopamine agonist apomorphine, which acts directly at post-synaptic dopamine receptors, after which dyskinesia was monitored. Preliminary results suggest that vortioxetine's direct and indirect actions at presynaptic 5-HT1A receptors reduce dopamine release following L-DOPA to suppress LID, while vortioxetine may directly influence dopamine receptor actions at striatal medium spiny neurons to further suppress dyskinetic behaviors. Such findings would support vortioxetine's multimodal features and implicate its repositioning for optimizing PD treatment.

Disclosures: M. Coyle: None. **E.R. Sarinick:** None. **C. Budrow:** None. **C.R. Bishop:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.06/J4

Topic: C.03. Parkinson's Disease

Support: NIH Grant RF1 NS113548-01A1
SRA from Seelos therapeutics
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Title: Snca-targeted epigenome therapy for parkinson's disease: pre-clinical proof of concept in a pd mouse model

Authors: *B. O'DONOVAN[1], S. UPADHYA[1], J. RITTINER[2], B. KANTOR[2], O. CHIBA-FALEK[1];
[2]Viral Vector Core, Dept. of Neurobio., [1]Duke Univ., Durham, NC

Abstract: Elevated SNCA levels are causative in Parkinson's Disease (PD) pathogenesis, while normal physiological levels of SNCA are essential to maintain neuronal function. Patients with SNCA triplication and duplication suffer from familial early onset from of PD, suggesting a therapeutics window of <30%. We aim to translate mechanistic knowledge of SNCA dysregulation towards the development of epigenome therapy for PD targeting SNCA expression. Towards this goal we developed all-in-one lentiviral vector (LV) carrying the deactivated CRISPR/(d)Cas9, a selected gRNA targeted at SNCA-intron1 and synthetic repressor molecules. Previously we provided in vitro proof-of-concept for the efficacy and efficiency of our LV-dCas9-repressor system in human iPSC-derived 'aged' dopaminergic neurons from a PD-patient with the SNCA triplication. We showed downregulation of SNCA-mRNA and protein levels that led to the rescue of disease-related pathological phenotypes including, mitochondrial dysfunction, neuronal-cell death, DNA damage and nuclear deficits. We have now moved forward into in vivo validation studies. Our PD mouse model was generated by inducing expression of human SNCA with an AAV-A53T-human SNCA vector that comprised of the mutated human-SNCA coding sequence fused with its native promoter/intron 1 region. We performed bilateral stereotactic injection of the AAV-A53T-human SNCA vector into the mouse substantia nigra (SN), the left SN was co-injected with the therapeutic LV-dCas9-repressor and the right SN was co-injected with the control inactive LV-dCas9 vector. Analysis of 10 mice demonstrated a significant 53% reduction in human SNCA protein. Pathological examinations showed a robust reduction in Ser129-phosphorylated SNCA (nearly 80%) and in aggregated SNCA protein (over 70%). In addition, we observed significantly higher expression of tyrosine hydroxylase (TH +24%), suggesting a greater retention of dopaminergic neurons. We also collected safety measures. Monitoring daily behaviors showed no abnormalities in well-being criterions and no weight loss. In addition, safety measures demonstrated no issues for blood counts, serum chemistry and liver histology. In conclusion, our novel CRISPR/dCas9-based technology offers the unprecedented tool to modify a particular epigenetic mark resulting in effective fine-tuned reduction of SNCA expression levels sufficient for reversing PD-associated perturbations. This study provided an in vivo proof-

of-concept for advancing our innovative epigenome editing-based system to a clinical trial as the next-generation PD epigenome therapy.

Disclosures: **B. O'Donovan:** None. **S. Upadhya:** None. **J. Rittiner:** None. **B. Kantor:** F. Consulting Fees (e.g., advisory boards); Seelos Therapeutics Inc. **O. Chiba-Falek:** F. Consulting Fees (e.g., advisory boards); Seelos Therapeutics Inc.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.07/J5

Topic: C.03. Parkinson’s Disease

Support: Branfman Family Foundation
Robert Crooks Stanley Fellowship
NIH R01 DC014044

Title: Effects of Gamma Stimulation on Neuroinflammation at the Tissue-Electrode Interface

Authors: *E. BOLTCREED[1], A. ERSOZ[3], M. HAN[3], G. MCCONNELL[2];
[1]Biomed. Engin., Stevens Inst. of Technol., Jersey City, NJ; [2]Biomed. Engin., Stevens Inst. of Technol., Hoboken, NJ; [3]Biomed. Engin. Dept., Univ. of Connecticut Biomed. Engin. Dept., Storrs, CT

Abstract: *Chronic neural electrodes can remain implanted for over a decade as part of a treatment protocol such as deep brain stimulation (DBS) for Parkinson’s Disease (PD). However, the long-term reliability of the electrodes, particularly as recording devices, is hampered by the tissue response to the electrode. Prior studies in an Alzheimer’s disease mouse model found that flickering light at gamma frequencies (i.e., 20-50Hz) results in an enhanced microglial recruitment. We hypothesized that DBS in the gamma frequency band increases microglial recruitment and reduces astrogliosis at the tissue-electrode interface.**Male Long Evans rats (n = 3) were implanted with silicon microelectrode arrays (two shanks with five contacts per shank) into the motor cortex. Following implantation, rats received 1 hour of 4 Hz stimulation at a constant current of 10µA using charge-balanced biphasic pulses. One of the two shanks on each electrode served as a non-stimulated control. Post mortem, horizontal tissue sections were stained with ED1 for activated microglia, GFAP for astrocytes, and DAPI for nonspecific nuclei. Fluorescent intensity and cell number as a function of distance from the tissue-electrode interface (0-500µm from the interface) were used to quantify all stained sections. **Fluorescent intensity for ED1 increased by 30% in the stimulated samples vs. non-stimulated control samples ($p<0.05$). Fluorescent intensity for GFAP decreased by 40% in the stimulated samples vs. non-stimulated control samples ($p<0.05$). No differences were observed in DAPI-stained sections between conditions. These results suggest that acute gamma stimulation modulates glial recruitment in the immediate vicinity of the microelectrode. Future studies will investigate the

long-term effects of gamma stimulation on the glial recruitment at the tissue-electrode interface as a strategy to improve chronic recording reliability.**

Disclosures: **E. Boltcreed:** None. **A. Ersoz:** None. **M. Han:** None. **G. McConnell:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR328.08/J7

Topic: C.03. Parkinson's Disease

Support: The National Science and Technology Council of Taiwan, ROC Grant MOST 111-2314-B-016- 051-MY3, MOST 111-2314-B-016-057, and MOST- 109-2314-B-016 -019 -MY2; MOST- 110-2314-B- 034
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The National Research Foundation, Republic of Korea Grant NRF-2014M3A9B5073868

Title: Pt320, a sustained-release glp-1 receptor agonist, ameliorates l-dopa-induced dyskinesia in a mouse model of parkinson's disease

Authors: *Y.-H. CHEN[1], K.-Y. TSENG[1], L. OLSON[2], B. J. HOFFER[3], T.-T. KUO[1]; [1]Tri-Service Gen. Hospital/National Def. Medi, Taipei, Taiwan; [2]Karolinska Inst., Karolinska Inst., Solna, Sweden; [3]NIDA/NIH, NIDA/NIH, Lyndhurst, OH

Abstract: This study investigated the efficacy of PT320 in mitigating L-DOPA-induced dyskinetic behaviors and neurochemical changes in a progressive Parkinson's disease (PD) MitoPark mouse model. PT320 was administered biweekly, starting at 5 (early treatment) or 17 (late treatment) weeks of age. The early treatment group received L-DOPA from 20 to 22 weeks of age and underwent longitudinal evaluations, while the late treatment group received L-DOPA from 28 to 29 weeks of age. Fast scan cyclic voltammetry (FSCV) was used to measure presynaptic dopamine (DA) dynamics in striatal slices after drug administration, exploring dopaminergic transmission. Early PT320 treatment of dyskinesia in L-DOPA-primed mice significantly reduced abnormal movements, while late PT320 treatment had no effect. Early

PT320 treatment increased tonic and phasic DA release in striatal slices, not only in L-DOPA-naïve MitoPark mice but also in L-DOPA-primed animals. Early PT320 treatment ameliorated L-DOPA-induced dyskinesia in MitoPark mice, potentially due to the progressive level of DA denervation in PD.

Disclosures: Y. Chen: None. **K. Tseng:** None. **L. Olson:** None. **B.J. Hoffer:** None. **T. Kuo:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.09/J8

Topic: C.03. Parkinson's Disease

Support: NRF-2022R1A2C2006061
NRF-2017R1A5A2014768

Title: Mitochondrial transplantation exhibits neuroprotective effects and improves behavioral deficits in an animal model of Parkinson's disease

Authors: *S.-H. YU;
Paean Biotech. Inc., Seoul, Korea, Republic of

Abstract: Mitochondrial Transplantation Exhibits Neuroprotective Effects and Improves Behavioral Deficits in an Animal Model of Parkinson's Disease Shin-Hye Yu[1,*], Hyeyoon Eo[2,*], Soomin Kim[1], Yujin Choi[2], Yujin Kim[1], Young Cheol Kang[1], Myung Sook Oh[2,#] and Chun-Hyung Kim[1,#1]Paean Biotechnology, Inc. 5 Samil-daero8-gil, Jung-gu, 04552, Seoul, Korea [2] Department of Biomedical and Pharmaceutical Sciences, Graduate School, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, 02447, Seoul, Korea *Contributed equally Mitochondria are fundamental for the survival of cells and their functions by providing energy in the form of ATP through oxidative phosphorylation. Mitochondrial dysfunction is associated with a number of human diseases, including metabolic syndromes, rare mitochondrial diseases, cancer, aging, and neurodegenerative diseases. Recently, mitochondrial transfer between cells has been shown to occur naturally, and exogenously mitochondrial transplantation is beneficial for treating mitochondrial dysfunction. In this study, mitochondria isolated from human stem cells showed the neuroprotective effect against MPP^+, 6-OHDA, and rotenone in human LUHMES cells and mouse MN9D cells, both of which were differentiated into dopaminergic cells. In Parkinson's disease (PD) mice model induced by MPTP, intravenously mitochondrial treatment demonstrated the clinical benefits including protective effect on the dopaminergic nigrostriatal neurons and behavioral improvements in grip strength, locomotion in open field, and rotarod test. Since there are currently no effective drugs for PD, mitochondrial transplantation would be a novel therapeutic strategy.

Key words: mitochondria, transplantation, stem cell, Parkinson’s disease, therapeutics*Support Contributed By: NRF-2022R1A2C2006061 and NRF-2017R1A5A2014768*

Disclosures: S. Yu: None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.10/J9

Topic: C.03. Parkinson’s Disease

Title: SUVN-I7016031: A Novel M1-receptor Positive Allosteric Modulator (M1-PAM) Alleviates Parkinson’s Disease Dementia (PDD)

Authors: *V. R. GRANDHI, R. MEDAPATI, R. ABRAHAM, A. K. SHINDE, A. R. MOHAMMED, K. BOJJA, R. NIROGI;
Pharmacol., SUVEN LIFE SCIENCES LIMITED, Hyderabad, India

Abstract: Parkinson's disease (PD) is a neurodegenerative disease affecting the dopaminergic neurotransmission in the nigrostriatal pathway and thereby movement. PD is characterized by abnormal microscopic deposits composed chiefly of alpha-synuclein, called Lewy bodies. As the disease progresses, PD patients experience a gradual decline in cognitive function and studies have reported that the average time from onset of PD to developing dementia is about 10 years. Haloperidol is a typical prototype antipsychotic drug that has the potential to induce symptoms of PD dementia by the blockade of striatal dopamine D2 receptors and further the downstream antagonism of M1 receptors in the signaling cascade. SUVN-I7016031 was evaluated in haloperidol-induced PD dementia. Haloperidol was supplied in potable water feeder bottles, in such a way that each animal consumed dissolved haloperidol at a dose of ~2 mg/kg to induce PD dementia. After one week of haloperidol supplementation, treatment was begun with either SUVN-I7016031 or positive control, rivastigmine and the dosing was continued up to seven weeks. During the second and sixth week, a social recognition task (SRT) was conducted. In SRT, familiarization and recognition trials were conducted with a trial delay of 30 min. Duration of social investigation time by an adult rat with either a familiar or novel conspecific juvenile rat was assessed during the recognition trial. The treatments were compared using discriminative index and examined using statistical analysis. Haloperidol treated control group did not discriminate the novel juvenile from the familiar one and showed cognitive dysfunction. Further, rivastigmine alleviated haloperidol-induced PD dementia on testing week-2 and week-6, as these rats significantly spent more time socially investigating the novel juvenile relative to familiar juvenile rat. SUVN-I7016031 dose dependently reversed haloperidol-induced PD dementia. Further, rivastigmine and SUVN-I7016031 also showed significant improvement of discriminative index relative to haloperidol control group. Observed effects with SUVN-I7016031 were more prominent during week-7 relative to week-2. SUVN-I7016031

showed potential in alleviating haloperidol-induced Parkinson's disease associated cognitive impairment in a preclinical model reflecting PD dementia.

Disclosures: V.R. Grandhi: None. **R. Medapati:** None. **R. Abraham:** None. **A.K. Shinde:** None. **A.R. Mohammed:** None. **K. Bojja:** None. **R. Nirogi:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR328.11/Web Only

Topic: C.03. Parkinson's Disease

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Additional funding from Anne M. and Phillip H. Glatfelter, III Family Foundation and Ron and Pratima Gatehouse Foundation

Title: Combining cresyl violet and tyrosine hydroxylase stain to quantify nigral neuronal loss in the paraquat and lectin model of Parkinson's Disease

Authors: *I. NESTER[1], C. SWAIN[1], V. PESHATTIWAR[1], K. LE[1], J. KALBUS[1], K. SAUTER[4], K. VENKITESWARAN[2], T. SUBRAMANIAN[3];
[1]Neurol., [2]Neurol. and Neurosci., [3]Neurology, Neuroscience, and Bioengineering, Univ. of Toledo Col. of Med. and Life Sci., Toledo, OH; [4]Col. of Med., Penn State Univ., Red Lion, PA

Abstract: Parkinson's Disease (PD) is caused by deterioration of the dopamine-producing pathway called the nigrostriatal pathway; in previous studies, exposure to paraquat, an environmental toxin, has been associated with development of PD. Our previous study showed that, when combined with lectin, a protein commonly found in the human diet, oral administration of paraquat and lectin (P+L) in Sprague Dawley rats over seven days showed decreased counts of tyrosine hydroxylase positive (TH+) neurons in the substantia nigra (SN), caused ascending synucleinopathy and parkinsonism that is levodopa responsive and could be prevented by performing subdiaphragmatic vagotomy. In another study, we showed that oral treatment with squalamine (S) for 30 days after P+L treatment prevented the animals from developing PD symptoms. This data was presented in the Society for Neuroscience 2022 conference (Swain et al., 2022). In an extension of this study, cresyl violet (CV) stain, a Nissl stain, was used to assess the total neuronal content of the SN. TH+ immunohistochemistry staining was used to determine the deterioration of dopaminergic neurons in the SN. As noted in our previously published work, combined CV and TH staining confirm that there has indeed been loss of dopaminergic neurons in the SNpc in P+L treated rats while they are spared in P+L+S treated rats on qualitative analysis. Unbiased stereological quantitative analysis using

systematic random sampling and the optical fractionator method of estimation of TH+ and CV+ neurons are ongoing to confirm these qualitative findings.

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Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.12/J10

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1ZIAAG000936

Title: Immunotherapy with an antibody against CD1d modulates neuroinflammation in an α-synuclein transgenic model of Lewy body like disease

Authors: ***M. IBA**[1], S. KWON[1], C. KIM[1], M. SZABO[1], C. R. OVERK[3], R. RISSMAN[3], E. MASLIAH[1,2];
[1]Lab. of Neurogenetics, [2]Div. of Neurosci., NIH, Natl. Inst. on Aging, Bethesda, MD; [3]UCSD, La Jolla, CA

Abstract: α-synuclein (α-syn) is a presynaptic protein which progressively accumulates in neuronal cells and propagates to glial cells leading to aberrant immune activation and neurodegeneration in synucleinopathies of the aging population such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB). The inflammatory process involves microglial activation as well as infiltration of the CNS by T cells including CD4, CD8 and NKT's. To investigate the role of NKT's in neuroinflammation we treated α-syn transgenic (tg) mice (eg: Thy1 promoter line 61) with an antibody against CD1d which is a glycoprotein expressed in antigen presenting cells (APC's). CD1d-presented lipid antigens activate NKT cells through the interaction with T cell receptor in NKT's resulting in the production of cytokines. Thus, we hypothesized that blocking the APC-NKT interaction with an anti-CD1d antibody might reduce neuroinflammation and neurodegeneration in models of DLB/PD. Treatment with the anti-CD1d antibody did not have effects on CD3 (T cells), slightly decreased CD4 and increased CD8 lymphocytes in the

mice. Moreover, double labeling studies showed that compared to control (IgG) treated α-syn tg, mice treated with anti-CD1d displayed decreased numbers of CD3/interferon γ (IFNγ) positive cells consistent with NKT's. Further double labeling immunohistochemical experiment showed that the CD1d positive cells co-localized with the astroglial marker GFAP and that anti-CD1d antibody reduced this effect. While CD3 positive cells localized near the astroglia cells in control α-syn tg mice, this was modified by the treatment with the anti-CD1d antibody. Levels of IFNγ, CCL4, and IL6 determined by qPCR were increased in the IgG treated α-syn tg mice. Treatment with CD1d antibody blunted this cytokine response that was associated with reduced astrogliosis and microgliosis in the CNS of the α-syn tg mice. Flow cytometric analysis of immune cells in α-syn tg mice revealed that CD1d-tet+ T cells were also increased in the spleen of α-syn tg mice and treatment with the CD1d antibody reduced the levels. Reduced neuroinflammation in the anti-CD1d treated mice was associated with amelioration of the neurodegenerative pathology. These results suggest that reducing infiltration of NKT cells with an antibody against CD1d might be a potential therapeutical approach for DLB/PD.

Disclosures: **M. Iba:** None. **S. Kwon:** None. **C. Kim:** None. **M. Szabo:** None. **C.R. Overk:** None. **R. Rissman:** None. **E. Masliah:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.13/K1

Topic: C.03. Parkinson's Disease

Support: NIH Grant 1ZIAES103310

Title: Dbs-based chemogenetic gene-therapy rescues motor deficits in parkinsonian mice

Authors: ***J. LI**[1], J. ZHOU[1], B. HE[1], A. B. PAPANERI[1], N. P. KOBZAR[1], A. C. YEH[1], Y. ZHANG[2], G. CUI[1];
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Abstract: Deep brain stimulation (DBS) is currently the most effective treatment for alleviating motor symptoms in patients with advanced Parkinson's Disease (PD). However, the mechanisms underlying its therapeutic effects remain elusive. Here we use genetically encoded fluorescent sensors to show that therapeutic DBS in the subthalamic nucleus (STN), the most common target for treating PD, induces persistent activation in the afferent axon terminals while inhibiting postsynaptic neurons in the STN. These differential effects on pre- and postsynaptic activities are likely caused by the depletion of synaptic vesicles because prolonged DBS causes a decrease in the levels of local neurotransmitters in the STN, with a larger decrease observed in glutamate than in GABA. Based on these results, we hypothesize that chemogenetic inhibition of STN neurons should achieve the same therapeutic outcomes as DBS. To test this hypothesis, we first use viral vectors to express inhibitory (Gi) or excitatory (Gq) Designer Receptors Exclusively

Activated by Designer Drugs (DREADDs) in the STN of 6-OHDA PD model mice and show that DREADD agonist Clozapine N-oxide (CNO) rescues the motor deficits in Gi-expressing mice but worsens the motor symptoms in Gq-expressing mice. We further confirm the acute and long-term therapeutic effect of chemogenetic inhibition of STN in MitoPark PD mice. These findings elucidate the neural mechanisms of therapeutic DBS, and point to a chemogenetic gene-therapy treatment that is less invasive, more affordable for treating advanced PD.

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Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.14/K2

Topic: C.03. Parkinson’s Disease

Support: Korea Health Technology R&D Project through the Korea Health Industry Development Institute (HF21C0053)
National Research Foundation of Korea (2021R1A4A1025662)

Title: Protective effect of Korean herbal medicine Sihosogan-tang on dopaminergic neurons in the nigrostriatal pathway of a Parkinson’s disease mouse model

Authors: *J. SEO, H.-Y. KIM, C.-H. BAE, H. LEE, S. KIM;
Pusan Natl. Univ., Yangsan-si, Mulgeum-eup, Korea, Republic of

Abstract: Parkinson’s disease (PD) is a neurological disorders and is characterized by the loss of the dopaminergic neurons in the substantia nigra (SN). The present study investigated the protective effect of the Korean herbal medicine *Sihosogan-tang* (SS) in 1-methyl-4-phenyl-1,2,3,6-tetrahydrophridine (MPTP)-induced PD mouse model. Male 8-week-old C57BL/6 mice were injected with vehicle or 30 mg/kg of MPTP at 24h intervals for 5 days, and orally treated SS *(*82 mg/kg, or 247 mg/kg) or intraperitoneally injected selegiline (2 mg/kg) once a day for 12 consecutive days from the first MPTP injection. The pole test and rotarod test were performed to assess the motor function of the mice, tyrosine hydroxylase-immunohistochemistry was performed to determine the destruction of the SN and striatum, and the expressions of glial fibrillary acidic protein (GFAP) and ionized calcium binding protein (Iba1) were measured to determine the activation of astrocytes and microglia in the striatum. MPTP administration caused motor dysfunction, dopaminergic neuronal death in the SN and striatum, and increased GFAP and Iba1 expressions in striatum; however, SS dose-dependently alleviated the MPTP-induced changes. These results suggest that SS suppresses MPTP-induced dopaminergic neuronal death in the nigrostriatal pathway and the activation of astrocytes and microglia in the striatum.

Disclosures: J. Seo: None. **H. Kim:** None. **C. Bae:** None. **H. Lee:** None. **S. Kim:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.15/K3

Topic: C.03. Parkinson's Disease

Support: NIH R33 NS112441
NIH U01 AG074960

Title: Engineered, Programmable, Microbiome-Based Levodopa Live-Bacterial Therapeutic Achieves Highly Favorable Pharmacokinetics and Pharmacodynamics and Improves Motor and Non-Motor Symptoms of Parkinson's Disease Without Levodopa-Induced Dyskinesia in a Preclinical Model of Progressive Dopaminergic Neurodegeneration

Authors: *P. PADHI[1], I. J. SCHEIBE[2], A. A. OTTO[2], J. P. THOMAS[2], A. JANG[2], N. BACKES[5], G. ZENITSKY[1], H. JIN[1], V. ANANTHARAM[1], A. KANTHASAMY[1], K. ALLENSPACH-JORN[3], J. MOCHEL[3], G. J. PHILLIPS[4], A. G. KANTHASAMY[1];
[1]Ctr. for Neurolog. Dis. Research, Physiol. and Pharmacol., [2]Dept. of Physiol. and Pharmacol., [3]Dept. of Pathology, [4]Ctr. of Neurolog. Dis. Research, Depaartment of Infectious Dis., Univ. of Georgia, Athens, GA; [5]Vertex Pharmaceuticals, Boston, MA

Abstract: To overcome the limitations of non-continuous, pulsatile delivery of L-DOPA (a gold-standard dopamine replacement therapy for Parkinson's disease (PD)) imposed on the brain by repeated fixed doses, we bioengineered a novel human probiotic, *E. coli* Nissle 1917 (EcN), that continuously produces and dose-dependently releases L-DOPA in the gut. By utilizing genome engineering and synthetic biology techniques, our lead levodopa bacterial live-biotherapeutic (LDBL), $EcN_{L\text{-}DOPA}$, achieved favorable pharmacokinetics, pharmacodynamics, and gastrointestinal (GI) kinetics when administered orally twice daily, resulting in steady and non-fluctuating dopamine supplementation to the brains of both rodents and canines. To comprehensively evaluate the therapeutic efficacy of $EcN_{L\text{-}DOPA}$ compared the current standard-of-care (SOC), we conducted extensive preclinical assessments in MitoPark PD rodent model of progressive degeneration. Our evaluation included phenotypic assessments of gross and fine motor coordination, gait, and GI function after 12 weeks of chronic administration of $EcN_{L\text{-}DOPA}$ with benserazide. Additionally, we examined neurochemical, microbiome, GI, and brain toxicological alterations with plasma metabolome, fecal metagenome, gut inflammatory and histological metrics. Our findings suggest that the sustained, non-pulsatile delivery of L-DOPA by $EcN_{L\text{-}DOPA}$ improves both gross and fine motor skills over longer period as compared to conventional L-DOPA. Notably $EcN_{L\text{-}DOPA}$ MitoPark displayed greater horizontal activity score in generalized locomotor activity assessment and exhibited delayed latency to fall in Rotarod test. Furthermore, $EcN_{L\text{-}DOPA}$ improved step sequence regularity index and cadence gait measures in MitoPark using CatWalk gait analysis. While also enhancing motor function, chronic $EcN_{L\text{-}DOPA}$ administration was well tolerated and improved gastrointestinal function, including gastrointestinal transit time and intestinal permeability. Importantly, we next evaluated whether

$EcN_{L\text{-}DOPA}$ prevented the generation of LID in the MitoPark after 8 weeks of chronic administration compared to SOC. Through blinded analysis of the cylinder test, $EcN_{L\text{-}DOPA}$ ameliorated hind-paw and three-paw dyskinesia compared to the conventional chemical L-DOPA therapy. We further validated these findings by assessing the well-established molecular markers of LID, including ΔFosB, pDARPP-32, and pERK. Overall, our engineered LDBL represents a novel L-DOPA treatment modality that ensures long-term effectiveness with minimal side effects, distinguishing it from conventional anti-Parkinsonian therapeutic approaches.

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Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.16/K4

Topic: C.03. Parkinson’s Disease

Title: HCW9218 reduces alpha synuclein and improves physical performance in a paraquat model of Parkinson’s Disease

Authors: *C. M. JANNEY, V. GEORGE, X. ZHU, N. SHRESTHA, H. C. WONG;
Res. & Develop., HCW Biologics, Miramar, FL

Abstract: Early in the progression of Parkinson’s Disease (PD) neuroinflammation begins in and around the brain, as well as the buildup of damaging alpha-synuclein aggregates. Clearance of alpha-synuclein and reduction of inflammation involves resident immune cells such as microglia and astroglia, along with the blood brain barrier (BBB) and glymphatic system. The peripheral immune system is also involved, including NK cells, T-cells, and other lymphocytes. Senescence markers are also increased in aging and aging-related neurodegenerative diseases such as Parkinson’s Disease. We are exploring whether our clinical-stage immunotherapeutic HCW9218, a bifunctional fusion molecule comprising a tetrameric TGF-β RII trap and IL-15/IL-15R domain which has been shown to neutralize TGF-β and to stimulate NK cells and $CD8^{+}$T cells in experimental mouse models and humans, is able to (1) reduce neuroinflammation by removing senescence in the brain, (2) improve waste clearance of the brain and removal of the pathological alpha-synuclein, and (3) improve physical performance in a chemically induced Parkinson’s mouse model. The chemically induced model consisted of an intranasal application

of paraquat of 5 doses at 7.5 mg/kg each over 14 days (n = 10). With only two doses of HCW9218 30 days apart (Untreated n = 5, Treated n = 5), we were able to reduce alpha synuclein at 2 months after the second dose ($p < 0.01$) and show physical performance maintenance or improvements up to six months after the second dose across multiple measures in the Open Field Test (Distance Traveled ($p < 0.0001$), Velocity ($p =< 0.001$), Rearing Frequency ($p < 0.05$), Rearing Duration ($p < 0.05$)). Dopamine Transporter (DAT) ($p = 0.0648$) and Tyrosine Hydroxylase (TH) ($p = 0.1637$), both critical for physical performance in PD, were also increased in the nigrostriatal pathway at 2 months after the second dose, though did not reach significance; Glial Fibrillary Acidic Protein (GFAP) , an astrocytic marker generally associated with the neuroinflammatory response, was significantly decreased ($p < 0.01$) while another astrocytic marker, S-100 Calcium Binding Protein beta (S100β), was decreased ($p = 0.1299$) but did not reach significance. Currently, we are evaluating whether a regimen with more frequent dosing could further improve the clinical outcomes of HCW9218 in this chemical-induced Parkinson's disease model. In summary, we demonstrated the potential of an immunotherapeutic approach to treat Parkinson's Disease.

Disclosures: C.M. Janney: A. Employment/Salary (full or part-time):; HCW Biologics, Inc.. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Michael J. Fox Foundation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HCW Biologics, Inc. **V. George:** A. Employment/Salary (full or part-time):; HCW Biologics, Inc. **X. Zhu:** A. Employment/Salary (full or part-time):; HCW Biologics, Inc. **N. Shrestha:** A. Employment/Salary (full or part-time):; HCW Biologics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HCW Biologics, Inc. **H.C. Wong:** A. Employment/Salary (full or part-time):; HCW Biologics, Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); HCW Biologics, Inc..

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR328.17/K5

Topic: C.03. Parkinson's Disease

Support: National Research Foundation of Korea(NRF) Grant 2017R1A5A2014768

Title: Lactobacillus LBM-01 has neuroprotective and anti-inflammatory effects in Proteus mirabilis-treated PD like mice.

Authors: *Y. CHOI[1], J. CHOI[1], E. HUH[1], J. KIM[1], S. LEE[1], H. LEE[1], M. PARK[2], M. OH[1]; [1]Kyunghee Univ., Seoul, Korea, Republic of; [2]MetaCen therapeutics company, Suwon, Korea, Republic of

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease that accompanies motor dysfunction and unregulated dopaminergic system. Recent studies have shown that its pathogenesis is strongly related to microbiota gut brain axis. LBM-01 is a strain of *Lactobacillus* bacteria that has been known for immunomodulatory and anti-inflammatory effects in genomic study and fatty liver mouse models. In this study, we discovered the effects of LBM-01 on PD pathologies in mice treated with *Proteus mirabilis* (PM), a specific gut bacterium that causes PD phenotypes like motor impairment, dopaminergic neuronal damage and inflammation in the brain and colon. PM and LBM-01 were orally administered to C57BL6J mice. Results showed that LBM-01 attenuated motor deficit and dopaminergic neuronal death induced by PM treatment. In addition, LBM-01 regulated the microglial activation in SNpc, colonic inflammatory responses and alteration of fecal microbiota. Here, our findings implicate that LBM-01 has neuroprotective and anti-inflammatory effects via gut-brain axis.

Disclosures: Y. Choi: None. **J. Choi:** None. **E. Huh:** None. **J. Kim:** None. **S. Lee:** None. **H. Lee:** None. **M. Park:** None. **M. Oh:** None.

Poster

PSTR328. Parkinson's Disease: Therapeutic Strategies and Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR328.18/K6

Topic: C.03. Parkinson's Disease

Support: The National Research Foundation of Korea funded by the Korean government (2022M3A9B6017813).

Title: Microneedle stimulus to acupuncture points ameliorates motor function and neuroinflammation in the mouse model of Parkinson's disease

Authors: *J. KIM[1], I. JU[2], J. KIM[1], Y. CHOI[1], H. LEE[1], H.-J. PARK[3], M. OH[1,2]; [1]Dept. of Biomed. and Pharmaceut. Sci., [2]Dept. of Oriental Pharmaceut. Sci. and Kyung Hee East-West Pharmaceut. Res. Instit, [3]Dept. of Anat. & Information Sciences, Col. of Korean Med., Kyung hee Univ., Seoul, Korea, Republic of

Abstract: Parkinson's disease (PD) is caused by loss of dopaminergic neurons in the substantia nigra (SN) and depletion of dopamine in the striatum (ST), resulting in movement disorders such as tremors and rigidity. Previous studies have shown that acupuncture for specific acupoints alleviates PD. Needles with a length of 25 to 1000 μm are called microneedles, which are recently used for stimuli or drug delivery to the skin without penetrating the dermal layer in various diseases. This study aimed to evaluate the effect of attaching microneedle patch (MP) to specific acupoints on motor function, dopaminergic neuron death and neuroinflammation in 1-

methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced PD mice. Mice were treated with MPs to the acupuncture points GB20 and GB34 which are known to be related to PD for a total of 14 days. As a result, MPs attachment has shown to improve motor functions such as bradykinesia and endurance. In addition, in brain tissue analysis, the MPs had a protective effect against MPTP-induced dopaminergic neuron death in the SN area. Furthermore, MPTP-induced neuroinflammation was suppressed after MP attachment in the ST. Collectively, this study suggests that MP could improve motor function by protecting dopaminergic neurons in SN area and inhibiting neuroinflammation in PD mouse model.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.01/K7

Topic: C.03. Parkinson's Disease

Support: CJD Foundation
NIH-R01

Title: The Inhibitory Effect of Cellulose Ether TC-5RW on Misfolded Proteins

Authors: M. GERASIMENKO[1], T. GILLILAND[1], S. SHAH[1], M. DING[1], K. DOH-URA[3], W. ZOU[1], *Z. WANG[2];
[1]Case Western Reserve Univ., Cleveland, OH; [2]Pathology, Case Western Reserve Univ., CLEVELAND HTS, OH; [3]Tohoku Univ. Grad. Sch. of Med., Sendai, Japan

Abstract: Neurodegenerative diseases, including Alzheimer's, Parkinson's, and prion diseases, are characterized by the progressive degeneration of neurons, often correlated with the accumulation of misfolded protein aggregates. Previous research has suggested that cellulose ethers, particularly TC-5RW, commonly employed in food and pharmaceuticals, extend the lifespan of prion-infected mice and hamsters. This study expands upon these findings by demonstrating that TC-5RW profoundly reduces the seeding activity and fibrilization of alpha-synuclein and tau proteins. Strikingly, TC-5RW also displayed an inhibitory effect on the in vitro amplification of tissues with Parkinson's Disease and Alzheimer's Disease as measured by Real-Time Quaking-Induced Conversion (RT-QuIC). Additionally, our research revealed that TC-5RW can directly reduce aggregated alpha-synuclein and tau, as well as oligomeric alpha-synuclein in vitro by western blotting. In a Parkinson's Disease cell model (SH-SY5Y-derived neurons), TC-5RW improved cell viability and reduced Reactive oxygen species (ROS) in a dose-dependent manner. Our results indicate the potential of TC-5RW in inhibiting protein aggregation and promoting disaggregation of misfolded proteins, suggesting its promising

therapeutic efficacy for prion diseases, and potentially extending its applications to other related neurodegenerative disorders.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR329.02/K8

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01NS129788
National Institute on Aging R21AG075189
Cancer Prevention and Research Institute of Texas RP200655
Mission Connect TIRR Foundation 022-105

Title: Human midbrain organoids to unravel astrocyte lipid dysregulation in Parkinson's Disease

Authors: *A. TAHANIS[1], T. NGUYEN[1], M. PATEL[1,2], S. OJI[1], R. KRENCIK[1];
[1]Ctr. for Neuroregeneration, Dept. of Neurosurg., Houston Methodist Res. Inst., Houston, TX;
[2]Dept. of Med. Sci., Texas A&M Sch. of Med., Bryan, TX

Abstract: Objective: Mutations in the GBA gene represent the highest risk factor for developing Parkinson's Disease (PD) and Lewy Body Dementia. This gene encodes glucocerebrosidase (GCase), an enzyme primarily located within lysosomes that catalyzes glycolipids. Recent studies have emphasized the vital role of astrocytes in breaking down otherwise toxic fatty acid derived from hyperactive neurons. However, the dynamics of lipid trafficking and metabolism in the context of PD pathology remains obscure. Our objective is to utilize a novel midbrain organoid model to understand the consequences of astrocyte lipid dysregulation in the development and progression of PD. **Methods:** We genetically engineered human induced pluripotent stem cells (hPSCs, iPSCs) derived from two PD patients with GBA mutations and one healthy control, to be rapidly induced into functional astrocytes (Cvetkovic et al.). We also targeted GBA with CRISPR-Cas9 and generated a complete knockout (KO) line. Separately, we engineered lines to express transcription factors that induce direct differentiation into dopaminergic neurons and cocultured astrocytes and neurons together in the form of neural organoids. **Results:** All cell lines efficiently differentiated into astrocytes, which was verified by the presence of protein markers GFAP, CD44, S100B, and RNA sequencing. We also successfully generated organoids containing dopaminergic neurons. Mutant GBA astrocytes exhibited reduced GCase activity, while GBA KO astrocytes had no activity, as expected. KO astrocytes had larger lysosomes, reduced uptake of exogenous fatty acids, and higher levels of sphingolipids, while having lower levels of baseline triglycerides, similar to inflammatory astrocytes. We are currently investigating the consequence of dysfunctional lipid trafficking on

synaptic network activity within the neural organoids. **Conclusions:** The findings suggest that astrocyte lipid metabolism may play a crucial role in the pathology of PD and is expected to be an important target for the development of new therapeutic approaches.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.03/K9

Topic: C.03. Parkinson's Disease

Support: Center for Regenerative Nanomedicine at the Simpson Querrey Institute

Title: Supramolecular Nanostructure Mimic of Glial Cell Line-Derived Neurotrophic Factor (GDNF) and its biological effects on human dopaminergic neurons

Authors: *O. CARBALLO, A. EDELBROCK, M. ALVAREZ-SAAVEDRA, Z. ALVAREZ, T. PEREZ-ROSELLO, S. CHIN, J. SURMEIER, S. STUPP;
Northwestern Univ., Chicago, IL

Abstract: Parkinson´s Disease (PD) is the second most common neurodegenerative disorder. The administration of glial cell line-derived neurotrophic factor (GDNF) has been shown to improve motor dysfunctions in animal models of PD. However, GDNF has not shown the desired results in clinical outcomes, partially because of the short half-life and the uncontrolled diffusion of this trophic factor. To overcome those obstacles, here we report the incorporation of a GDNF-mimetic peptide on the surface of peptide-based nanofibers. Peptide amphiphiles (PAs) are self-assembling molecules that form nanostructures that have shown the ability to incorporate bioactive peptide sequences into their structure. We show that the nanostructure displaying the GDNF mimetic peptide sequence activates the canonical GDNF receptor RET *in vitro* as well as, the upregulation of genes involved in dopamine synthesis, neuronal development, and neuroprotection. We demonstrate that GDNF mimetic PA enhanced the cell viability of dopaminergic neurons derived from human induced pluripotent stem cells and promoted their morphological, synaptic, and electrophysiological maturation. Like native GDNF, this bioactive nanostructure provides a neuroprotective effect against the neurotoxin 6-OHDA. In addition, we evaluated the biological effects of this bioactive nanostructure in midbrain organoids derived from human embryonic stem cells. We demonstrated a significant dopaminergic axonal extension in the organoids embedded in the GDNF mimetic PA relative to the control. These results indicate that GDNF mimetic PA is a bioactive matrix capable of mimicking the main properties of GDNF on dopaminergic neurons. This bioactive nanostructure could improve cell replacement therapy in PD by enhancing the cell viability of grafted neurons and accelerating its maturation.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Program #/Poster #: PSTR329.04/K10

Topic: C.03. Parkinson's Disease

Title: Theranostic potential of nitrogen-doped graphene quantum dot nanoparticles in targeting alpha-synuclein protein aggregation in Parkinson's Disease

Authors: ***M. PALANIVEL**, K. K. GHOSH, P. PADMANABHAN, K.-L. LIM, B. GULYAS; Lee Kong Chian Sch. of Med., Nanyang Technological Univ., Singapore, Singapore

Abstract: Parkinson's disease (PD) is one of the frequently occurring progressive neurodegenerative disorders in the world, in which the misfolding and aggregation of a critical physiological protein, α-synuclein (α-syn), into toxic oligomers and eventually fibrils is widely theorized to trigger the death of dopaminergic neurons, leading to motor abnormalities. In this study, we evaluated the theranostic potentials of nitrogen-doped graphene quantum dot (NGQD) nanoparticles against α-syn aggregation, as well as their biocompatibility through in-vitro studies and biophysical assays. We have adapted a microwave-assisted synthesis protocol of NGQD and thoroughly characterized their properties through extensive spectrometric methods and transmission electron microscopy. The NGQDs were in the nanometre size range of 5.808 ± 1.272 nm and exhibited UV-Visible and near infrared region fluorescence emissions, suggesting their potential biocompatibility with cells and tissues, together with usage as an optical probe for α-syn detection. Most importantly, Thioflavin T assays demonstrated that NGQDs at concentrations of 250ug/mL, 125ug/mL, and 62.5ug/mL vastly reduced the aggregation of α-syn proteins by 28-fold, 9-fold and 2.5-fold respectively. Additionally, the NGQDs themselves exhibited an increase in fluorescence upon heightened inhibition of α-syn aggregation, further pointing to their utility as a diagnostic probe to detect α-syn monomers and fibrils. These demonstrated the theranostic potential of NGQD of both detecting α-syn and reducing its aggregation. Moreover, through *in vitro* MTT assays with the SH-SY5Y human neuroblastoma cell line, the NGQDs displayed very limited cytotoxicity to the cells, exhibiting more than 80% cell viability for concentrations of 250mg/mL up to 120 hours of treatment. Confocal microscopy revealed significant uptake of the NGQDs by SH-SY5Y cells and bright fluorescence in the green to orange region of the UV-Vis spectrum within the cells. We have also expressed an A53T mutant α-syn-containing SH-SY5Y cell line to further investigate the theranostic potentials against α-syn aggregation *in vitro.* Further investigations including the functionalization of NGQDs with α-syn aggregation targeting small molecules, specifically, curcumin and rosmarinic acid, and testing of their α-syn anti-aggregative efficacies are underway. Our current roadmap includes the experimentation of anti-aggregative activity of NGQDs *ex vivo* and *in silico.* This work paves the way for the development of theranostic

NGQD nanoparticles that can both detect and monitor α-syn aggregation, while simultaneously inhibiting the phenomenon.

Disclosures: M. Palanivel: None. **K.K. Ghosh:** None. **P. Padmanabhan:** None. **K. Lim:** None. **B. Gulyas:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.05/L1

Topic: C.03. Parkinson's Disease

Support: NINDS UH3NS116921
MJFF-001006

Title: Selection of small molecules that reduce the intrinsically disordered protein alpha-synuclein using iPSC-derived dopaminergic neurons from Parkinson's disease patients

Authors: *J. LIU[1], Y. TONG[2], J. L. CHILDS-DISNEY[2], S. MADDILA[1], K. HASSANZADEH[1], M. D. DISNEY[2], M. MOURADIAN[1];
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Abstract: Parkinson's disease (PD) is a common progressive neurodegenerative disorder currently with no cure. Alpha-synuclein (α-syn) is a key protein in the pathogenesis of PD since it misfolds and forms fibrils that can propagate across neurons, a process that underlies the progressive nature of the disease. A strong driver of pathological α-synuclein aggregation is its concentration in the brain, as individuals with multiplication of the SNCA gene locus develop early onset PD. Thus, reducing α-syn protein expression is a plausible disease modifying strategy. We previously discovered small molecules that directly target a structured iron-responsive element (IRE) in the 5' untranslated region (5' UTR) of the SNCA mRNA, which controls its translation. To improve drug-like properties, the lead compound Synucleozid was used as a prototype for further optimization. Here, by using a successive series of innovative cell-based biological approaches, we screened and identified additional analogues as well as diverse scaffolds that reduce α-syn protein levels in Hela cells. We then explored the potential of combining two screen hits. We found that the activity of certain compound-combinations (combos) was concentration dependent, with substantial reductions in α-syn levels occurring at considerably lower concentrations for certain compound pairs. To maximize the predictability of the activity of these compounds in clinical trials, we then tested top performers in human iPSCs-derived dopaminergic (DA) neurons to evaluate therapeutic candidates for PD. Using PD patient-derived DA neurons, combos had a significant and potent α-syn-lowering effect compared with single compounds. Importantly, the amount of high-molecular-weight insoluble species of α-syn and phosphorylated α-syn aggregates were significantly reduced in α-syn pre-formed fibrils-

challenged neurons when incubated with combos. Cell toxicity studies further demonstrated that potent combos provide a significant cytoprotective effect in PD iPSC-derived neurons. Altogether, our studies provide a promising compound selection strategy for PD and establish a path towards identifying effective disease-modifying small molecules for α-synucleinopathies.

Disclosures: **J. Liu:** None. **Y. Tong:** None. **J.L. Childs-Disney:** None. **S. Maddila:** None. **K. Hassanzadeh:** None. **M.D. Disney:** None. **M. Mouradian:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Program #/Poster #: PSTR329.06/L2

Topic: C.03. Parkinson's Disease

Title: Inhibition of Stearoyl-CoA Desaturase 1 rescues α-Synuclein pathology and dopamine neuron death in models of induced Synucleinopathy

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Merck & Co, Inc, West Point, PA

Abstract: α-synuclein (αSyn) accumulation and lipid dysregulation are strongly associated with the underlying pathology of Parkinson's disease (PD). Mutations in the lipid binding domain of αSyn are causative for PD and genome-wide association studies suggest that lipid-associated pathways attribute risk for developing PD. Based on this, modulation of cellular αSyn-lipid membrane interactions and lipid homeostasis may represent a therapeutic approach for treating PD. Reduced activity of stearoyl-CoA desaturase (SCD) enzymes; SCD1 and SCD5, which desaturate palmitic and stearic fatty acids, are reported to ameliorate αSyn related pathology and toxicity in preclinical models. Our data confirm that small molecule SCD inhibitors rescue αSyn pathology and are neuroprotective in additional disease relevant neuronal models including a recombinant αSyn pre-formed fibril (PFF) model and an αSyn oligomer induced dopaminergic neuron neurodegeneration model. Among SCD enzymes, SCD5 does not contribute functional desaturase activity, suggesting a dominant role for SCD1 in modulating membrane saturation state to alleviate αSyn toxicity. SCD inhibitors may modulate αSyn through multiple mechanisms relevant to disease pathophysiology, such as inducing autophagy and reducing lipotoxicity via lipid droplet formation. Overall, our findings corroborate and expand on the previous published data identifying SCD1 as a modulator of αSyn pathology and neurodegeneration in cellular models and suggest lipid modulation as relevant therapeutic target for rescuing synucleinopathy in PD.

Disclosures: **D. Toolan:** None. **S. Jinn:** None. **H. Andaluz:** None. **B. Shyong:** None. **C. Harrell:** None. **N. Hatcher:** None. **V. Shurtleff:** None. **C. Burgey:** None. **S.M. Smith:** None. **J.N. Marcus:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Program #/Poster #: PSTR329.07/L3

Topic: C.03. Parkinson's Disease

Support: Intramural grant from Fondazione Italiana Fegato to S.J. and S.G
The Indonesia Endowment Fund for Education (Lembaga Pengelola Dana Pendidikan/LPDP) from the Indonesian Ministry of Finance to S.J

Title: Protective effect of bilirubin-loaded nanobubbles in an ex vivo model of Parkinson's disease

Authors: *S. JAYANTI[1,2], S. ANSARI[3], E. FICIARÀ[5], C. GUIOT[3], R. CAVALLI[4], C. TIRIBELLI[2], S. GAZZIN[2];
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Abstract: Parkinson's disease (PD) still faces an unmet clinical need for disease-modifying therapy. We have shown that low concentrations of unconjugated bilirubin (UCB) prevent dopaminergic neuron (DOPAn) loss in an *ex vivo* model of PD (organotypic brain cultures of the substantia nigra of Wistar rats, OBCs-SN (Jayanti et al., 2022)). Since the protection requires a precise amount of UCB, we explored the use of nanobubbles (NBs) as a potential approach to deliver UCB. In step 1, the safety of three different polymeric shells of nanobubbles (NBs, about 300 nm), consisting of glycol-chitosan (GC), GC-deferoxamine (GC-DFO), and GC-DFO-superparamagnetic iron oxide nanoparticles (GC-DFO-SPIONs), was tested in OBCs-SN. The OBCs-SN was exposed to a range of dilutions of each NBs formulation and underwent viability tests. In step 2, the formulations of NBs were loaded with UCB (GC-UCB and GC-DFO-UCB) and used on rotenone-treated OBCs-SN (PD model) to test their protective effect. Both safety and protection were evaluated by the MTT test, lactate dehydrogenase (LDH) release, and the DOPAn count (tyrosine hydroxylase positive in immunofluorescence staining). Dimethyl sulfoxide (DMSO)-exposed OBCs-SN was used as a control in both steps. The encapsulation efficiency of NBs loaded with UCB is more than 98%. After safety evaluation (step 1), GC-DFO-SPIONs were excluded due to agglomeration. NBs were toxic at 1:8 dilution for both GC and GC-DFO (1.2±0.1-fold, $p<0.01$ and 1.6±0.4-fold, $p<0.05$ vs. DMSO, respectively). GC at 1:8 dilution, as well as GC-DFO at 1:8 and 1:64 induced a significant DOPAn loss. Dilutions 1:8 and 1:64 were excluded in step 2. In step 2 (protection evaluation), the DOPAn number in the PD model was 61±9.1% *vs.* DMSO ($p<0.001$). The GC-DFO-UCB NBs already showed a protective effect at 1:1524 dilution, increasing by 27% the number of DOPAn compared to the PD model (88±0.91% *vs.* DMSO; $p<0.001$ vs. rotenone). In conclusion, at specific formulations and concentrations, NBs-UCB showed protection in the PD model. Further studies are needed to

understand the number of NBs entering the tissue and their capacity to release UCB to maximize protection and validate the effect in an *in vivo* setting.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.08/L4

Topic: C.03. Parkinson's Disease

Support: Branfman Family Foundation

Title: Identification of small-molecule modulators of seeded aSyn aggregation in cellular preformed fibril models

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Abstract: Parkinson's disease (PD) is a progressive neurodegenerative disorder for which there are no disease-modifying treatments. Neuropathological hallmarks of PD include the loss of dopaminergic neurons and the formation of cytoplasmic inclusions enriched with aggregated forms of the presynaptic protein alpha-synuclein (aSyn), known as Lewy bodies and Lewy neurites. Although dopaminergic therapies remain the gold standard in PD treatment, current therapeutic approaches only provide symptomatic relief and cannot reverse the degeneration of dopaminergic neurons. Accordingly, there is a high demand for identifying new agents that could serve as disease-modifying therapies. Misfolded aSyn can propagate from cell to cell, and widespread deposition of aSyn aggregates is observed in PD brains. aSyn oligomers and aggregates are toxic, inducing mitochondrial dysfunction, endoplasmic reticulum (ER) stress, and impairment of the autophagy-lysosomal pathway (ALP), ultimately resulting in neuronal death. Cellular models with aSyn aggregate-induced neuropathology are critical for understanding the formation and spread of aggregates and testing the efficacy of drugs in rescuing disrupted cellular pathways. Here, we used preformed fibrils (PFFs) prepared from recombinant aSyn as seeds to induce aSyn aggregation in HEK293T FRET biosensor cells that stably express CFP- and YFP-fused A53T aSyn, as well as primary cortical neurons. Aggregate formation was monitored by measuring the FRET signal between the CFP and YFP modules in HEK293T FRET biosensor cells, and by immunostaining primary cortical neurons with an antibody specific for aSyn phosphorylated on serine residue 129, a form of the protein that serves as a marker of aSyn pathology. Compounds that target mitochondrial free radicals, previously shown to alleviate dopaminergic neuron death elicited by mitochondrial toxins or aSyn-encoding

viruses in primary midbrain cultures or in *C. elegans*, were tested for their effects on seeded aSyn aggregation in cell culture models. Unexpectedly, we found that aSyn aggregate levels were increased in neurons or HEK293T cells treated with PFFs plus compound compared to PFFs alone. These results demonstrate that our cellular PFF aSyn models can be used to screen for drug candidates that target mitochondria and potentially other subcellular compartments affected by aSyn aggregates. Moreover, the small molecules examined in the current study could serve as powerful tool compounds to characterize cellular phenomena that modulate the spread of aSyn pathology in the brains of PD patients.

Disclosures: W. Qi: None. **S. Sammi:** None. **J. Cannon:** None. **J. Rochet:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR329.09/L5

Topic: C.03. Parkinson's Disease

Support: NIH/NIEHS, 5 R01 ES024745-07
NIH/NIEHS, 5 R01 ES033462-02
MJFF-020697

Title: Characterizing an Interaction between Acyl-CoA: Cholesterol Acyltransferase 1 and the NLRP3 Inflammasome in Microglia

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Abstract: Increasing prevalence, debilitating symptoms, and lack of effective treatments for neurodegenerative diseases necessitate the characterization of cellular and molecular mechanisms relevant to these disorders. Recent work has implicated inflammation in neurodegeneration and the NLRP3 inflammasome has emerged as a target of translational importance. Inflammasomes are protein oligomers functioning in the innate immune system to initiate pro-inflammatory responses. Activation leads to the assembly of functional inflammasomes that drive the enzymatic cleavage and subsequent secretion of various pro-inflammatory cytokines. Inflammasome activation can also cause pyroptosis, a form of pro-inflammatory cell death. Lipids have been established as mediators of inflammasome activity, but the molecular basis of this interaction is poorly understood. Cholesterol metabolism and intercellular cholesterol concentrations regulate components and downstream effectors of the NLRP3 inflammasome including, but not limited to, IL1β and Caspase 1. NLRP3 inflammasome function is modulated by the availability of cholesterol at the endoplasmic reticulum (ER) which

serves as a site of cholesterol biosynthesis and a sensor for responding to and maintaining cellular cholesterol levels. The enzyme Acyl-CoA: Cholesterol Acyltransferase 1 (ACAT1) is a membrane-bound protein located at the ER that is responsible for intracellular cholesterol esterification and storage. ACAT1 loss reduces amyloid plaque burden in mouse models of Alzheimer's disease but its function in neuroinflammation is less understood. We tested ACAT1 inhibitors in wild-type and *Nlrp3*$^{-/-}$ primary mouse microglia cultures based on the prediction that increasing the pool of available intracellular cholesterol would suppress NLRP3 inflammasome activity. Inhibition of ACAT1 decreased the release of the activated forms of IL1β and Caspase 1 in wild-type but not *Nlrp3*$^{-/-}$ microglia. These data suggest that ACAT1 inhibition blocks the function of the NLRP3 inflammasome. Our results identify an interaction between two druggable targets: ACAT1 and the NLRP3 inflammasome, providing an inroad to test hypotheses related to cholesterol homeostasis pathways modulating inflammasome-mediated neuroinflammation.

Disclosures: E.N. Fikse: None. **K. Biggs:** None. **C. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Application Entitled: Method for Attenuating Neuroinflammation, Amyloidopathy and Tauopathy. **T. Chang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Application Entitled: Method for Attenuating Neuroinflammation, Amyloidopathy and Tauopathy. **M.C. Havrda:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

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Topic: C.03. Parkinson's Disease

Support: Campbell Foundation
GVSU P. Douglas Kindschi Undergraduate Research Fellowship in the Sciences

Title: Effect of microRNA mimics on alpha-synuclein gene expression in SH-SY5Y cells

Authors: *S. KHOO, Z. WALTERS;
Grand Valley State Univ., Grand Rapids, MI

Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder with no cure. Although the etiology of PD remains unclear, the presence of alpha-synuclein (a-syn) aggregates as hallmark of PD is suggested to cause dopaminergic neuron degradation that leads to PD pathogenesis. Thus, one key therapeutic strategy is to target a-syn. Here, we evaluated the effects of 5 known a-syn-related microRNA mimics (miR-7, miR-153, miR-34b, miR-34c, and miR-214) on a-syn gene expression in SH-SY5Y cells, using quantitative real-time PCR (qRT-PCR) assay. We hypothesized that these mimics would bind to a-syn mRNA to lower a-syn gene

expression. First, we treated the cells with retinoic acid, followed by brain-derived neurotropic factor, before treated with neurotoxin rotenone for neuron degradation like early PD. One day after rotenone treatment, we transfected the cells with 50 pmol of each miRNA mimic. We then extracted total RNA and used Taqman qRT-PCR assay to quantitate a-syn expression. Interestingly, we found up-regulation of α-syn gene expression for all miRNAs, except miR-34b. The results were not what we expected and there are three possible explanations. One, since the mimic negative control also showed up-regulation of the gene, we cannot rule out the possibility of the mimic fragment itself, not its specific sequences, can somehow affect gene expression by unexplained reasons. Second, although these miRNAs target α-syn, they also target other genes (multiple targeted genes are the nature of miRNAs) and thus may also bind to other genes that lead to α-syn up-regulation. Third, it is possible that after transfection, there was an initial down-regulation of α-syn gene expression. However, the effect triggers the negative feedback loop of the regulatory system to increase α-syn gene expression to reach its original equilibrium, which perhaps is a more plausible explanation. We showed miR-34b mimic can lower α-syn gene expression, similar with another study using precursor miR-34b. One reason why miR-34b mimic may be more efficient in suppressing α-syn expression is because of it targeting 2 specific binding sites on α-syn mRNA instead of one.

Disclosures: S. Khoo: None. **Z. Walters:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Topic: C.03. Parkinson's Disease

Support: NIH Grant NS070577
NIH Grant OD024622
Parkinson's Cell Therapy Research Fund at McLean Hospital and Massachusetts General Hospital
Masson Family Endowment in Neurosurgery

Title: Intra-striatal co-transplantation of autologous T_{REG} in Parkinson's disease cell therapy

Authors: *J. JEON[1,2], T.-Y. PARK[1,2], N. LEE[1,2], Y. CHA[1,2], M. KIM[1,2], P. LEBLANC[1,2], T. M. HERRINGTON[3], B. S. CARTER[4], J. S. SCHWEITZER[4], K.-S. KIM[1,2,4];
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Abstract: The specific loss of midbrain dopamine (mDA) neurons causes major motor dysfunction in Parkinson's disease (PD), rendering cell replacement a promising therapeutic approach. However, poor survival of grafted mDA neurons remains a major obstacle to successful clinical outcomes. Here we show that the surgical procedure itself (referred to here as

"needle trauma") triggers a profound host response characterized by acute neuroinflammation, robust infiltration of peripheral immune cells, and brain cell death, even without cell implantation. When human induced pluripotent stem cell (hiPSC)-derived mDA cells were transplanted into the rodent striatum, only a small percentage (<10%) of implanted tyrosine hydroxylase (TH)+ mDA neurons survived 2 weeks post-transplantation. In contrast, TH- grafted cells mostly survived. Remarkably, transplantation of autologous regulatory T cells (T_{REG}) greatly modified the response to needle trauma, suppressing acute neuroinflammation and immune cell infiltration. Furthermore, we carefully analyzed the effects of intra-striatal co-transplantation of T_{REG} and hiPSC-derived mDA cells using several rodent models including rat (Fischer 344), immunodeficient NSG, and humanized NSG mouse models. We found that T_{REG} significantly protected grafted mDA neurons from needle trauma-associated death and substantially improved therapeutic outcomes in these 6-OHDA lesioned PD rodent models. Co-transplantation with T_{REG} also suppressed undesirable proliferation of TH- grafted cells, resulting in more compact grafts with a higher proportion and higher absolute numbers of TH+ neurons. Taken together, these data emphasize the importance of the initial inflammatory response to surgical injury in differential survival of cellular components of the graft and suggest that co-transplantation of autologous T_{REG} effectively reduces needle trauma-induced death of mDA neurons, suggesting a potential strategy to achieve better clinical outcomes of PD cell therapy.

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Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.12/L7

Topic: C.03. Parkinson's Disease

Title: High-throughput electrophysiology tools to measure TMEM175 proton conductance

Authors: *D. PAU, S. RICE, D. DALRYMPLE, I. MCPHEE;
SB Drug Discovery, Glasgow, United Kingdom

Abstract: TMEM175 is a novel, constitutively active lysosomal channel involved in regulating lysosomal pH and autophagy. Mutations in this gene have been shown to impair normal lysosomal and mitochondrial function, and as a result, increase aggregation of insoluble proteins such as phosphorylated α-synuclein, a hallmark of Parkinson's Disease. Such protein aggregation can lead to cell toxicity and death, resulting in the degenerative physical and cognitive symptoms typical of PD. Consequently, there is significant potential for TMEM175 to play a key role in the treatment of Parkinson's disease. By increasing the activity of this channel, it may be possible to enhance the efficiency of the cellular recycling process, leading to increased breakdown of toxic aggregates such as α-synuclein. However, the lack of specific pharmacological tools has

hampered further investigation into the exact role of TMEM175 in normal lysosomal function and its role in such pathological processes.
Although TMEM175 is commonly reported as a potassium “leak” channel, under normal lysosomal pH (4.5-5.0) TMEM175 has been shown to be much more permeable to protons than to potassium or sodium, with >90% of the ion flow via TMEM175 mediated by protons.
To facilitate the detection of TMEM175-induced proton conductance, we have successfully developed an automated electrophysiology assay to measure proton-activated, proton conductance using plasma membrane localized TMEM175. By introducing a proton gradient across the membrane, we successfully detected large inward proton currents which increased in relation to the lowering of the extracellular pH. This proton conductance was modulated by known TMEM175 activators and blocked by the reference inhibitor 4-AP.
The assay described here can be used to identify and characterize novel pharmacological tools for TMEM175 drug discovery and should help to further investigate into the role of this exciting target in normal physiology and its involvement in neurodegenerative diseases.

Disclosures: **D. Pau:** None. **S. Rice:** None. **D. Dalrymple:** None. **I. McPhee:** None.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

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Program #/Poster #: PSTR329.13/L8

Topic: C.03. Parkinson’s Disease

Support: National Institutes of Health [R01-NS117757 (D.K.C.)]

Title: Living deep brain stimulation for external control of striatal dopamine.

Authors: *K. N. A. YANKSON[1,2,5], D. CHOUHAN[3,5], D. K. CULLEN[4,2,5];
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Abstract: Parkinson's disease (PD) is the world's second most common neurodegenerative disorder. The premature death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the loss of their axonal connections to the striatum reduce dopamine levels in the striatum to sub-functional levels, causing debilitating motor symptoms like postural instability, rigidity, tremor, and bradykinesia. Current gold standard treatments for PD, such as dopamine replacement therapy and deep brain stimulation (DBS), lose efficacy over time, are nonspecific, and do not replace lost dopaminergic neurons in the brain. In addition, DBS utilizes inorganic microelectrodes that can cause neuronal loss, glial scarring, and inflammation. We are advancing an alternative strategy to control striatal dopamine levels featuring implantable, living axonal tracts projecting from a discrete population of optically controlled dopaminergic neurons which

we refer to as Dopaminergic Tissue Engineered Living Electrodes (dTELEs). The dTELEs consist of aggregated dopaminergic neurons and long-distance axonal tracts enclosed within hydrogel micro-columns. We have shown that these "living electrodes" fabricated using primary rat neurons can be externally controlled using light after being transduced to express channelrhodopsins and they can establish connections with existing neural circuitry after implantation into the rat brain. Our current objective is to optimize the transduction efficiency of human induced pluripotent stem cell (iPSC)-derived dopaminergic neurons using AAV vectors to allow for optical control while maintaining cell health. Using immunocytochemistry and confocal microscopy, the aggregates were confirmed to be dopaminergic neurons through the co-expression of tyrosine hydroxylase (TH) and β-tubulin III. We found higher transduction efficiency with the 48hr incubation time, and the highest titer as indicated by the fluorescent reporter intensity and extent of coverage throughout the neuronal somata and axonal tracts. However, cell viability and health metrics decreased with increases in viral titer, and differential health metrics were found across the various vectors at matched titers. Further comparisons based on light- versus electrical-induced evoked dopamine release are currently ongoing. These efforts are advancing a novel biologically based treatment for the debilitating symptomology of PD based on a tissue engineering approach that replaces lost dopaminergic neurons, directs axonal growth and connection to the striatum, and ultimately allows for specific, controlled external activation to regulate striatal dopamine levels.

Disclosures: K.N.A. Yankson: None. **D. Chouhan:** None. **D.K. Cullen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent App. 62/758,203 titled “Engineered neural networks in tailored hydrogel sheaths and methods for manufacturing the same”., U.S. Patent App. 16/093,036 titled “Implantable living electrodes and methods for the use thereof”, Patent App. 15/032,677 titled “Neuronal replacement and reestablishment of axonal connections”, Scientific co-founder of Innervace Inc. and Axonova Medical LLC.

Poster

PSTR329. Therapeutic Strategies: Cellular Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR329.14/M1

Topic: C.03. Parkinson’s Disease

Title: Development and characterization of human iPSC-derived 3D neurospheres for drug discovery

Authors: *C. FORMICA, I. ONOFRE, C. VAN BERKEL, K. PITSA, T. WÜST, S. JAIN; Ncardia, Leiden, Netherlands

Abstract: Background. Drug attrition rates remain high, with 90% of drugs tested in Phase I trials failing to reach the market. This is even more pronounced for central nervous system (CNS) targeting drugs. One major reason for this is the use of inappropriate preclinical models,

which fail to predict successfully the outcome of clinical trials in terms of efficacy and toxicity.However, induced pluripotent stem cells (iPSCs) and iPSC-derived 3D models offer potential solutions. iPSC-derived 3D neurospheres can be generated from human cells and better recapitulate the biology, morphology and molecular dynamics observed in human *in vivo*. Additionally, these models can be developed from patient derived iPSC, thus increasing the relevance and predictability of the model. **Methods.** We describe the generation of 3D neurospheres from a co-culture of our in house developed hiPSC-derived Ncyte cortical neurons with Ncyte astrocytes and microglia. We characterized the model in respect of cell composition, maturation and function. We then adapted the protocol to use iPSCs cells carrying either a mutation in MAPT or SNCA genes to generate models for Frontotemporal dementia and Parkinson's disease respectively. **Results.** Using whole mount immunofluorescence, we confirm the presence of mature neurons and astrocytes by expression of MAP2 and GFAP, and of synaptic markers SYP and PSD-95. Particularly, we observe improved spatial organization in 3D compared to 2D. Using a calcium assay, we show that our 3D neurospheres have spontaneous and synchronized calcium oscillations, which can be modulated by activating or inhibiting compounds, such as glutamate and GABA. We also observe that 3D neurospheres generated from mutant iPSCs present a dysregulation of calcium homeostasis and increased vulnerability to stress *in vitro*. **Conclusions.** We generated a 3D CNS models that can efficiently model CNS morphology and function *in vitro*, both in physiological and pathological conditions. By integrating this system with hiPSC-derived microglia cells and/or combining it with Tau or alpha-Synuclein PFF we aim at modelling neurodegenerative diseases like Alzheimer and Parkinson. Moreover, our protocol can be easily adapted to high throughput screening platform and liquid handling robots, thus increasing its applicability to drug discovery.

Disclosures: **C. Formica:** A. Employment/Salary (full or part-time):; Ncardia Services BV. **I. Onofre:** A. Employment/Salary (full or part-time):; Ncardia. **C. van Berkel:** A. Employment/Salary (full or part-time):; Ncardia. **K. Pitsa:** A. Employment/Salary (full or part-time):; Ncardia. **T. Wüst:** A. Employment/Salary (full or part-time):; Ncardia. **S. Jain:** A. Employment/Salary (full or part-time):; Ncardia.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.01/M2

Topic: C.06. Neuromuscular Diseases

Title: Assessing the Impact of ALS and NEIMY Causative KIF5A Mutations on Cargo Transport and Cytoskeletal Structure

Authors: *O. STERLING-ANGUS, J. R. BRENT, H.-X. DENG;
Northwestern Univ., Chicago, IL

Abstract: KIF5A, a member of the kinesin superfamily, is a neuron-specific motor protein, and is essential to intracellular transport and regulating cytoskeletal dynamics. Mutations in this gene are associated with a broad range of neurodevelopmental and neurodegenerative diseases. We chose to focus on Amyotrophic Lateral Sclerosis (ALS) and Neonatal Intractable Myoclonus (NEIMY) because these diverse clinical phenotypes are caused by distinct mutations in a similar location of the C-Terminal domain. We developed *in vitro* models of the ALS and NEIMY variants of KIF5A to explore the impact on cargo transport and the cytoskeleton. Our results reveal that both mutations cause disruption of the protein's autoregulation, resulting in over-accumulation of the protein in the axon tip. However, the accumulation of the NEIMY variant is much more severe, causing extreme aggregation of mutated KIF5A. These aggregates seem to also sequester kinesin light chain 1 (KLC1) suggesting that the entire Kinesin-1 complex is affected. Further, the aggregates cause a physical blockage in the axon, resulting in major defects in the cytoskeleton, and obstructing transport to the distal axon. Our findings suggest that the primary mechanism for both diseases is gain of function. However, we found that truncating mutations in the region of the NEIMY mutation cause a partial phenotypes of the disease, suggesting a secondary mechanism of loss of function. Our findings further elucidate the mechanisms of disease-causative KIF5A mutations and characterize the underlying differences in the divergent clinical phenotypes of ALS and NEIMY.

Disclosures: O. Sterling-Angus: None. **J.R. Brent:** None. **H. Deng:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.02/M3

Topic: C.06. Neuromuscular Diseases

Support: 2021 AAN Clnical Research Training Scholarship in ALS
2022 Clinician Scientist Retention Award From the Doris Duke and Walder Foundations

Title: Als causative mutations in kif5a disrupt autoinhibition leading to toxic gain of function

Authors: *J. BRENT, O. STERLING-ANGUS, H.-X. DENG;
Northwestern Univ., Chicago, IL

Abstract: Objective:The objective of this study was to elucidate the pathogenic mechanisms of ALS causative mutations in motor protein KIF5A. Background:KIF5A is a neuronal specific subunit of Kinesin-1, which is a microtubule motor protein that plays roles in axonal transport and cytoskeletal regulation. Mutations in distinct regions of Kinesin-1 lead to a broad range of neurologic diseases. KIF5A enables Kinesin-1 to walk along microtubules and transport various cargos such as mitochondria and RNA granules into the distal axon. ALS-causative mutations in KIF5A affect the structure of the domain of the protein that functions in cargo binding and

autoregulation. However, the consequences of ALS-causative mutations on disease pathogenesis are not fully understood. Our central hypothesis was that KIF5A ALS mutations disrupt cargo binding and/or autoregulation leading to neurodegeneration.
Design/Methods:We generated *in vitro* models of KIF5A ALS using transient transfection of disease causative variants into neuroblastoma cell lines. We performed immunofluorescence staining and confocal imaging to visualize the distribution of motor proteins KIF5A, KIF5B, and Dynein. We utilized Drosophila S2 cell lines to study mitochondrial motility using live-imaging.
Results:Whereas wild type (WT) KIF5A displayed relatively homogeneous distribution, ALS mutant KIF5A developed dramatic accumulation within distal neurites. Co-expression of the ALS mutant protein with the WT caused it to accumulate as well. Mitochondria displayed similar mislocalization to distal neurites. No change was seen in the distribution of KIF5B or Dynein.Conclusions:We found that ALS mutant KIF5A caused dramatic accumulation of mutant and WT protein as well as mitochondrial within distal neurite tips. These findings suggest that ALS mutation disrupts its autoinhibition causing mislocalization of its cargos through gain of function. These changes in distribution are specific to KIF5A containing motors. Our findings establish dysregulation of KIF5A activity as the underlying pathogenic mechanism for KIF5A ALS.

Disclosures: **J. Brent:** None. **O. Sterling-Angus:** None. **H. Deng:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.03/M4

Topic: C.06. Neuromuscular Diseases

Support: Takeda Science Foundation
Japan Society for the Promotion of Science (JSPS) KAKENHI (Grant No. JP17K08311)
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API Research Foundation
Smoking Research Foundation

Title: Activation of α7nACh receptor mediated neuroprotection against mutant copper-zinc superoxide dismutase 1-mediated toxicity

Authors: *T. ITO, K. OHUCHI, H. KURITA, I. HOZUMI, M. INDEN;
Gifu Pharmaceut. Univ., Gifu-shi / Gifu, Japan

Abstract: Amyotrophic lateral sclerosis (ALS) is an adult-onset neurological disorder that is characterized by muscle weakness and atrophy, paralysis, and eventual death by respiratory

failure. Symptoms result from the selective degeneration of upper and lower motor neurons. Currently, there is no clearly effective treatment or cure for this disease. Although many drugs have entered clinical trials, few have shown effectiveness in the treatment of ALS. A pathological hallmark of ALS is the presence of cytoplasmic inclusions or protein aggregates in affected motor neurons, suggesting that impairment of protein degradation may play a role in the disease pathology. Mutant copper-zinc superoxide dismutase 1 (SOD1) aggregates are present in both sporadic and familial ALS. Thus, removal of SOD1 aggregates may be a potential therapeutic approach for ALS treatment. As a strategy to remove mutant SOD1 aggregates, activation of autophagy has been reported to be effective in previous studies. In this study, we focused on the activation of α7 nicotinic acetylcholine receptor (nAChR) as an inducer of autophagy. Other studies have shown that the activation of α7 nAChR have neuroprotective effects in some models of neurodegenerative disease, as well as prevent glutamate-induced motor neuronal death. However, the effect of α7 nAChR agonists on ALS-associated mutant SOD1 aggregates in motor neurons remains unclear. In this study, we examined whether α7 nAChR activation had a neuroprotective effect against SOD1 (G85R)-induced toxicity in a cellular model for ALS. We found that α7 nAChR activation by PNU282987, a selective agonist of α7 nAChR, exhibited significant neuroprotective effects against SOD1 (G85R)-induced toxicity via the reduction of intracellular protein aggregates. This reduction also correlated with the activation of autophagy through the AMP-activated protein kinase (AMPK)-mammalian target of rapamycin (mTOR) signaling pathway. Furthermore, the activation of α7 nAChRs was found to increase the biogenesis of lysosomes by inducing translocation of the transcription factor EB (TFEB) into the nucleus. These results support the therapeutic potential of α7 nAChR activation in diseases that are characterized by SOD1 (G85R) aggregates, such as ALS.

Disclosures: **T. Ito:** None. **K. Ohuchi:** None. **H. Kurita:** None. **I. Hozumi:** None. **M. Inden:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR330.04/M5

Topic: C.06. Neuromuscular Diseases

Support: Dept of Defense W81XWH-22-1-0218

Title: Modulation of G-quadraplex formation as a novel strategy to inhibit TDP-43 aggregation in amyotrophic lateral sclerosis

Authors: ***M. E. S. HERDA**, K. REYNOLDS CAICEDO, D. A. LINSEMAN, S. HOROWITZ; Biol. Sci., Univ. of Denver, Denver, CO

Abstract: Modulation of G-quadraplex formation as a novel strategy to inhibit TDP-43 aggregation in amyotrophic lateral sclerosis McKenna Spaeth Herda, Kevin Reynolds Caicedo,

Daniel A. Linseman, Scott Horowitz Many different proteins, including TAR DNA-binding protein-43 (TDP-43), can cause amyotrophic lateral sclerosis (ALS); a debilitating neurodegenerative disorder driven by aberrant protein aggregation. The variety of proteins that drive different forms of ALS make it exceptionally difficult to treat with one type of therapeutic, but it has been observed that nearly all of these protein aggregates bind a specific nucleic acid structure: G-quadraplexes. G-quadraplexes are nucleic acid-based guanine tetrads that form in RNA and DNA and that act as protein chaperones in the cell. We hypothesize that these unique protein chaperones may drive pathological protein aggregation in ALS. To test this hypothesis, we have developed a series of in vitro ALS models including overexpression of TDP-43 in transfected HEK293 cells, NSC34 motor neuron-like cells, and spinal motor neurons differentiated from mouse embryonic stem cells. Each of these cell systems can be treated with G-quadruplexes or G-quadruplex stabilizing molecules and the effects on TDP-43 aggregation measured. Preliminary results show that incubation of cells with G-quadruplex stabilizing molecules significantly blocks TDP43 aggregation induced by oxidative stress. In addition, using live and fixed cell imaging, we observed large quantities of G-quadraplexes accumulating outside of the nucleus in HEK cells incubated with G-quadruplex stabilizers. These results suggest that modulating G-quadruplex formation in cells could be a therapeutic avenue for inhibiting TDP-43 aggregation in ALS.

Disclosures: M.E.S. Herda: None. **K. Reynolds Caicedo:** None. **D.A. Linseman:** None. **S. Horowitz:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.05/M7

Topic: C.06. Neuromuscular Diseases

Title: Sod1g93a mutation connects mitochondrial dysfunctions, neuromuscular junction disruption and increased sensitivity to glutamatergic stress in in vitro models of als.

Authors: *A. HENRIQUES[1], L. ROUVIÈRE[2], E. STEIDL[2], P. POINDRON[2], N. CALLIZOT[2]; [1]Neuro-Sys, GARDANNE, France; [2]Neuro-Sys, Gardanne, France

Abstract: Amyotrophic lateral sclerosis (ALS) is a rare motor neuron disease affecting people between 50 and 70 years of age and is characterized by degeneration and loss of upper (motor cortex) and lower (spinal cord and brainstem) motor neurons and muscle denervation. Neurodegeneration of spinal motor neurons and loss of neuromuscular junctions in ALS are caused by complex and multifactorial pathological events, including mitochondrial stress and defects in axonal transport driving neuromuscular disruption. Toxic gain-of-function mutations of superoxide dismutase type-1 (SOD1), an antioxidant enzyme whose activity is preserved in most mutant forms, have been linked to familial cases of ALS. Mutants SOD1 animal and cellular models are useful tools to study the disease.

Primary spinal motor neurons (MNs) from SOD1 G93A transgenic (Tg) or wild type (WT) rats were cultured for 14 days. Neuronal maturation and functional mitochondria were assessed under physiological condition. Neuronal survival, integrity of the neurite network and abnormal cytoplasmic accumulation of TDP43 were also evaluated after glutamate stress. In addition, rat spinal explants were co-cultured with human myoblasts to form in vitro motor units. The formation of these motor units was evaluated using spinal explants from both SOD1 G93A transgenic and WT rat embryos.
Our results showed that under normal conditions, SOD1 G93A Tg MNs had a lower number of functional mitochondria, higher levels of mitochondrial reactive oxygen species, and delayed neuronal maturation compared to WT MNs. Furthermore, in vitro NMJs derived from SOD1 G93A spinal explants exhibited delayed maturation and a shorter neurite network.
When exposed to glutamate-induced injury, there was a clear loss of spinal MNs and abnormal cytoplasmic accumulation of TDP43, with the effects being more pronounced in SOD1 G93A MNs. Similarly, glutamatergic stress caused disruption of NMJs, which was exacerbated in the SOD1 G93A conditions. The neuroprotective effects of the FDA-approved drugs riluzole and edaravone, commonly used for treating ALS patients, were observed but were relatively less effective in SOD1 G93A cultures compared to wild-type cultures.
Altogether, these findings clearly demonstrate that the expression of the SOD1 G93A transgene leads to mitochondrial stress, impaired neuronal maturation in basal conditions and increased sensitivity to glutamatergic stress, in two in vitro models of ALS.

Disclosures: A. Henriques: None. **L. Rouvière:** None. **E. Steidl:** None. **P. Poindron:** None. **N. Callizot:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

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Program #/Poster #: PSTR330.06/M8

Topic: C.06. Neuromuscular Diseases

Support: United States Department of Veterans Affairs Grant IK2RX002688
Research Grant from Neurodegenerative Disease Research Inc.

Title: Carboxyl-terminal modulator protein (CTMP) upregulation as a mechanism of NFkB-mediated muscle atrophy

Authors: *C. WALKER, V. PHILLIPS, C. MUMAW, J. WANG, Z. ESTAKI, L. GOLDMAN; Indiana Univ., Indianapolis, IN

Abstract: Carboxyl-terminal modulator protein (CTMP) upregulation as a mechanism of NFκB-mediated muscle atrophy

Chandler L. Walker[1,2], Victoria Phillips[1], Christen Mumaw[1], Junmei Wang[1], Zohreh Estaki[1], & Lillian Goldman[1]

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Amyotrophic lateral sclerosis (ALS) causes gradual paralysis and atrophy of skeletal muscles due to a loss of motor neurons and impaired neuromuscular connections. Developing effective therapies is challenging due to the intricate nature of the disease and the lengthy diagnostic process. Neuroinflammation and inflammatory responses in peripheral skeletal muscle play an important role in disease progression and exacerbates its severity. The inflammatory protein, tumor necrosis factor-α (TNF-α), triggers a cascade of inflammatory signals by activating nuclear factor kappa B (NFκB), a key contributor to inflammation in atrophic muscle. Anabolic Akt signaling is downregulated and atrogene expression is induced upon denervation and activation of NFκB. However, the mechanism by which NFκB promotes muscle atrophy in ALS is not well understood. We have previously shown that carboxyl-terminal modulator protein (CTMP) increases in skeletal muscle in ALS, after nerve injury, and in response to elevated TNF-α treatment. CTMP is known as an antagonist of Akt signaling, and our prior work has linked CTMP to the downregulation Akt/mammalian target of rapamycin (mTOR) signaling, and the promotion of muscle breakdown in different muscle atrophy models. Still, the mechanism for its increase is unknown. In our recent work, we investigated whether NFκB inhibits anabolic signaling by upregulating CTMP expression in models of inflammatory muscle atrophy. We found that inhibiting NFκB activation significantly attenuates CTMP expression ($p<0.05$) and elevates Akt/mTOR activity (both $p<0.01$) and downstream markers of translation (ribosomal protein S6 and 4EBP1 phosphorylation ($p<0.05$ & $p<0.01$, respectively) in myotubes under inflammatory conditions. Our results thus far suggest that NFκB triggers CTMP upregulation resulting in a decrease in anabolic signaling in our inflammatory muscle atrophy model. Since NFκB is known to increase CTMP expression through transactivation, we are investigating this as a potential mechanism. These findings are likely to provide new insights into the molecular mechanisms underlying inflammation-associated muscle atrophy, such as that observed in ALS.

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Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR330.07/M9

Topic: C.06. Neuromuscular Diseases

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R01GM099836

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R21AG065854

Title: Nuclear import receptors as modifiers of TDP-43 pathology in ALS/FTD disease models

Authors: A. GADGIL[1], A. M. LY[2], C.-W. TSAI[3], B. KHALIL[2], M. LINSEMEIER[5], F. LIU[1], M. WREN[1], C. SMITH[1], D. CHHANGANI[6], D. MORDERER[1], Y. ZHANG[2], D. W. DICKSON[4], S. H. YOSHIMURA[8], S. BARMADA[9], D. E. RINCON-LIMAS[7], J. SHORTER[10], *W. ROSSOLL[1];
[2]Neurosci., [3]Neurosci. Res. Dept., [4]Pathology & Neurosci., [1]Mayo Clin., Jacksonville, FL; [5]Univ. of Pennsylvania, Philadelphia, PA; [6]McKnight Brain Inst., Gainesville, FL; [7]McKnight Brain Inst., Gainsville, FL; [8]Kyoto Univ., Kyoto, Japan; [9]Neurolog. Disorders, Univ. of Michigan Dept. of Neurol., Ann Arbor, MI; [10]Dept. of Biochem. & Biophysics, Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Cytoplasmic mislocalization and aggregation of TAR DNA-binding protein-43 (TDP-43) is a hallmark of the amyotrophic lateral sclerosis and frontotemporal dementia (ALS/FTD) disease spectrum. While most ALS cases are sporadic, mutations in TDP-43 can directly cause ALS, likely via a combination of nuclear loss of function and cytoplasmic toxic gain of function phenotypes. Here we follow up on previous findings that karyopherin beta-1 (KPNB1) and other members of the nuclear import receptor (NIR) protein family can rescue the hallmarks of TDP-43 proteinopathy, by restoring its solubility and nuclear localization, and reducing neurodegeneration in cellular and animal models of ALS/FTD. We propose that importins, analogous to their canonical role in dissolving the diffusion barrier formed by phenylalanine and glycine-rich nucleoporins (FG-Nups) in the nuclear pore, are recruited into TDP-43 aggregates present in TDP-43 proteinopathies. Here we show that expression of importins or their active fragments can therapeutically reverse the cytoplasmic mislocalization and deleterious phase transition of TDP-43 and coaggregation with FG-Nups, mitigating neurodegeneration in cellular and animal models of ALS/FTD.

Disclosures: A. Gadgil: None. **A.M. Ly:** None. **C. Tsai:** None. **B. Khalil:** None. **M. Linsemeier:** None. **F. Liu:** None. **M. Wren:** None. **C. Smith:** None. **D. Chhangani:** None. **D. Morderer:** None. **Y. Zhang:** None. **D.W. Dickson:** None. **S.H. Yoshimura:** None. **S. Barmada:** None. **D.E. Rincon-Limas:** None. **J. Shorter:** F. Consulting Fees (e.g., advisory boards); JS is a consultant for Dewpoint Therapeutics, ADRx, and Neumora. **W. Rossoll:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.08/M10

Topic: C.06. Neuromuscular Diseases

Title: Nuclear transport receptors as novel modifiers of C9orf72 pathology

Authors: *F. LIU, S. VETTLESON-TRUTZA, J. WAIDMANN, W. ROSSOLL;
Dept. of Neurosci., Mayo Clin., Jacksonville, FL

Abstract: The expansion of the intronic G4C2 repeat within the C9orf72 gene is the most common genetic cause of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The underlying pathomechanism involves both loss of function of the C9orf72 protein and the toxic gain of function arising from repeat RNA foci and distinct dipeptide repeat proteins (DPRs) generated by repeat-associated non-AUG translation (RAN). Among the DPRs, poly-GA is prominently expressed and forms detergent-insoluble cytoplasmic aggregates in post-mortem human brain samples. However, the precise triggers initiating the pathological cascade and the potential for therapeutic intervention to reverse or alleviate these aberrant processes remain poorly understood. Previous studies have implicated nucleocytoplasmic import receptors in C9ALS/FTD pathology. Here we conducted a comprehensive screen of nuclear transport receptors (NTRs) to investigate the impact of importins and exportins on the formation of poly-GA aggregates. We discovered several NTRs as potential modifiers of poly-GA pathology. Moreover, we explore the activity of NTRs on arginine-rich DPRs and observed their ability to reduce the aggregation of both poly-GR and poly-PR, indicating a broader chaperone role in DPR pathology. To gain insights into the mechanism by which NTRs reduce poly-GA aggregation, we generated a series of NTR fragment constructs with N-terminal or C-terminal truncations by systematically dissecting the HEAT repeats, to identify minimal regions that are necessary and sufficient for the reduction of poly-GA aggregates in the cytoplasm. These findings provide mechanistic insights into the novel role of NTRs as modifiers of DPR aggregation, highlighting potential targets for future therapeutic strategies. Ongoing investigation will focus on exploring the involvement of NTRs in the RNA foci and DPR pathology using ex vivo brain slice culture and in vivo C9 mouse models.

Disclosures: F. Liu: None. **S. Vettleson-Trutza:** None. **J. Waidmann:** None. **W. Rossoll:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.09/N1

Topic: C.06. Neuromuscular Diseases

Title: Nucleoporin 50 as a modifier of TDP-43 proteinopathy in FTD/ALS models

Authors: *C. L. SMITH[1], B. KHALIL[2], A. RAFFELHUESCHEN[2], J. LEE[1], T. COMYN[2], E. MASON[2], A. LY[2], M. WREN[2], W. ROSSOLL[2];
[1]Mayo Clin. Grad. Sch. of Biomed. Sci., Jacksonville, FL; [2]Neurosci., Mayo Clin., Jacksonville, FL

Abstract: TDP-43 proteinopathy, the mislocalization of the predominantly nuclear protein to the cytoplasm where it forms hyperphosphorylated and ubiquitinated aggregates, is the characteristic pathological hallmark of 97% and 50% of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), respectively. The pervasiveness of this pathology, in addition to the genetic link between TDP-43 and ALS/FTD, suggests it plays a critical role in the disease pathogenesis. We and others have shown that nucleoporins are lost from the nuclear pore complex and coaggregate with TDP-43 in patient tissue. Multiple nucleoporins have been identified to mitigate cytotoxicity in modifier screens in drosophila C9orf72 models. These data suggest a key role for nucleoporins in the disease; however, the link between nuclear pore defects, TDP-43 proteinopathy, and neurodegeneration is not well defined. In a screen for modifiers of TDP-43 proteinopathy, nucleoporin 50 (NUP50) restored solubility and nuclear localization of TDP-43, suggesting an important role for NUP50 in the disease process. This premise is further supported by the recent discovery of rare NUP50 variants as a risk factor for ALS, and a critical role for NUP50 in neuronal survival. These findings lead us to hypothesize that NUP50 has a non-canonical function in reducing TDP-43 mislocalization and aggregation. We are using cell models to define the mechanism of this and define its smallest active fragment. These results provide mechanistic insights into the novel activity of NUP50 in resolving TDP-43 proteinopathy and highlight its potential as a target for FTD/ALS therapy development.

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Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

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Program #/Poster #: PSTR330.10/N2

Topic: C.06. Neuromuscular Diseases

Support: DOD Grant W81XWH-22-1-0218

Title: Synthetic protein mimetics antagonize TDP-43 aggregation in diverse cell culture models of amyotrophic lateral sclerosis

Authors: *K. M. REYNOLDS CAICEDO[1,2], N. H. STILLMAN[1,2], M. HERDA[1,2], R. A. DOHONEY[1,2], D. A. LINSEMAN[1,2], S. KUMAR[1,2];
[1]Univ. of Denver, Denver, CO; [2]Knoebel Inst. for Healthy Aging, Denver, CO

Abstract: The pathological aggregation of TAR DNA-binding protein-43 (TDP-43) is a hallmark of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). In this study, we developed several in vitro systems (HEK293t cells, differentiated NSC-34 motor neuronal cells, and mouse embryonic stem cell-derived spinal motor neurons) expressing fluorescently tagged versions of either wild type TDP43 or a disease-linked point mutant (Q331K). These cell models were then subjected to

either oxidative stress (sodium arsenite), proteasome stress (proteasome inhibitor, MG132), or endoplasmic reticulum stress (ER-to-golgi transport inhibitor, Brefeldin-A) to induce TDP-43 aggregation and cytotoxicity. Using live-cell confocal imaging, we monitored the kinetics of TDP-43 aggregation and observed that oxidative stress treatment induced robust inclusions within 8 hours. We then screened a library of synthetic protein mimetics to identify potent antagonists of intracellular TDP-43 aggregation. We identified multiple antagonists which significantly reduced the extent of TDP-43 inclusions, suggesting that they bind different domains of the protein. These protein mimetics are structurally and chemically stable in biological milieu and efficiently cross the cell membrane. Our results demonstrate the utilization of this novel array of cellular models to identify potent antagonists of intracellular TDP-43 aggregation. This work will aid in expediting the discovery of lead therapeutics for ALS and FTLD.

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Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR330.11/N3

Topic: C.06. Neuromuscular Diseases

Title: Unraveling the Role of Astrocytes in C9orf72 ALS using a Novel Primary Rat Astrocyte Model

Authors: *J. HILL[1,2], C. MEURICE[2], D. SOOD[3], R. PASSARO[3], A. LEE[3], P. BATHALA[4], M. HOSSAIN[4], D. GHOSH[4], G. TOMASSY[2], M. GOULET[2], M. LEVIT[4], J. DODGE[3], S. RODRIGUEZ[3];
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Abstract: Mutations resulting in a G4C2 hexanucleotide repeat expansion (HRE) in the C9orf72 gene accounts for approximately 40% of familial Amyotrophic Lateral Sclerosis (fALS) cases. The HRE is hypothesized to cause both a loss-of-function (LOF) by reducing levels of C9orf72 protein and a toxic gain-of-function (GOF) through the generation of sense and antisense RNA transcripts. These RNA transcripts are believed to sequester RNA binding proteins to generate RNA foci, and when translated, make toxic dipeptide repeat proteins (DPRs). C9orf72 is expressed in most cell types in the CNS and the non-cell autonomous consequences of the HRE in glia have not been well-characterized. Here, we generated primary astrocytes from a recently described 80 G4C2 HRE Knock-In (KI) rat model to investigate the role of astrocytes in C9orf72 ALS. Astrocytes were characterized for LOF and GOF disease relevant mechanisms and were also co-cultured with human induced pluripotent stem cell-derived (hiPSC) motor neurons to

assess their neurotoxicity. Notably, we found a marked decrease in motor neuron neurite length and viability during co-culture with diseased astrocytes. Furthermore, transcriptomics, proteomics, immunostaining, and western blot analysis revealed significant autophagy dysregulation in the diseased astrocytes compared to wild-type. Altogether, these data suggest that astrocytes play an important role in C9orf72 disease pathogenesis and that these primary astrocytes can be used to further our understanding of non-cell autonomous and cell autonomous mechanisms of disease to identify new treatments for ALS.

Disclosures: **J. Hill:** A. Employment/Salary (full or part-time):; Sanofi. **C. Meurice:** A. Employment/Salary (full or part-time):; Sanofi. **D. Sood:** A. Employment/Salary (full or part-time):; Sanofi. **R. Passaro:** A. Employment/Salary (full or part-time):; Sanofi. **A. Lee:** A. Employment/Salary (full or part-time):; Sanofi. **P. Bathala:** A. Employment/Salary (full or part-time):; Sanofi. **M. Hossain:** A. Employment/Salary (full or part-time):; Sanofi. **D. Ghosh:** A. Employment/Salary (full or part-time):; Sanofi. **G. Tomassy:** A. Employment/Salary (full or part-time):; Sanofi. **M. Goulet:** A. Employment/Salary (full or part-time):; Sanofi. **M. Levit:** A. Employment/Salary (full or part-time):; Sanofi. **J. Dodge:** A. Employment/Salary (full or part-time):; Sanofi. **S. Rodriguez:** A. Employment/Salary (full or part-time):; Sanofi.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.12/N4

Topic: C.06. Neuromuscular Diseases

Support: NHMRC Program Grant

Title: Exploring Cell Senescence in ALS Patient iAstrocytes and iMicroglia

Authors: ***A. MAXIMOVA**[1], M. A. SULLIVAN[1], E. L. WERRY[1], M. KASSIOU[2];
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Abstract: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative condition with no cure, resulting in upper and lower motor neuron death, and an average lifespan of 3-5 years following diagnosis. Cell senescence is a non-proliferative state that cells enter upon exposure to increased stress, while remaining metabolically active. Post-mortem motor and prefrontal cortices from ALS patients display upregulated senescence-associated proteins such as p16, p21, and p53, which are additionally correlated with region-specific motor neuron death. Similar trends are seen when measuring senescence using senescence-associated beta galactosidase (SA-β-Gal) staining in cells derived from ALS patients, and in animal models of ALS *in vivo*, such as the SOD1 ALS mouse model. Furthermore, several cytokines involved in inflammation and oxidative damage that are characteristic of the senescence-associated secretory phenotype (SASP) are elevated in blood plasma of ALS patients. Although this evidence implicates senescence in ALS, whether this occurs secondary to motor neuron degeneration or is cell

autonomous is unclear, as are the cell types and molecular pathways involved.
This study aims to characterise senescent ALS astrocytes and microglia in the absence of motor neuron degeneration, and to explore molecular pathways that are involved in ALS-associated motor neuron death, with a long-term aim of identifying a potential drug target for a new senolytic therapeutic. The project utilised three experimental assays to characterise cell viability, proliferation, and senescence expression in iPSC-derived astrocytes and microglia that were developed from fibroblast lines donated by ALS patients carrying *c9orf72* hexanucleotide repeats and age-matched healthy controls. ALS iAstrocytes showed 2-fold and 4-fold significantly reduced cell viability and proliferation ($p<0.05$), respectively, while displaying 2-fold significantly more senescent cells compared to healthy control iAstrocytes ($p<0.05$). In contrast, no significant differences were identified between the microglial cultures. To explore some molecular pathways underlying iAstrocyte senescence in ALS, we performed western blotting for the BCL-xL protein, which helps senescent cells evade apoptosis. ALS iAstrocytes showed 2-fold significantly higher BCL-xL expression compared to controls ($p<0.05$). Future direction will involve SASP-linked cytokine characterisation to further the understanding of molecular pathways involved in ALS-associated senescence.

Disclosures: A. Maximova: None. **M.A. Sullivan:** None. **E.L. Werry:** None. **M. Kassiou:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.13/N5

Topic: C.06. Neuromuscular Diseases

Support: R00NS123242

Title: Exploring emerging connections: CHMP2B and the Disruption of Nuclear Pore Complex, Nuclear Envelope and CHMP7 Pathophysiology.

Authors: *A. CHANDIA CRISTI, A. N. COYNE;
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Abstract: Defects in nucleocytoplasmic transport have been associated with several neurodegenerative disorders such as Frontotemporal Dementia (FTD), Alzheimer's disease (AD), and Amyotrophic Lateral Sclerosis (ALS). The nuclear pore complex (NPC) and its nucleoporin components together with nuclear transport receptors mediate controlled, bidirectional transport of proteins, RNA and RNP complexes between the cytoplasm and nucleus. Abnormalities in specific nucleoporins have been identified in sporadic ALS and familial C9orf72 ALS/FTD. Previously, with multimodal imaging of ALS patient-derived induced pluripotent stem cell (iPSC) derived neurons (iPSNs), we demonstrated that nuclear accumulation of CHMP7, an ESCRT-III protein, triggers NPC injury, linking CHMP7 and the

ESCRT-III nuclear surveillance pathway to early ALS pathogenesis. Notably, the loss of nuclear TDP-43 function and localization, characteristic of ALS, occurs downstream of CHMP7-mediated NPC injury. Moreover, recent studies showed that the knockdown of some ESCRT-III proteins, including CHMP2B, rescues nucleoporin levels, normalizes NCT, and suppresses C9orf72 repeat RNA-mediated neurodegeneration. Given that mutations in the closely related ESCRT-III protein, CHMP2B are causative of familial FTD, we sought to determine whether CHMP2B mutations also disrupt the NPC and downstream TDP-43 function. In this study, using isogenic mutant CHMP2B iPSNs and multiple imaging approaches, we observed significant defects in the nuclear membrane, nuclear pore complex, and TDP-43 function. Currently, further studies are being conducted to understand this pathological cascade in the context of CHMP2B mutations and to investigate whether antisense oligonucleotides (ASOs) targeting CHMP7 can repair this injury, as previously demonstrated in ALS.

Disclosures: A. Chandia Cristi: None. **A.N. Coyne:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.14/N6

Topic: C.06. Neuromuscular Diseases

Support: FAPESP
CNPq
Abel Tasman - Groningen University

Title: Enhanced Global Protein Synthesis and Neuroprotection: IFNy Safeguards Motor Neurons against Oxidative Stress in FUS-associated Amyotrophic Lateral Sclerosis

Authors: *A. FARIA ASSONI[1,2], R. WARDENAAR[2], E. GUERRERO[3], D. F. OLIVEIRA[1], P. BAKKER[2], O. K. OKAMOTO[1], M. ZATZ[1], F. FOIJER[2];
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Abstract: Amyotrophic lateral sclerosis type 6 (ALS6) is a familial form of ALS associated with a specific genetic mutation in the Fused in Sarcoma (*FUS*) gene. The *FUS* gene mutation leads to a decline in global protein synthesis, which is crucial for various cellular processes. However, the precise mechanism through which FUS regulates global protein synthesis remains unclear. In this study, we employed induced pluripotent stem cells (iPSCs) derived from ALS6 patients to investigate the impact of the *R521H FUS* mutation on protein synthesis during the progression of the disease. Our findings demonstrate that *R521H FUS* motor neurons (MNs) exhibit reduced viability due to impaired protein synthesis compared to MNs derived from healthy donors. Additionally, we observed a diminished transcriptome associated with TGF-β and mTORC signaling in *R521H FUS* MNs upon induction of oxidative stress. Importantly, we found that

treatment with IFNy rescues ALS6 MNs from apoptosis induced by oxidative stress and enhances translation rates in these cells. Collectively, these results suggest that IFNy plays a significant role in FUS-mediated protein synthesis, potentially involving FUS nuclear translocation in the context of ALS6.

Disclosures: **A. Faria Assoni:** None. **R. Wardenaar:** None. **E. Guerrero:** None. **D.F. Oliveira:** None. **P. Bakker:** None. **O.K. Okamoto:** None. **M. Zatz:** None. **F. Foijer:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.15/N7

Topic: C.06. Neuromuscular Diseases

Support: Laurence and Sandi Gluck Charitable Foundation

Title: Apolipoprotein B-100-treated astrocyte conditioned media protects against human motor neuron degeneration

Authors: *A. E. MCDERMOTT, R. P. GRIFFIN, I. GAO, J. K. WONG, S. A. SADIQ; Tisch MS Res. Ctr. of NY, New York, NY

Abstract: Amyotrophic lateral sclerosis (ALS) is a fatal progressive neurodegenerative disease characterized by motor neuron degeneration. We previously identified apolipoprotein B-100 (ApoB) as a neurotoxic factor significantly upregulated in sporadic ALS cerebrospinal fluid (CSF) compared to healthy control CSF and familial ALS CSF. When intrathecally injected in mice, ApoB induces motor disability, motor neuron degeneration and other hallmark ALS pathology. We also found that ApoB-treated human iPSC-derived motor neurons undergo degeneration. However, ApoB-induced motor neuron degeneration did not occur when motor neurons were co-cultured with healthy human astrocytes. It is unknown whether this neuroprotection against ApoB-induced motor neuron degeneration is mediated via direct interaction between astrocytes and motor neurons, or via soluble factors secreted by astrocytes. The goal of this study was to determine whether conditioned media from ApoB-treated astrocytes can attenuate ApoB-induced motor neuron degeneration.
Human primary astrocytes were cultured for 1 week in astrocyte growth media then treated with 1 ng/µl ApoB for 24 hours. Astrocyte conditioned media was then collected from ApoB-treated astrocytes (ApoB-ACM) or untreated astrocytes (ACM). Human iPSC-derived motor neurons were cultured for 8 days then treated with 1 ng/µl ApoB or incubated with either ACM or ApoB-ACM for 1 hour before ApoB treatment. After 24 hours, cells were fixed with 4% paraformaldehyde for ChAT and ApoB immunocytochemistry. Areas of $ChAT^{+}$ motor neuron clusters were quantified as a measure of motor neuron death and ApoB staining intensities were quantified. All analyses were performed blinded.
ChAT cluster sizes were significantly smaller in ApoB-treated motor neurons compared to motor

neurons grown in media. However, motor neurons treated with ApoB-ACM prior to ApoB had significantly larger clusters than ApoB-treated motor neurons. Cluster sizes of motor neurons treated with ACM then ApoB were not significantly different from ApoB-treated motor neurons. ApoB immunostaining intensities were significantly upregulated following ApoB treatment but significantly reduced in motor neurons incubated with ApoB-ACM prior to ApoB treatment. This study shows that neuroprotective factors secreted by ApoB-treated astrocytes can attenuate motor neuron death by reducing ApoB uptake by motor neurons. Future studies will aim to identify neuroprotective factors present in ApoB-treated astrocyte conditioned media.

Disclosures: **A.E. McDermott:** None. **R.P. Griffin:** None. **I. Gao:** None. **J.K. Wong:** None. **S.A. Sadiq:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.16/N8

Topic: C.06. Neuromuscular Diseases

Support: 2022-MSCRFF-5833

Title: Modeling corticospinal tract pathophysiology with iPSC-derived corticospinal motor neurons

Authors: *K. L. MARSHALL, **K. RUST**, S. LIU, R. SINGH, A. CHARALAMPOPOULOU, Y. ZHAO, C. O'KEEFE, C. W. HABELA, T.-H. WANG, A. VENKATESAN, N. J. MARAGAKIS;
Johns Hopkins Univ., Baltimore, MD

Abstract: iPSC-derived cell subtypes are powerful tools for generating relevant human models of disease pathophysiology. While differentiation protocols for spinal motor neurons (SPMNs) have been capable of producing relatively enriched populations of SPMNs, cortical neuron differentiation protocols produce many neuronal and glial subtypes, of which corticospinal motor neurons (CSMNs) account for a relatively small proportion. Though some motor neuron diseases, like ALS and hereditary spastic paraplegia, are characterized by specific degeneration of the corticospinal tract (CST) and early cortical dysfunction, the impacts of specific disease-causing mutations on CSMNs are understudied, especially in human cells. We are generating a fully human, iPSC-based model of the CST using compartmentalized co-culture to specifically interrogate corticospinal connections. Modeling motor neuron disease with a focus on corticospinal connectivity is particularly important in determining mutation specific impacts on disease progression in the CST and may provide a future platform for investigating potential therapeutics. Human iPSC-derived CSMNs and SPMNs (hiPSC-CSMN and hiPSC-SPMN, respectively), co-cultured with astrocytes (hiPSC-A), were maintained in microfluidic devices for long-term compartmentalized co-culture, where the cortical and spinal neuron populations

were connected through axons traversing microchannels. Combination of microfluidic devices with multielectrode array (MEA) plates allowed for assessment of neuronal firing and functional connectivity of hiPSC-CSMNs and hiPSC-SPMNs in spatially separated cortical and spinal compartments. Following transduction of cortical neurons with Camkii-driven channelrhodopsin, optic stimulation of the co-culture resulted in firing of both cortical and spinal compartments, suggesting synaptic connection of the cortical and spinal compartments.Differentiation of CSMNs from patients with neurodegenerative disease represents a precision medicine approach for testing drugs suitable for future clinical trials. Compartmentalized co-cultures of regionally defined neurons and astrocytes provide a highly relevant and manipulatable model that will allow us to better understand CST pathophysiology and test therapeutic strategies to impact CST health and connectivity.

Disclosures: K.L. Marshall: None. **K. Rust:** None. **S. Liu:** None. **R. Singh:** None. **A. Charalampopoulou:** None. **Y. Zhao:** None. **C. O'Keefe:** None. **C.W. Habela:** None. **T. Wang:** None. **A. Venkatesan:** None. **N.J. Maragakis:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.17/O1

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

Support: NIH-NINDs (R01NS116143)
ALS Association (18-11A-418)

Title: CREB pathway dysfunction in ALS/FTD C9ORF72 iPSC derived cortical neurons

Authors: *M. GREGOIRE[1], L. DONATELLI[2], R. SIRTORI[3], C. FALLINI[1];
[1]Cell and Mol. Biol., [2]Interdisciplinary Neurosci., [3]Univ. of Rhode Island, Kingston, RI

Abstract: Activation of the transcription factor cAMP-response element binding protein (CREB) via phosphorylation at serine133 influences neuronal survival and maturation via a wide range of downstream targets. CREB pathway dysfunction has been implicated in several neurodegenerative diseases which demonstrate cognitive decline including Alzheimer’s and Huntington’s Disease, and it has been well documented that CREB activation impairment can reduce dendritic branching which is critical for the integration of synaptic inputs. Synapse loss affects the process of long term potentiation, which is important in memory formation and maintenance. Synapse loss is also observed in cells of patients with the fatal neurodegenerative disease ALS/FTD (amyotrophic lateral sclerosis/frontotemporal dementia). Interestingly, down-regulation of CREB phosphorylation and sequestration of the CREB cofactor CREB-binding-protein (CBP) have also been observed in ALS/FTD C9ORF72 (C9) cells. However, a gap of knowledge still remains in understanding the role of CREB pathway dysfunction in ALS/FTD pathology. To address this, we sought to determine if induced pluripotent stem cell (iPSC)-

derived cortical neurons carrying mutations in the C9 gene would demonstrate impaired activation of the CREB pathway following stimulatory signals. Through immunofluorescence, western blotting, and high throughput RNA sequencing assays we found early and stimulation specific impairment in the activation of CREB in C9 mutant neurons. However, we also found a convergence of data implicating dysfunctional CREB activation as having a key role in neuronal cell focal adhesion pathways. Altogether, our data points to the role of early transcriptional alterations in C9 mutant cells as a key and early driver of pathology.

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Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.18/O2

Topic: C.06. Neuromuscular Diseases

Support: MNDA grant 943-793
Battelle Memorial Institute Wadsworth PhD Fellowship

Title: C9orf72 repeat expansion reduces mitochondrial function and mitophagy in neurons

Authors: *J. A. K. LEE[1], S. ALLEN[1], L. FERRAIUOLO[1], P. J. SHAW[2], H. MORTIBOYS[1]; [1]Neurosci., Univ. of Sheffield, Sheffield, United Kingdom; [2]Academic Neurol. Unit, Univ. Sheffield, Sheffield S10 2RX, United Kingdom

Abstract: Amyotrophic Lateral Sclerosis (ALS) is an adult-onset neurodegenerative disease characterised by progressive loss of motor neurons. Mitochondrial dysfunction is a hallmark of ALS pathology, with previous studies reporting a range of different metabolic phenotypes in different models, including changes to mitochondrial morphology, function and turnover (mitophagy). Greater clarity is needed on mitochondrial dysfunction across a range of ALS genotypes, to allow us to pursue targeted therapeutic strategies.
We hypothesised that in neurons from ALS patients with the C9orf72 repeat expansion mutation, the most common mutation in Europe, we would observe increases in mitochondrial fragmentation and mitochondrial membrane potential (MMP), alongside decreases in mitophagy. Mitochondrial phenotype was assessed in neurons generated from 3 directly reprogrammed induced neural progenitor cell lines (iNPC's) from ALS cases with a C9orf72 mutation and 3 aged matched controls. For each experiment, 3 biological repeats were obtained from each cell line. Direct reprogramming to iNPC's retains age-related epigenetic changes, an advantage over iPSC-derived neurons when studying conditions with age as a risk factor. Mitochondrial morphology, membrane potential and mitophagy were assessed by live and fixed confocal imaging. Respirometry was assessed using the Seahorse XFe96 Bioanalyser. In C9orf72-ALS neurons, mitochondrial morphology was unaffected. We observed a significant reduction in basal mitophagy ($p=0.004$, Mann-Whitney test) and MMP ($p=0.0273$, Wilcoxon test). There were no

significant changes in basal oxygen consumption or spare respiratory capacity in C9orf72-ALS neurons. There was a significant decrease in LC3-positive autophagosomes in C9orf72-ALS neurons (p=0.0078, Mann-Whitney Test). There was no significant difference in upregulation of mitophagy by the PINK1/Parkin-dependent pathway when induced with oligomycin/antimycin A. Induction of BNIP3-dependent mitophagy revealed no significant differences between control and C9orf72-ALS neurons.
This work provides further evidence for the role of C9orf72 in autophagy via autophagosome production. Moreover, these findings help confirm previous work highlighting mitochondrial dysfunction in C9orf72-ALS and begin to explore a deficit in mitophagy, a deficit not previously reported in C9orf72-ALS patient cells. The mechanisms underlying the mitophagy deficit in C9orf72-ALS remain poorly understood. Further work is currently underway to identify if this deficit is driven primarily by an autophagosome deficit and characterise the potential disruption of mitophagy pathways.

Disclosures: **J.A.K. Lee:** None. **S. Allen:** None. **L. Ferraiuolo:** None. **P.J. Shaw:** None. **H. Mortiboys:** None.

Poster

PSTR330. Motor Neuron Disease: Models and Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR330.19/O3

Topic: C.06. Neuromuscular Diseases

Title: Drastically Accelerated Differentiation of Human Pluripotent Stem Cells into Motor Neurons

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Abstract: Motor neurons are involved in the voluntary control of muscular contraction, and are the primary cell type affected in amyotrophic lateral sclerosis (ALS), an incurable and deadly neurological disease resulting in paralysis. Given their unlimited potential for self-renewal and differentiation, it has been a longstanding goal to model ALS patient-specifically using human pluripotent stem cell (hiPSC)- derived motor neurons for drug discovery to develop life-saving treatments for this disease. While innumerable methods have been published to differentiate motor neurons from hiPSCs, all protocols to date demonstrate substantial clone-to-clone and donor-to-donor variability, failing to produce motor neurons at high yields and purity from each hiPSC line. Many of these failings can be attributable to the protracted time in culture required to generate motor neurons, often extending beyond 30 days in vitro. Here we report a drastically accelerated, developmentally guided, directed differentiation protocol based on small molecules and growth factors to generate motor neurons in only 7 days. Owing to its speed, the protocol is highly efficient and reproducible, manufacturing motor neurons of high purity and yield for several donor hiPSC lines tested. These resulting motor neurons express MNX1, ISLET1, and

ChAT by immunocytochemistry and innervate muscle while in co-culture using a microphysiological system.

Disclosures: **P. Walsh:** A. Employment/Salary (full or part-time):; Anatomic Incorporateed. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Anatomic Incorporated. **M. Dau:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. **V. Truong:** A. Employment/Salary (full or part-time):; Anatomic Incorporated. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Anatomic Incorporated.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.01/O4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MWU Intramural Funding

Title: 8-methoxymanzamine a, but not manzamine a, induced neurite outgrowth in murine cortical cultures in a dose-dependent manner

Authors: ***M. L. PIERCE**[1], S. SEO[2], J. ABURAS[1], S. SHAH[2], J. DRAVES[2], O. ALAM[3], A. HASAN[3], M. T. HAMANN[4], A. M. MAYER[5];
[1]Pharmacol., [2]Chicago Col. of Osteo. Med., [3]Biomed. Sci., Midwestern Univ., Downers Grove, IL; [4]COP Drug Discovery and Biomed. Sci., Med. Univ. of South Carolina, Charleston, SC; [5]Pharmacol., Midwestern University, CCOM, Downers Grove, IL

Abstract: INTRODUCTION
Marine organisms produce a wide variety of primary and secondary metabolites with unique scaffolds that are biologically active. Manzamines are marine-derived polycyclic alkaloids. Approximately 100 manzamine-type alkaloids have been isolated from 16 marine sponge species, and some of these compounds are reported to have anti-inflammatory and neuritogenic properties. Previous neurite outgrowth screens have identified the following compounds for further analyses: manzamine A, 8-methoxymanzamine A, 12-propoxymanzamine A, 12-isobutoxymanzamine A, ATL 2-97, ircinin-1, and palinurin.
METHODS
In order to understand the neuritogenic potential of these manzamine compounds, this study conducted neurite outgrowth assays to build dose-response curves for manzamine A and 8-methoxymanzamine A in murine primary cortical cultures.
RESULTS
Results from the neurite outgrowth analyses showed that 8-methoxymanzamine A induced neurite outgrowth in a statistically significant manner at the 1 micromolar dose. Likewise, that

dose and others also demonstrated a statistically significant increase in neuronal complexity via number of processes and number of branches. However, at the 10 micromolar dose, 8-methoxymanzamine appears to be largely toxic, showing reduced neurite outgrowth, number of processes and number of branches as well as some nuclear blebbing indicative of apoptosis. In contrast, manzamine A does not show increased neurite outgrowth or complexity at any dose, appears potentially toxic at the 10 micromolar dose.
CONCLUSIONS
Together, these data suggest that 8-methoxymanzamine A has the potential to increase neurite outgrowth, although it shows similar indications of toxicity at the 10 micromolar dose as manzamine A. Further studies will be performed with fluorescein diacetate and propidium iodide to assess live and dead cells. Together, these studies will help identify novel neuroactive manzamine compounds for the marine natural products preclinical pipeline.

Disclosures: **M.L. Pierce:** None. **S. Seo:** None. **J. Aburas:** None. **S. Shah:** None. **J. Draves:** None. **O. Alam:** None. **A. Hasan:** None. **M.T. Hamann:** None. **A.M. Mayer:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.02/Web Only

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: This research project was supported by CONAHCYT Grant: 319578. We are grateful for the Postdoctoral Fellowships from CONAHCYT (Dr. María del Carmen Silva-Lucero) and DGAPA (Dr. Laura Gómez-Virgilio).

Title: Exploring Metformin effects on autophagy pathway vs mTOR in olfactory neuroepithelial cells from two different groups of age. Implications in its pharmacological potential for neurodegenerative diseases.

Authors: *L. GÓMEZ-VIRGILIO, O. R. LORA-MARIN, M.-D.-C. SILVA-LUCERO, M.-D.-C. CÁRDENAS-AGUAYO;
Fisiologia, Facultad de Medicina, UNAM, Ciudad De México, Mexico

Abstract: *Background*. Several longitudinal population-based studies have shown that the rate of cognitive decline is accelerated in elderly people with type 2 diabetes mellitus (DM2). There is also evidence for an elevated risk of vascular dementia and Alzheimer's disease in DM2. On the other hand, it had been reported that metformin (MET), widely used for treating type 2 diabetes mellitus (T2DM), could alleviate age-related cognitive dysfunction (Madhu et al., 2022). In this sense, we use human Neural Stem/Progenitor Cells Derived from the Olfactory Epithelium (NS/PCs-OE) exfoliated from healthy subjects of different ages. The aim is to evaluate the ability of MET to modulate adverse changes like increased mTOR signaling and reduced

autophagy, that likely underlies its beneficial effect on cognitive function. *Method.* Human NS/PCs-OE were exfoliated from the anterior region of the medial-lateral turbinate using a special brush and circular movements to obtain cells from the lateral wall of the nasal cavity and septum. The Ethical Committee of the UNAM, School of Medicine approved this study (FMED/CEI/PMSS/074/2021). Prior to nasal exfoliation, participants provided written informed consent for all procedures. Those cells have been subcultured and characterized by immunodetection. Cells were harvested in DMEM/F-12 supplemented with 10% fetal bovine serum, 4 mM L-glutamine, 100 g/ml streptomycin, and 100 IU/ml penicillin. All experiments were performed on cells from passages 5 to 10 from young and adult normal subjets. We performed Western blotting to evaluate the expression of p62, LC3, Lamp2, Atg5, Beclin-1 and pmTOR with or without MET. We also evaluate the expression of neuronal markers. We performed an autophagy colorimetric assay (CYTO-ID). *Results.* Human NS/PCs-OE express proliferation (Ki67) and precursor stage (Nestin) markers. These cells also expressed βIII-tubulin, but they did not express markers of mature neurons and glial cells. Moreover, hNS/PCs-OE show the capability to differentiate into mature olfactory neurons. Human NS/PCs-OE isolated from aged subjects show decreased autophagy and increased mTOR signaling in comparison with younger subjects. MET treatment enhanced the expression of autophagy markers. In addition, MET suppressed mTOR signaling. *Conclusion.* In NS/PCs-OE from old age subjects non-diabetic, MET treatment inhibits mTOR signaling and enhances autophagy.

Disclosures: L. Gómez-Virgilio: None. **O.R. Lora-Marin:** None. **M. Silva-Lucero:** None. **M. Cárdenas-Aguayo:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

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Program #/Poster #: PSTR331.03/O5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NRF-2022R1l1A1A01069717

Title: Diosgenin induces antihyperalgesic effect via antagonism of transient receptor potential vanilloid 1 in pain model

Authors: *M. RAHMAN[1], H. JO[1], C. PARK[1], Y. KIM[2];
[1]Gachon Univ., Incheon, Korea, Republic of; [2]Dept. of Physiol., Col. of Medicine, Gachon Univ., Incheon, Korea, Republic of

Abstract: Diosgenin is a botanical steroidal saponin with anti-inflammatory, antioxidant, anti-thrombotic, anti-apoptotic, anti-depressant, and anti-nociceptive properties. However, it's unknown how diosgenin affects anti-nociception. TRPV1, a transient receptor potential vanilloid, is crucial for nociception. Therefore, we investigated whether TRPV1 antagonism mediates the anti-nociceptive effects of diosgenin. In vivo mouse experiments were performed to

examine nociception-related behavior, while in vitro experiments were per-formed to examine calcium currents in dorsal root ganglion (DRG) and Chinese hamster ovary (CHO) cells. The duration of capsaicin-induced licking (pain behavior) was significantly reduced following oral and intraplantar administration of diosgenin, approaching levels observed in mice treated with the TRPV1 antagonist BCTC. Additionally, oral administration of diosgenin reduced capsaicin-induced thermal hyperalgesia. Further, diosgenin reduced capsaicin-induced Ca2+ currents in a dose-dependent manner in both DRG and CHO cells. Oral administration of diosgenin also improved thermal and mechanical hyperalgesia in the sciatic nerve constriction injury-induced chronic pain model by reducing the expression of TRPV1 and inflammatory cytokines in DRG cells. Overall, our findings imply that diosgenin has analgesic benefits in a mouse model of neuropathic pain by inhibiting TRPV1 and reducing inflammation in the DRG.

Disclosures: **M. Rahman:** None. **H. Jo:** None. **C. Park:** None. **Y. Kim:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.04/O6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NINDS CounterACT GRANT 5U01NS105058-05

Title: Disease modifying effect of delayed intramuscular atenolol and levetiracetam therapy on cardiac and brain functions following organophosphate induced status epilepticus

Authors: R. E. BLAIR, E. HAWKINS, R. J. DELORENZO, ***L. S. DESHPANDE**; Neurol., Virginia Commonwealth Univ., Richmond, VA

Abstract: Organophosphate (OP) compounds include pesticides and chemical warfare nerve agents. Lethal OP exposure leads to status epilepticus (SE) and survival despite the standard-of-care treatment is associated with cardiac irritability, neuronal injury, chronic memory impairment, and spontaneous recurrent seizures (SRS). We recently showed that treatment with a beta-adrenergic blocker atenolol (AT) and a neuronal calcium-induced calcium release inhibitor levetiracetam (LV) administered after the onset of OP paraoxon (POX)-induced SE significantly reduced mortality. Here, we investigated whether AT+LV would protect the heart and the brain to lower the mortality and neurobehavioral morbidities following POX-induced SE. Male Sprague-Dawley rats were injected with POX (2 mg/kg, s.c). One minute later, atropine (0.5 mg/kg, i.m.) and 2-PAM (25 mg/kg, i.m) were injected. At 1-h post SE onset, midazolam (1.78 mg/kg, i.m.) was used to terminate SE. Following POX-SE, rats were treated with AT+LV (AT, 5 mg/kg, LV 50 mg/kg, i.m., b.i.d for 7 days) or saline (CON). Separate groups of rats were used for ECG, EEG, behavior, and histopathology. OP intoxication produced rapid SE and increased survival was noted with AT+LV. ECG parameters (QTc and QTd) were significantly increased following POX-SE which were normalized with AT+LV. AT+LV also lowered the cardiac

damage index from 2.65 ± 0.19 in POX SE rats to 0.76 ± 0.18 (n= 6, p<0.001, Tukey-test). On the Barnes Maze, during the acquisition-phase, latency to escape and the number of errors were significantly lower in AT+LV rats compared to CON and, during the probe trial, AT+LV rats spent significantly more time in the target quadrant and made significantly more visits to the escape zone compared to CON rats. Video-EEG monitoring revealed the presence of POX SE SRS in 73.3% of CON rats while only 56.2% of AT+LV rats exhibited SRS. Additionally, AT+LV resulted in a significant decrease in seizure frequencies from 7.5± 9.6 to 1.7±2.2 SZs/day when compare to CON (n=8, p≤ 0.01, t-test). AT+LV treatment resulted in significant neuroprotection in the dentate gyrus hilus when compared to CON represented by an increase to 135.6±8.4 from 103.7±11.2 cells/mm2 respectively (n=11, p≤0.05, Tukey-test). Our results indicate that decrease in POX-SE mortality following AT+LV could be due to normalization of the QTc prolongation and a reduction in myocardial injury. Our results also indicate AT+LV was neuroprotective, it significantly improved memory outcomes and lowered SRS occurrence in POX-SE rats. AT+LV therapy could improve survival and provide significantly better neurological outcomes following OP toxicities.

Disclosures: R.E. Blair: None. **E. Hawkins:** None. **R.J. DeLorenzo:** None. **L.S. Deshpande:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.05/O7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R21NS125201
NJDOH CBIR20PIL017

Title: Delayed Leukemia Inhibitory Factor administration improves the metabolic profile of injured cells and tissue after mild traumatic brain injury.

Authors: B. SWIETEK[1], V. D'MELLO[1], S. ALI[2], E. MACODIYO[2], Y. WANG[3], S. W. LEVISON[1], ***J. R. BERLIN**[1];
[1]Rutgers-New Jersey Med. Sch., Newark, NJ; [2]Rutgers-School of Grad. Studies, Newark, NJ; [3]Rutgers-Robert Wood Johnson Med. Sch., New Brunswick, NJ

Abstract: Leukemia Inhibitory Factor (LIF) is a cytokine in the Interleukin-6 superfamily that reduces gliosis and improves neurological function after injury and is neuroprotective. One of our labs recently reported (D'Mello et al. Neurotrauma Reports, 4:236-250, 2023. DOI: 10.1089/neur.2021.0075) that intranasal LIF administered after mild closed-head traumatic injury reduced subsequent astrogliosis, microgliosis, decreased axonal damage and improved sensorimotor function in juvenile mice. As a follow up, we conducted a series of *in vitro* and *in vivo* studies to investigate how LIF might produce its neuroprotective effects in the brain. *In vitro*

studies used mixed neuron-glial neocortical cultures established from P17 embryonic rats that were cultured on a distensible poly-D-lysine-treated silicone substrate. Injury, produced by a rapid bilateral stretch of the substrate, was followed by functional and biochemical assays. 24 hrs after stretch injury, cell viability was unchanged compared to non-injured controls; however, the rate of spontaneous intracellular Ca^{2+} responses (due to neuronal bursting activity) and neuronal mitochondrial membrane potential ($\Delta\Psi_{mito}$) were decreased while S100B levels in the culture media were increased, all an indication of cellular and/or neuronal damage. Addition of LIF (10 ng/ml) to the media after stretch injury decreased spontaneous Ca^{2+} responses more than injury alone. In addition, LIF prevented the injury-induced reduction of $\Delta\Psi_{mito}$ and decreased the elevated levels of S100B. *In vivo* studies were conducted on 8-week old CD-1 mice that were subjected to a mild, midline closed-head injury anterior to bregma (3 mm flat impactor, 4 m/s, depth of 1.3 mm). LIF (40 ng) or vehicle (water) was administered intranasally twice daily during weeks 7 and 8 after injury. Sensorimotor function was tested one week later and then the corpus collosum was extracted for analysis of metabolic intermediates with LC-MS/MS. Compared to injury with vehicle administration, LIF administration reduced injury-induced sensorimotor deficits. LIF also increased tissue levels of NAD and TCA cycle intermediates (α-ketoglutarate, fumarate), components of pyrimidine synthesis (cytidine, uracil) and increased levels of several amino acids when compared to injury with vehicle administration. Altogether these data suggest that LIF reduces delayed neurodegeneration by improving metabolism, energy production, and transcriptional activity in injured tissue.

Disclosures: **B. Swietek:** None. **V. D'Mello:** None. **S. Ali:** None. **E. Macodiyo:** None. **Y. Wang:** None. **S.W. Levison:** None. **J.R. Berlin:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.06/O8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant AOD22011-001-00000; MOA-AI-21002-01
ORISE

Title: Physiologic characterization of acute hydrazine exposure in rats, and midazolam as a therapeutic treatment.

Authors: *B. J. TRAVIS, J. C. HILL, M. B. PETTOVELLO, G. E. CAPACIO, M. A. WOODSON, T. M. WHITTY, D. M. CRAIG, H. S. MCCARREN;
US Army Med. Res. Inst. of Chem. Def. (USAMRICD), APG, MD

Abstract: Hydrazines are a highly toxic, highly reactive class of chemicals used by the military, aviation, and aerospace industries in fuels. Occupational exposures to hydrazines carry a high degree of risk because multiple organ systems are rapidly affected. In the brain, hydrazines

disrupt synthesis of GABA by inhibiting pyridoxal kinase, which is thought to be the cause of neurological symptoms like seizures. This two-part study characterizes the clinical presentation and neurotoxic effects of acute exposure to hydrazine and methylhydrazine in a simulated intensive care unit (sim-ICU) in rats, and tests midazolam as a treatment against the neurotoxic effects. Adult male and female Sprague Dawley rats were challenged with a subcutaneous dose of either hydrazine (n=12) or methylhydrazine (n=12), based on existing toxicologic literature. The animals were monitored for 24 hours post-exposure in the sim-ICU, including continuous electroencephalogram (EEG) recording and intervaled measurements of temperature, oxygen saturation (SpO2), blood pressure, and blood chemistry to identify disruptions in homeostatic function. Part two followed the same structure; however, midazolam was administered within one-minute following seizure onset. Exposure to either hydrazine or methylhydrazine resulted in neurotoxic effects, causing seizures in all animals (n=24). Methylhydrazine at 20mg/kg (n=6) resulted in rapid onset of seizure (t=67±2min) and death (t=110±9min, n=5). At 35mg/kg (n=6) the toxic effect was significantly more rapid for both seizure onset (t=44±3min; p<.0001) and death (t=79±6min; p=.0001). A 20mg/kg dose treated with midazolam increased survival, with 10/12 rats surviving until the 24h end point and the remaining two rats surviving 6-9h. Midazolam controlled seizures for one rat for the 24hr duration of the study and provided initial temporary control (t=126±40min) for the remaining (n=11), but they eventually recurred. Hydrazine at 50mg/kg (n=6) resulted in rapid-onset seizures (t=44±41min) but no lethality with all rats surviving until 24h. At 100mg/kg (n=6) seizures were no more rapid in their onset (t=56±58min; p=.6744), but lethality was significantly increased with all rats dying 146±35min post-exposure. At a 100mg/kg dose treated with midazolam (n=12) initial seizure control (t=107±41min) was observed in 10/12 rats, 3/12 rats survived until 24h, and mortality was delayed (t=383±90min, n=9).

Disclosures: **B.J. Travis:** None. **J.C. Hill:** None. **M.B. Pettovello:** None. **G.E. Capacio:** None. **M.A. Woodson:** None. **T.M. Whitty:** None. **D.M. Craig:** None. **H.S. McCarren:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.07/Web Only

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Neuroprotective significance of Chebulinic acid against ethidium bromide induced multiple sclerosis in experimental rats.

Authors: *R. ARORA[1,2];
[1]Neuropharm., ISF Col. of Pharm., Moga, India; [2]Fac. of Pharmaceut. Sci., PCTE group of institutes,, Ludhiana, India

Abstract: Multiple Sclerosis (MS) is an autoimmune progressive neurodegenerative disease characterized by behavioral and biochemical alterations following demyelination of the central

nervous system, affecting about 2.8 million people worldwide but still lacking therapeutic interventions. Chebulinic acid (ChA), a flavonoid obtained from Terminalia Chebula has been shown to have antioxidant and anti-inflammatory potential in several experimental models of neurodegeneration. Herein, ChA (25, 50, and 100 mg/p. o) was assessed for its neuroprotective potential and mechanisms against ethidium bromide (EB)-induced experimental demyelination in female Wistar rats (10 animals in each group). EB was infused bilaterally at the dose of (0.1%/10µl/ICP) on days 1st to 7th to induce MS-like symptoms and ChA was administrated from day 7th to 21st. Behavioral activities were assessed through locomotor activity, Morris-water maze test, rotarod test, and narrow beam walking test on days 1st, 7th, 14^{th,} and 21st. On day 22nd, rats were sacrificed, and cortex brain regions were used to identify biochemical, neurochemical, and neuroinflammatory alterations. EB-infused rats showed significant learning and memory deficit which was associated with an increase in oxidative stress (lipid peroxidation and nitrite), compromised antioxidant defense (reduced glutathione), neurotransmitter alterations (AChE, dopamine, noradrenaline, 5-hydroxytryptamine, gamma amino butyric acid, and glutamate), and elevation in neuroinflammatory cytokine (IL-1 β, IL-6, and TNF-α) levels in comparison to sham control. ChA dose-dependently attenuated EB-induced cognitive deficit (spatial cognition, memory, grip, and motor coordination) and biochemical alterations and restored neurochemical levels. The observed protective effect might be attributed to the antioxidant and anti-inflammatory potential of ChA and its ability to restore neurochemistry. Together, these findings suggest the therapeutic potential of ChA in cognitive disorders such as MS-related motor neuron dysfunctions.

Disclosures: R. Arora: None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.08/P1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01EY028916
NIH R01NR015639
The Paul and Evanina Bell Mackall Foundation Trust
Research to Prevent Blindness

Title: Micronutrient regulation as a therapeutic target in CNS bacterial infection

Authors: *S. FOSHE[1,2], H. ROSSMILLER[2], J. STERLING[4,5,6], E. WHITE[3], R. ASTLEY[7], M. CALLEGAN[7,8,9], E. GRICE[3], J. DUNAIEF[2];
[1]Neurosci., Univ. of Pennsylvania, Philadelphia, PA; [2]FM Kirby Ctr. for Mol. Ophthalmology, [3]Dept. of Dermatol., Univ. of Pennsylvania Perelman Sch. of Med., Philadelphia, PA; [4]Dept. of Med., [5]Dept. of Ophthalmology, [6]Feinberg Cardiovasc. and Renal Res. Inst., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL; [7]Dept. of Ophthalmology, [8]Dept. of Microbiology and

Immunol., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK; [9]Dean McGee Eye Inst., Oklahoma City, OK

Abstract: Treating bacterial infections of the central nervous system is complicated by increased antibiotic resistance and the lack of neuronal regeneration. Bacterial endophthalmitis, an intraocular infection, can rapidly cause retinal degeneration and permanent vision loss. *Staphylococcus aureus* is a particularly virulent pathogen that is well-known for its ability to develop antibiotic resistance. Since iron is an essential nutrient for bacterial survival and proliferation, iron chelation has shown promising anti-bacterial effects in models of other infections. In this study, we evaluated the potential of the Fe^{2+} chelator 2,2'-bipyridine (BP) in *ex vivo* and *in vivo* models of *S. aureus* endophthalmitis. *Ex vivo*, we cultured *S. aureus* in vitreous fluid isolated from rabbit eyes and measured the bacterial growth over 12 hours. *In vivo*, we injected *S. aureus* into the eyes of wild-type C57BL/6J mice (n=5 per group) and observed signs of infection after 24 hours. We found that treatment with BP significantly inhibits *S. aureus* growth in both isolated vitreous and the mouse eye. To evaluate the safety of intraocular BP injection, we examined its effect on murine retinal structure and function. Through *in vivo* imaging and electroretinography, we determined that a low dose (2 mM) of BP does not cause overt retinal damage up to one month post-injection. These data show promising support for the clinical application of iron chelation in the treatment of nervous system infection.

Disclosures: S. Foshe: None. **H. Rossmiller:** None. **J. Sterling:** None. **E. White:** None. **R. Astley:** None. **M. Callegan:** None. **E. Grice:** None. **J. Dunaief:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.09/P2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: IBRO Early Career Awards 2020 grant
CONACYT-Mexico grant No. 256878 to JLGA
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I. Parra was supported by a scholarship from CONACYT-Mexico (1024175).
L. Vásquez-Celaya was supported with a postdoctoral fellowship from PRODEP-SEP (Official document 511–6/2020–2912, ref. CCPIyV/043/20)

Title: Neuroprotective and Immunomodulatory Effects of Probiotics in a Rat Model of Parkinson's Disease

Authors: *I. PARRA[1], L. VÁSQUEZ-CELAYA[2], I. MARTÍNEZ[1], V. ALATRISTE[1], F. LUNA[1], Y. TIZABI[3], J. GÓNGORA[2], L. MENDIETA[1];

[1]Benemérita Univ. Autónoma De Puebla, Puebla, Mexico; [2]Univ. Autónoma de Yucatán, Mérida, Yucatán, Mexico; [3]Howard Univ. Col. of Med., Col. of Med., Washington, DC

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by motor symptoms and neuroinflammation. It is now well recognized that a bidirectional relationship between gut microbiota and the brain, referred to as the gut-brain axis, plays a prominent role in maintaining homeostasis and that a disruption in this axis can result in neuroinflammatory response and neurological disorders. The gut-brain axis has been implicated in the pathogenesis of PD, suggesting a potential role for probiotics in modulating disease progression. This study aimed to investigate the neuroprotective and immunomodulatory effects of probiotics in a rat model of PD. Male Wistar rats were administered a mixture of *Bifidobacterium animalis* ssp. lactis Bb12 and *Lactobacillus rhamnosus* GG [$1x10^9$ CFU each strain in 300 µL of Probiotic] orally for 15 days prior to the induction of hemiparkinsonism with lipopolysaccharide (LPS). Motor behavior was assessed using the cylinder and beam models. Neurodegeneration in the substantia nigra pars compacta (SNpc) and microglial activation in the dorsolateral striatum and SNpc were quantified. Probiotic treatment did not affect motor asymmetry in the cylinder model. However, it significantly improved gait deficits in the beam test. Probiotics also reduced microglial activation compared to LPS-induced inflammation. However, no significant effects on neurodegeneration in the SNpc were observed. The findings suggest that the probiotic mixture of *Bifidobacterium animalis* ssp. lactis Bb12 and *Lactobacillus rhamnosus* GG has neuroprotective and immunomodulatory effects in a rat model of PD. The improvement in motor coordination and reduced microglial activation highlight the potential therapeutic utility of probiotics in PD. Further research is needed to elucidate the underlying mechanisms and evaluate their translation into clinical applications.

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Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.10/P3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Evaluation of Retinal Pigment Epitheliumfrom Human Pluripotent Stem Cells with Low Immunogenicity

Authors: Q. WANG[1], Y. ZHOU[1], S. YUAN[2], J. WANG[3], *G. CHEN[1];
[1]Fac. of Hlth. Sci., Univ. of Macau, Taipa, Macao; [2]Nanjing Med. Univ., Nanjing, China; [3]Help Therapeut. Inc, Nanjing, China

Abstract: Age-related macular degeneration (AMD) impairs central vision in almost 9% of people globally. During the development of AMD, the degeneration of retinal pigment

epithelium (RPE) leads to vision loss associated with the loss or dysfunction of photoreceptors in the retina. While there is no effective therapy for most AMD patients, RPE transplantation is a promising approach to treat AMD by supplementing healthy RPE cells. However, RPE transplantation is limited by the lack of RPE sources and immune rejection after transplantation. In this project, we use human induced pluripotent stem cells (iPSCs) as starting materials to generate large amount of RPE through lineage specific differentiation. Meanwhile, we utilize genetic engineering approaches to suppress immunogenicity in the cells. We then examine the functionality on the cellular level, and then evaluate the toxicity and efficacy of RPE transplantation in a mouse model. This study will help establish the technical foundations to treat AMD with iPSC-derived RPE in the near future.

Disclosures: Q. Wang: None. **Y. Zhou:** None. **S. Yuan:** None. **J. Wang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Help Therapeutics Inc. **G. Chen:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.11/P4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Intranasal administration of hN for the therapy of Rett's syndrome

Authors: *L. DE FILIPPIS[1], D. POZZER[2], M. INDRIGO[2], M. BRECCIA[2], E. FLORIO[2], N. LANDSBERGER[2], G. MARTINO[2], A. ARAMINI[3], M. ALLEGRETTI[3];
[1]Dompè Farmaceutici S.p.A., Milano, Italy; [2]San Raffaele Hosp. (IRCCS), Milan, Italy; [3]Res. and Develop., Dompè Pharmaceuticals SpA, L'Aquila, L'Aquila, Italy

Abstract: Rett Syndrome (RTT) is a severe X-linked neurodevelopmental disorder that affects around 1:10000 girls worldwide and is mainly characterized by an apparently normal development for the first 6-18 months of age. At this time-point a regression phase occurs leading to the onset of progressive symptoms such as cognitive and motor defects, seizures, hand stereotypes, decrease in acquired speech and breathing problems. This syndrome is caused by mutations in the methyl-CpG-binding protein 2 (*MECP2*) gene, which translates for a multifunctional ubiquitously expressed protein (mostly abundant in the brain). The role of MECP2 is mainly transcriptional repression. Several molecular deficiencies characterize the RTT brain among which alterations in the levels of neurotrophins appear of particular therapeutic interest.In this study we evaluated the effect of a human neurotrophic molecule (hN) (that will be disclosed upon acceptance of the abstract for the presentation) treatment in a mouse model of RTT through intranasal administration. Specifically, the molecule is currently used in clinical studies. First we assessed the biodistribution of the molecule and optimized the administration procedure. Then we conducted longitudinal studies on both full MeCP2 knock-out male and heterozygous female RTT mice that were monitored and treated for at least 1 month.

Weight, phenotypic scores, motor and cognitive tests (pole, grid, NOR, marble test) have been carried out, alongside measurements of the breathing parameters and the survival rate. At the end of the treatment cortices have been dissected and bulk RNA sequencing has been performed to identify affected molecular pathways. We will provide several results proving the capacity of the molecule to exert positive effects in both genders, particularly in cognitive functions and interaction with the environment. Analysis of RNAseq suggested defective pathways related to mitochondrial functions and protein synthesis/sorting in the untreated RTT cortices that appear to be positively affected by the treatment. We suggest the proposed treatment as a promising approach for the therapy of Rett's syndrome, with special emphasis on the administration route which is non-invasive and on the use of a neurotrophic molecule which has being already used in clinical studies.

Disclosures: **L. De Filippis:** 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.; Grant n.74 PINNACOLO DM31/12/2021 Accordi Innovazione-Fondo Crescita Sostenibile Ministry of Enterprises and Made in Italy. **D. Pozzer:** None. **M. Indrigo:** None. **M. Breccia:** None. **E. Florio:** None. **N. Landsberger:** A. Employment/Salary (full or part-time):; Department of Medical Biotechnology and Translational Medicine, University of Milan. **G. Martino:** None. **A. Aramini:** None. **M. Allegretti:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.12/P5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: VitreoRetinal Surgery Foundation Research Fellowship
The Knights Templar Eye Foundation Pediatric Ophthalmology Career-Starter Research Grant
NIH NEI R01 EY032136

Title: Combination therapy of gene augmentation and pharmacological supplementation for CRX-associated retinopathies

Authors: *C. SUN, S. CHEN;
Washington Univ. in St. Louis, St. Louis, MO

Abstract: CRX is a transcription factor that is predominantly expressed in rod and cone photoreceptors and regulates the expression of many target genes during photoreceptor development and maintenance. Nonsense or frameshift mutations, such as CRX^{E168d2}, within the CRX activation domain often produce truncated CRX proteins that fail to transactivate the target genes. These mutations are typically associated with autosomal dominant Leber congenital

amaurosis (LCA) or early-onset cone-rod dystrophy (CRD). A hallmark of *CRX*$^{E168d2/+}$ mouse model is the complete loss of cone photoreceptors in young adulthood, resembling the CRD manifestation in human patients. Treatment is still unavailable. In order to validate if CRX augmentation can reduce the disease severity and rescue the phenotypic defects in *CRX* mutant models, we generated a transgenic system, *Tet-On-hCRX*, to express a human wildtype CRX in *CRX*$^{E168d2/+}$ mouse with the presence of doxycycline. As compared to the untreated controls, *Tet-On-hCRX* treated *CRX*$^{E168d2/+}$ retinas showed improved photoreceptor morphology and function as well as significantly reduced, but not fully prevented, photoreceptor degeneration. In order to extend the therapeutic effects and neuroprotect treated photoreceptors, we developed two types of pharmacological supplementations: Trichostatin A (TSA, a HDAC inhibitor) and resveratrol (an antioxidant reagent). The combination therapy of each type of treatments (for 2 weeks) along with *Tet-On-hCRX* gene augmentation promoted significantly enhanced survival of cone photoreceptors as compared to treated samples with each subset. Moreover, *Tet-On-hCRX* treated samples showed better survival of cone photoreceptors and retinal morphology than samples that only received drug treatments, implying gene augmentation as the driving force of photoreceptor recovery. These results collectively suggested a promising therapeutic potential of the combined force of CRX augmentation and neuroprotective supplementation for *CRX*-associated retinopathies. Future directions include the molecular analysis on the recovery mechanisms of the combination therapy.

Disclosures: C. Sun: None. **S. Chen:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.13/P7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Anxiolytic effects of Cannabidiol(CBD) in the rodent model of Chemo-brain

Authors: *B. POUDEL[1], J. L. CHEATWOOD[2], D. B. HALES[3];
[1]Southern Illinois Univ., Carbondale, IL; [2]Anat. and Physiol., [3]Physiol., SIU Sch. of Med., Carbondale, IL

Abstract: Chemotherapy-associated cognitive impairment (Chemo-brain) is one of the most detrimental neurological disorders highly reported among cancer patients. For this reason, the search for new therapeutic options for the treatment of “Chemo-brain” is a priority. Doxorubicin (Dox) is a cytotoxic anthracycline antibiotic widely used to treat many types of cancer including breast cancer. It has been reported to induce chemo-brain via DNA damage, disruption of hippocampal neurogenesis, oxidative stress, inflammation, and dysregulation of apoptosis which limits the clinical application of this drug. Cannabidiol (CBD) is a non-psychoactive compound derived from industrial hemp (*Cannabis sativa*) that has been identified as a possible therapeutic agent against many neurodegenerative disorders. However, studies regarding the neuroprotection

of CBD against chemo-brain remain obscure. For this purpose, Doxorubicin was administered intraperitoneally to eight-week-old female Long Evans hooded rats once a week for four consecutive weeks, with an associated oral administration of CBD (10 mg/kg) three times a week for the same period. The Elevated Plus Maze Test, Open Field Test, and Marble Burying Test as well as the Sucrose Preference Test were performed to assess anxiety and depression-like behaviors respectively. Results showed that the oral supplementation of CBD significantly mitigated the cognitive deficits induced by Dox. Hence, our study can provide insights on the possible mechanism by which Dox-induced cognitive dysfunction could be alleviated proving CBD as a potential therapeutic for Chemo-brain.

Disclosures: B. Poudel: None. **J.L. Cheatwood:** None. **D.B. Hales:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.14/P8

Topic: C.08. Ischemia

Support: Florida Department of Health # 20K09

Title: Estrogen receptor-beta agonist treatment confers ischemic protection via altered brain metabolism in aged female rats

Authors: *Z. Q. BASSETT[1], H. PRADHYUMNAN[1], S. H. PATEL[1], A. P. RAVAL[1,2]; [1]Peritz Scheinberg Cerebral Vascular Dis. Res. Labs. (CVDRL), Dept. of Neurol., Leonard M. Miller Sch. of Medicine, Univ. of Miami, Miami, FL; [2]Bruce W. Carter Dept. of Veterans Affairs Med. Ctr., Miami, FL

Abstract: One-fifth of postmenopausal women suffer stroke in the United States. Menopause is characterized by decline in endogenous estradiol-17β (E2), which is normally neuroprotective against ischemia. Despite safety concerns of E2 treatment, women appear to be naturally protected against ischemic neuronal damage during pre-menopausal life, suggesting an estrogen-influenced neuroprotective mechanism. In a published study, we demonstrated that pretreatment with a specific E2 receptor subtype-beta (ER-β) agonist reduces ischemic brain damage in reproductively senescent (RS) female rats. Here we hypothesize that ER-β agonist treatment induces metabolic changes in the brain of RS female rats resulting in neuroprotection. We randomly assigned female Sprague-Dawley retired breeder rats (9-10 months; n=6/group) to two cohorts. The first was exposed to either vehicle (DMSO) or ER-β agonist every 48 hours for a month then brains were analyzed for global metabolomic (Metabolon Inc) changes. The second cohort was exposed to transient middle cerebral artery occlusion (tMCAO; 90 min) or sham surgery. At 4.5h after tMCAO, they were treated with either DMSO or ER-β agonist, and then treated every 48 hours for one month. One-month post-surgery, we measured percent freeze-time in a hippocampal-dependent contextual fear conditioning model then perfused and collected

brains for infarction quantification. We observed significantly ($p<0.05$) reduced infarct volume in ER-β agonist-treated rats as compared to DMSO, as well as significantly increased freezing, suggesting cognitive improvement. Metabolomics data demonstrated significantly altered ($p<0.05$) levels of intermediates of histidine, glutathione, and purine metabolism, as well as intermediates in the gamma-glutamyl cycle. These observed metabolic changes may be targets for protecting the brain from oxidative damage in RS rats.

Disclosures: **Z.Q. Bassett:** None. **H. Pradhyumnan:** None. **S.H. Patel:** None. **A.P. Raval:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.15/P9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: National Research Foundation of Korea NRF-2020R1C1C1004107
Korea Health Industry Development Institute HF21C0018

Title: Change of circulating exosomal microRNA reveals the anti-depressive effect of acupuncture on neuroinflammation-induced depression animal model

Authors: K.-Y. CHUN, *S.-N. KIM;
Dongguk Univ., Goyang, Korea, Republic of

Abstract: Depression is one of the common psychological disorders and it affects an individual's physical condition, thoughts, mood, and life in general. Despite its high prevalence and a high rate of recurrence among global population, treatment options are limited due to its complex pathophysiology and incomplete understanding of the biological mechanisms. In recent years, prolonged inflammatory responses are associated with etiology of depression and animal studies in past decades have suggested role of microRNA in various neuropsychiatric disorders and inflammatory diseases. Meanwhile, acupuncture treatment was reported its effectiveness on neuropsychiatric diseases and alleviating depression-like behaviors, still its miRNA-related mechanism has yet to be clearly identified. Here, using the LPS-induced depression model, we applied acupuncture treatments and analyzed changes of exosomal miRNAs in mouse serum. Using the exosomal miRNA microarray data, we analyzed differentially expressed miRNAs in LPS group and LPS+ST36 group and identified statistically significant ($p < 0.1$ and $|\log2FC| > 1$) miRNAs in each group with comparison to CON group. The identified miRNAs were then grouped and applied to bioinformatics prediction. Target genes of differentially expressed miRNAs were selected precisely by using overlapping genes in Target Scan (cumulative weighted context score++ < -0.3) and miRDB (target score > 70) and were analyzed in the Kyoto Encyclopedia of Genes and Genomes (KEGG) biological pathway and Gene Ontology (GO). Based on the findings, we were able to identify multiple miRNAs that were significantly

different from each experimental group and suggest its mechanism. Furthermore, additional analysis on each candidate exosomal miRNA could suggest them as clinical biomarker or novel diagnostic tools of depression.

Disclosures: K. Chun: None. **S. Kim:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.16/P10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: PAPIIT-IN201621

Title: Prolactin protects hippocampal neurons in vitro from H_2O_2-induced oxidative stress and reduces NOX4 activation

Authors: *F. MACÍAS[1], M. ULLOA[1], C. CLAPP[1], G. MARTÍNEZ DE LA ESCALERA[1], E. ARNOLD[1,2];
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Abstract: Oxidative stress has been linked to neuronal apoptosis and the progression of several neurodegenerative diseases. Oxidative damage elevates NADPH oxidases (NOX) activity, one of the main endogenous sources of ROS, which contribute for neuronal cell death. Efforts have been made to find NOX inhibitors as a neuroprotective strategy. Prolactin (PRL) is neuroprotective against glutamate excitotoxicity-induced oxidative damage in mouse hippocampal neurons and hydrogen peroxide (H_2O_2)-induced apoptosis of human retinal pigment epithelial cells. Furthermore, PRL regulates NOX activation in fish macrophages. Using primary cultures of mouse hippocampal neurons, we investigated the neuroprotective effect of PRL against H_2O_2-induced oxidative stress, cell death, and NOX activation. H_2O_2 treatment induced apoptotic cell death and increased ROS generation, lipoperoxidation and NOX activity in hippocampal neuronal cultures. In contrast, PRL pretreatment prevented H_2O_2-induced apoptotic cell death, reduced ROS levels, lipid peroxidation and NOX activity. PRL-induced protection against H_2O_2 was abolished by using a PRL receptor antagonist 1-9 G129R-hPRL. To assess the molecular mechanism involved in these PRL actions, we evaluated the mRNA expression of apoptosis mediators and NOX. PRL downregulated H_2O_2-induced BAX, BAD, and NADPH oxidase 4 (NOX4) expression. In summary, our results demonstrate that PRL is an antioxidant neuroprotective factor that reduces the expression of NOX4, ROS production and apoptosis in hippocampal neurons, that could serve as a potential therapeutical strategy for neurodegenerative diseases.

Disclosures: F. Macías: None. **M. Ulloa:** None. **C. Clapp:** None. **G. Martínez de la Escalera:** None. **E. Arnold:** None.

Poster

PSTR331. Neuroprotective Mechanisms: Pharmacological Approaches II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR331.17/Q1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: The Assistant Secretary of Defense for Health Affairs through the Congressionally Directed Gulf War Illness Research Program (GW210060)
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX005015))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (I01RX001520))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Rehabilitation Research and Development (IK2RX003253))
Department of Veterans Affairs (Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research and Development (I01BX004561))
The Veterans Bio-Medical Research Institute.

Title: Gulf War toxicant-induced effects on the hippocampal dendritic arbor were reversed by treatment with a Withania somnifera extract

Authors: *A. L. SHAIKH[1,3], K. E. MURRAY[1,3], V. DELIC[1,3,4], K. D. BECK[2,3,4], V. RAVINDRANATH[5], B. A. CITRON[1,3,4];
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Abstract: Gulf War Illness (GWI) is recognized as a multi symptom disorder that manifests with fatigue, sleep disturbances, mood-cognition pathologies, and musculoskeletal symptoms. This affects at least 25% of the military personnel that served in operations Desert Shield and Desert Storm from 1990-1991. We modeled their exposures during the Gulf War in C57Bl/6J mice by injecting them with a mixture of pyridostigmine bromide (an anti-sarin drug), chlorpyrifos (an organophosphate insecticide), and DEET (an insect repellent) for two weeks starting at 12 weeks old. CNS damage occurs in the GWI model, therefore investigation into its effects on dendrites and their synaptic spines may reveal mechanisms involved in the cognitive problems and, potentially, strategies to ameliorate them. We examined hippocampal neurons. We have previously observed that GWI model mice displayed dendritic arbor reduction and loss of spines

in granule cells of the dentate gyrus of the hippocampus at 14 weeks post-exposure. Traditional Ayurvedic medicine in India effectively tested a large variety of herbs over the millennia and found a few that seemed effective for neurological conditions, including *Withania somnifera,* commonly referred to as Ashwagandha. The root of this shrub contains bioactive molecules, many of which share chemical groups with modern pain, cancer, and anti-inflammatory drugs. We treated GWI mice with *W. somnifera* root extract by oral administration, 12 weeks post-toxicant exposure for a duration of 6 weeks. GWI mice were found to have 23% decreased dendritic length ($P<0.0001$) and dendritic spine density, with at least some of the density reduction involving stubby spines (14% decreased, $P<0.0001$) that can reflect neurodegeneration. GWI mice that had been treated exhibited dendritic lengths and spine densities close to normal. These findings demonstrate the efficacy of the Ayurvedic extract treatment for neuroprotection following these toxic exposures. We hope that the extract or the mechanisms influenced will open new avenues of research regarding treatment of neurodegenerative diseases.

Disclosures: A.L. Shaikh: None. **K.E. Murray:** None. **V. Delic:** None. **K.D. Beck:** None. **V. Ravindranath:** None. **B.A. Citron:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.01/Q2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: A novel mouse model to reveal the role and the impact of neuroimmune modulation in ALS with TDP-43 pathology

Authors: *E. ULUPINAR[1,3], A. AHRENS[1,3], O. GOZUTOK MOORE[1,3], Z. FITZGERALD[1,3], B. GENC[1,3], M. GAUTAM[1,3], H. OZDINLER[1,3,4,5,2];
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Abstract: Motor neuron loss is associated with neuroinflammation and accumulation of protein aggregation in amyotrophic lateral sclerosis (ALS). TDP-43 pathology is one of the most common forms of protein aggregation and the contribution of neuroinflammation is well-documented. However, we lack cell-based model systems that would bring cellular clarity and precision to our understanding for the mode of interaction, site of initiation and the underlying molecular basis of neuronal degeneration. To investigate the contribution of neuroimmune modulation in ALS pathogenesis, we generated a novel mouse model, prpTDP-43^{A315T}-MCP1-CCR2, by crossing prpTDP-43^{A315T}mice with MCP1-CCR2 reporter line, in which cells expressing MCP1 (monocyte chemoattractant protein-1) and CCR2 (CC chemokine receptor 2) are genetically labeled by monomeric red fluorescent protein-1 and enhanced green fluorescent

protein, respectively. Therefore, cells that are responsible for the initiation of innate immunity are fluorescent in one of the best-characterized mouse models of TDP-43 pathology, and can be traced, isolated and assessed with cellular precision. To investigate the interplay between the peripheral and the central nervous system, we studied both the peripheral immune system organs, including spleen, thymus and inguinal lymph nodes, as well as the brain and the spinal cord of prpTDP-43^{A315T}-MCP1-CCR2 mice at early symptomatic (P60) and late symptomatic (P100) stages. MCP1-CCR2 mice are used as healthy controls. Our initial investigations suggest an overall increase in the levels of CCR2+ cells around the blood vessels and meninges, suggesting a leakage from the BBB in the presence of TDP43 pathology, and robust infiltration of neuroimmune cells to the brain parenchyma early in the disease. Infiltrating cells were in close vicinity with Iba1+ microglial cells and GFAP+ astrocytes, which are present at high levels in layer V of the motor cortex of prpTDP-43^{A315T}-MCP1-CCR2 mice. Our observations suggest a utility for this novel mouse model, as it allows visualization and cellular assessment of neuroimmune axis within the context of TDP-43 pathology in both the CNS and the periphery. Since translation is at the cellular level, MCP1+ and CCR2+ cells in this novel TDP-43 model will shed light onto the cellular mechanism responsible for the initiation and progression of TDP-43 pathology, and will help develop therapeutic approaches related to neuroimmune modulation in ALS.

Disclosures: **E. Ulupinar:** None. **A. Ahrens:** None. **O. Gozutok Moore:** None. **Z. Fitzgerald:** None. **B. Genc:** None. **M. Gautam:** None. **H. Ozdinler:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.02/Q3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: UW Madison Anesthesiology Research and Development Grant

Title: Effects of DMT and fluvoxamine on lipopolysaccharide induced neuro-inflammation seen through EEG and pro-inflammatory cytokines

Authors: *Z. ZAHID[1], A. ZEIMET[1], S. M. GRADY[2], C. J. WENTHUR[1], R. D. SANDERS[3], M. I. BANKS[2];
[2]Anesthesiol., [1]Univ. of Wisconsin Madison, Madison, WI; [3]Anaesthetics, Univ. of Sydney, Sydney, Australia

Abstract: Introduction. Neuroinflammation has been linked to a range of psychiatric disorders, such as depression, acute disorders of consciousness, such as post-operative delirium, and neurological disorders, such as Alzheimer's disease. We recently showed that administration of the bacterial endotoxin lipopolysaccharide (LPS) to mice triggered neuroinflammation (increased brain levels of IL-6 and MCP-1) that was coupled to an increase in EEG slow wave activity

(SWA). The nonsteroidal anti-inflammatory drug piroxicam and the adenosine receptor antagonist caffeine ameliorated the increase in LPS-induced SWA without affecting changes in cytokine levels. The selective serotonin uptake inhibitor fluvoxamine (FLUV) and the serotonergic psychedelic N,N-Dimethyltryptamine (DMT) have been shown previously to exhibit anti-inflammatory activity both in vitro and in vivo, presumably via agonism at the sigma-1 receptor. Here, we tested the effects of FLUV and DMT on LPS-induced SWA and brain cytokine release in mice.**Methods.** Male and female C57BL/6J mice (2-5 months old) were instrumented with skull screw EEG electrodes and skull magnets to monitor movement. After >5-day recovery period, EEG and movement activity was recorded continuously for 5 hours from unrestrained mice. This included a during a 1-hour baseline period followed by (IP) injection of DMT (2.5 or 10 mg/kg), FLUV (20 mg/kg), the sigma-1 receptor antagonist BD1063 (1 mg/kg), or saline (5 ml/kg). Thirty minutes later, IP injection of LPS (25 μg/kg) occurred followed by a 4-hour passive recording. Movement was monitored by magnetometry. EEG SWA was obtained via standard time-frequency analysis. Propensity score weighting based on movement distributions during baseline compared to t = 1-2 hours following LPS injection was applied to SWA data to account for the effects of LPS-induced changes in movement. At t = 4 hours, animals were deeply anesthetized, and their brains were removed and flash frozen for subsequent ELISA analysis of cytokine levels.**Results.** LPS increased SWA and decreased movement compared to baseline and to saline. Neither FLUV nor DMT affected the LPS-induced decrease in movement. FLUV reversed the increase in SWA in both male and female mice, whereas DMT did so in female but not male mice. Surprisingly, BD1063 alone also attenuated the LPS-induced increase in SWA. LPS increased IL-6 and MCP-1 concentrations compared to saline, and neither FLUV nor DMT prevented that increase.**Conclusions**. Amelioration by FLUV and DMT of LPS-induced SWA without effects on movement or cytokine levels indicates a decoupling between inflammation and effects on neural activity.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.03/Q4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: CAS Graduate Student Research Award

Title: The Therapeutic Impact of CBD (Cannabidiol) in Hyperglycemic Zebrafish (Danio rerio)

Authors: *E. MCCARTHY[1], *E. MCCARTHY[2], K. AUGUSTINE[1], L. GERTNER[1], V. CONNAUGHTON[1];
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Abstract: The breakdown of the blood brain barrier and the blood retinal barrier in Diabetes Mellitus (DM) is the cause for many later life complications such as the cognitive decline and diabetic retinopathy. These complications are associated with inflammation and oxidative stress in both brain and retina. Current research suggests that cannabidiol (CBD), a non-psychoactive derivative of *Cannabis sativa* with potential medicinal effects, may have anti-inflammatory properties. To investigate this further, we performed a series of experiments to determine the potential of CBD as a possible treatment for the complications associated with DM in a zebrafish model. To conduct our study, we maintained 6 groups of fish (6 per group). Each group was maintained in glucose, water, or mannitol and received a drug treatment (5mg/L CBD, 5mg/L methanol, or water) for 20mins every other day, resulting in the following treatment groups: Water/Water, Glucose/Water, Mannitol/CBD, Water/Methanol, Water/CBD (stress, hyperglycemic, osmotic, drug, and vehicle control, respectively), and Glucose/CBD. To test for locomotion, anxiety, or vision effects we performed Novel Tank (anxiety and locomotion) and Optomotor Response (vision-based behavior) at the conclusion of every week for four-weeks. Brain and retina tissue were collected at four-weeks for further analysis. After one-week there was no significant difference in locomotion, anxiety, or vision between any of the groups. At the two-week checkpoint Novel Tank results revealed no significant difference in locomotion between any group. However, Water/CBD fish spent significantly less time at the bottom of the tank than Water/Methanol ($p = 0.033$), Glucose/Water ($p = 0.004$), Glucose/CBD ($p = 0.007$), Mannitol/CBD ($p = 0.002$;). This trend continued after three- and four-weeks of treatment, showing that CBD had a slight anxiolytic effect in the water-treated fish. OMR results at two-weeks showed no significant difference between the six groups. However, at week three OMR results show that Glucose/Water fish performed significantly fewer rotations than both Water/Water treated fish ($p = 0.003$) and Water/CBD fish ($p = 0.04$). Similarly, Glucose/CBD fish performed significantly fewer rotations than Water/Water treated fish ($p = 0.005$). This trend continued at four weeks. These data show that our dose of CBD, while not influencing locomotion, may be having a slight anxiolytic effect. Our results also validated our previous findings that hyperglycemia causes visual impairments; however, this preliminary study does not show that CBD was helpful in ameliorating the visual deficits.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

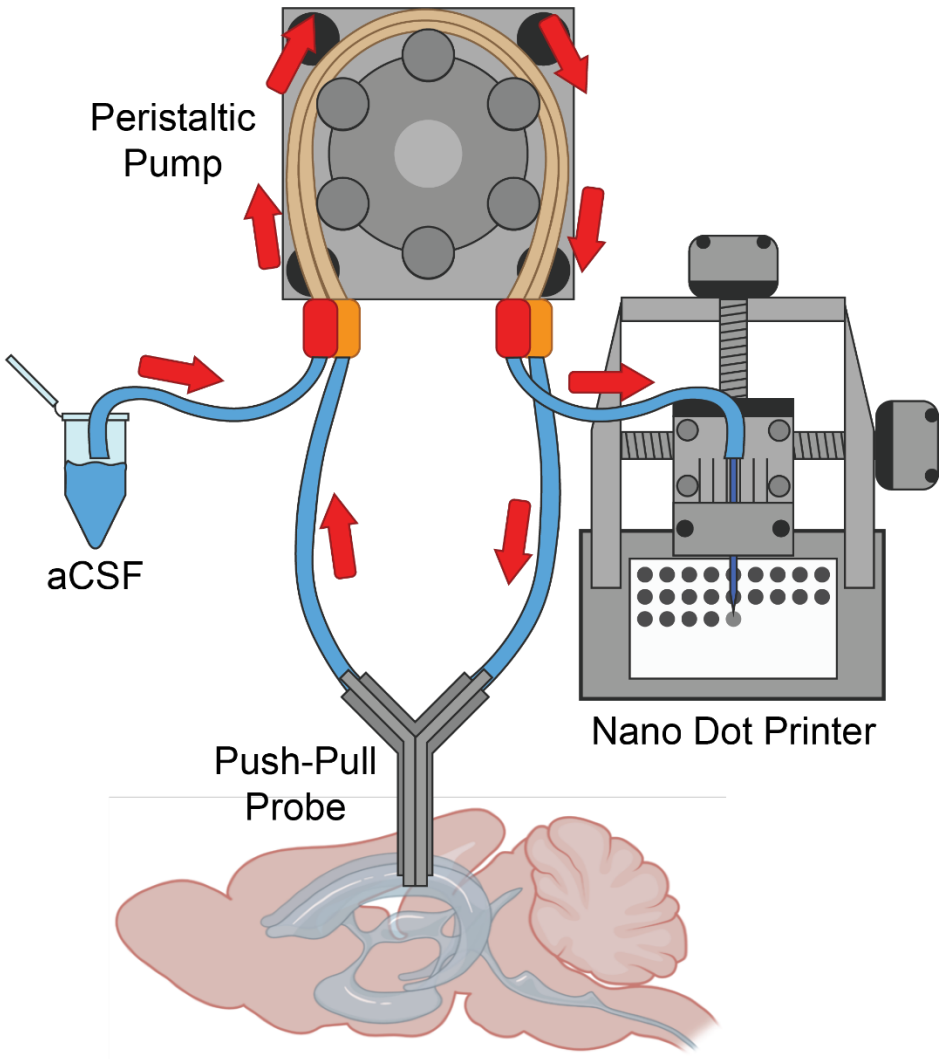
Program #/Poster #: PSTR332.04/Q5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Identification of rapid IL-1β release in cerebrospinal fluid during acute epileptiform activity induced by 4-aminopyridine using a novel methodology with high temporal resolution

Authors: *S. MARTINEZ GALLEGOS[1], A. MORALES-VILLAGRAN[1,2], J. ORTEGA-IBARRA[2], L. MEDINA-CEJA[1];
[1]Mol. and Celular Biol., Univ. of Guadalajara, Nextipac, Zapopan, Mexico; [2]MexBio Res. Innovations, El Salto, Jalisco, Mexico

Abstract: Neuroinflammation is an innate immunity mechanism that can be activated as a result of brain damage caused by seizures or status epilepticus. One of its hallmarks is the release of inflammatory cytokines, with IL-1β generally recognized as one of the main mediators during the acute phase. However, the dynamics of IL-1β release have not been studied with high temporal resolution due to the lack of a suitable detection method. In this study, a novel approach to quantify the levels of IL-1β by implementing the push-pull technique to collect cerebrospinal fluid (CSF) samples from the lateral ventricle was introduced. The samples were automatically deposited in a nitrocellulose membrane (0.3 µl / dot) with a sample rate of one minute and the concentration was determined by enhanced chemiluminescence immunodetection. To induce acute epileptiform activity, 4-aminpoyridine was administered through the push-pull probe (75 mM for 10 minutes at 0.3 µl/min). Preliminary results show non-detectable levels of IL-1β in basal samples; however, a rapid and statistically significant ($p<0.01$) increase in the concentration of IL-1β after the administration of 4-AP was identified. The increase began immediately after 4-AP reached the ventricle and lasted approximately 45 minutes, with no further increases observed over the next 6 hours. Given the rapid nature of this increase, it is possible that the source of IL-1β originates from an internal store, as it has been reported that IL-1β can be stored in lysosomes. Furthermore, 4-AP has been shown to increase intracellular Ca^{2+} by interacting with voltage-gated Ca^{2+} channels, potentially facilitating exocytosis of lysosomal contents.



Disclosures: **S. Martinez Gallegos:** None. **A. Morales-Villagran:** None. **J. Ortega-Ibarra:** None. **L. Medina-Ceja:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.05/Q6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RF1-AG-028271

Title: Post-surgical morphine induces long-lasting spatial memory deficits in aged female rats

Authors: *B. D. ALVAREZ, S. MUSCAT, M. BETTES, J. DEMARSH, M. BUTLER, R. BARRIENTOS;
The Ohio State Univ., Columbus, OH

Abstract: Background: Cognitive deficits of executive functions, memory, and attention, frequently occur proceeding several types of surgical procedures such as abdominal, orthopedic, cardiac, etc. These impairments can persist months after such surgeries and are accompanied by elevated risk of mortality and dementia in older individuals. Female patients are more susceptible to these longer lasting impairments, yet few studies investigate post-operative cognitive dysfunction (POCD) in female rodents. Although the mechanisms encompassing the sex-based prevalence differences are inconclusive, POCD results in exaggerated neuroinflammation in aged, male rats receiving opioids (i.e., morphine). Here, we examined POCD in female rats and hypothesized that the combination of aging, laparotomy, and morphine would impair hippocampal-dependent memory incited by neuroinflammation. **Methods:** To understand these differences, we performed either sham surgeries or laparotomies on young adult and aged female rats, followed by 7 days of saline or morphine (2mg/kg, i.p., 2x/day). Two weeks post-surgery, the open field task, novel object recognition/location tasks, and contextual fear conditioning were conducted. Neuroinflammatory markers (i.e., IL-1β, IL-6, and TNF-α) within the hippocampus were measured via ELISA. **Results:** Although no differences were observed in time spent in periphery/center in the open field, young rats exhibited excessive grooming compared to aged rats. Only the compounded factors of aging, laparotomy, and morphine resulted in a spatial memory deficit. A similar trend was observed with object recognition memory though this was not statistically significant. Contextual memory appeared intact in all rats, though excessive grooming in young rats may play a role in masking possible impairments. Hippocampal inflammation will be presented. **Conclusion:** Preliminary findings indicate that similar to what we have previously reported in males, aged female rats, but not their younger counterparts, are susceptible to long-lasting hippocampal-dependent memory deficits following surgery and morphine treatment, and these impairments may be the result of exaggerated neuroinflammation.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.06/Q7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: W81XWH1810166
W81XWH1810167
W81XWH2210461
W81XWH2210462

Title: Chronic inflammation impairs recovery after posthemorrhagic hydrocephalus

Authors: *B. G. VIJAYAKUMAR[1,2], T. HECK[1], Y. KITASE[1], S. ROBINSON[1,2,3], L. L. JANTZIE[1,2,3,4];
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Abstract: Acquired hydrocephalus or elevated intracranial pressure from abnormal accumulation of cerebrospinal fluid (CSF) commonly occurs in adults after intraventricular hemorrhage (IVH) and subarachnoid hemorrhage (SAH). Post-hemorrhagic hydrocephalus (PHH) can be life-threatening, and treatment typically requires surgical insertion of a shunt to divert CSF. Here, we hypothesized that sustained inflammation from IVH would preclude the recovery of CSF dynamics. To induce PHH secondary to systemic inflammation and IVH, young adult rats received 3 mg/kg LPS or saline control intraperitoneally on postnatal day 21 (P21) and P23. On P25, rats of both injury groups and both sexes underwent IVH via littermate lysed blood cells. Differences were examined using student's T-test, n=8-12/group. Rats with PHH have ventriculomegaly and elevated intracranial pressure in adulthood ($p<0.05$). These changes in pressure and ventricular volume occurs simultaneously with microstructural brain injury observed on diffusion tensor imaging as evidenced by decreased fractional anisotropy in major white matter tracts compared to controls ($p<0.01$). CCL2 (C-C motif chemokine ligand 2/monocyte chemoattractant protein 1/MCP-1), a pro- inflammatory chemokine essential to immune cell recruitment, is overexpressed in the choroid plexus of rats with PHH ($p<0.05$). In addition, adult rats with PHH have markedly higher levels of the pro-inflammatory cytokines IL-1β and IL-6, in their blood and CSF ($p<0.01$ for all). Those with hydrocephalus also have elevated CXCL1 (CXC Chemokine Ligand 1), a potent neutrophil trafficker and molecular mediator of ependymal injury concomitant with upregulated cerebral hepcidin and Transferrin Receptor mRNA compared to controls ($p<0.01$ for all). Taken together, PHH induces profound inflammation. Specifically, inflammation from blood products and direct initiation of inflammatory signal transduction within the ventricular system, exacerbates PHH pathophysiology and chronically alters the cerebral microenvironment. Subacute pharmacologic therapy for PHH that addresses this inflammatory microenvironment could prevent both the need for long-term access to neurosurgery and the need to live and work in proximity to advanced, urgent surgical care.

Disclosures: B.G. Vijayakumar: None. **T. Heck:** None. **Y. Kitase:** None. **S. Robinson:** None. **L.L. Jantzie:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR332.07/Q8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant U18DA052402
NIH Grant R01HL139492
NIH Grant R01HL158593

Title: Rat Perinatal exposure to cyclohexanone disrupts functional and anatomical brain connectivity causing significant behavioral disturbances in adulthood.

Authors: *Y. KITASE[1], E. M. CHIN[2,7], O. LIU[3], S. ROBINSON[2,4], D. GRAHAM[5], A. D. EVERETT[3], L. L. JANTZIE[2,7,4,6];
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Abstract: Background: Cyclohexanone (CXO) is an organic solvent sealer in IV bags and seeps through plastic tubing into infused medical fluids. Although circulating levels of CXO are independently associated with neurodevelopmental deficits after neonatal cardiac surgery, causality is unknown. Thus, using neonatal rats, we tested the hypothesis that exposure to clinically relevant levels of CXO would cause neurodevelopmental deficits, diminish functional connectivity and alter white matter microstructure. Methods: Beginning on postnatal day 7 (P7), Sprague-Dawley rats of both sexes were randomly allocated to receive CXO (0.7ul/g body weight/day, i.p.) or saline (CTRL) q12 h for 7 days. Beginning at P21 and assayed through adulthood, rats underwent behavioral testing including assessment of gait, coordination, and open field behavior. Brain imaging included In vivo diffusion tensor imaging and functional MRI assessment at P21. Functional connectivity was examined amongst 46 gray matter regions of interest (ROIs). Inter-ROI edge identity (46*45/2 edges) and group identity (CXO vs. CTRL) were included in a Type III ANOVA to distinguish connectivity patterns, brain-wide differences in connectivity magnitude, and differences between groups in specific connections. Comparisons were assessed with a student's t-test with $p<0.05$ considered statistically significant. Results: CXO exposure resulted in a functional hyperactive phenotype with increased distance traveled ($p<0.05$), time mobile ($p<0.01$), and center time ($p<0.05$) in an open field compared to CTRL. CXO exposure also resulted in significant abnormalities in gait, posture, and balance, including changes in mean breaking time ($p<0.05$), mean stance width ($p<0.01$), and mean paw angle ($p<0.01$) compared to CTRL. Imaging revealed that CXO exposure resulted in reduced fractional anisotropy was in major white matter tracts, including the corpus callosum ($p<0.001$) and external capsule ($p<0.05$), concomitant with increased radial diffusivity ($p<0.01$). CXO exposure significantly reduced global network connectivity and network topology, particularly in the cortex and basal ganglia($p<10^{-6}$). Conclusion: CXO exposure had profound effects on neurodevelopment, behavior, anatomical and functional connectivity that persisted into adulthood. Network dysfunction has been implicated in executive function deficits and may be key to CXO-induced impairments in neurodevelopment observed in humans. Additional studies are urgently needed to establish the mechanism of this injury and necessary avenues for neurorepair and CXO reduction in medical environments.

Disclosures: Y. Kitase: None. **E.M. Chin:** None. **O. Liu:** None. **S. Robinson:** None. **D. Graham:** None. **A.D. Everett:** None. **L.L. Jantzie:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.08/R1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Rudi Schulte Research Institute

Title: Infantile Post-infectious Hydrocephalus Causes Gait Deficits in Juveniles in a Preclinical Rat Model

Authors: *X. JIA[1,2,3], T. HECK[2], A. ODUKOYA[3], L. L. JANTZIE[2,3,4,5], S. ROBINSON[3,4]; [1]Johns Hopkins Univ., Baltimore, MD; [2]Pediatrics, [3]Neurosurg., [4]Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD; [5]Kennedy Krieger Inst., Baltimore, MD

Abstract: Infantile post-infectious hydrocephalus (PIH) is the most common cause of hydrocephalus in the world. PIH is particularly common in low- and middle-income countries with little access to neurosurgical care. Investigation of non-surgical pharmacological strategies to treat PIH requires a clinically relevant preclinical model. The objective of this study was to define functional deficits in juvenile rats with infantile onset PIH. We hypothesized that infantile onset PIH would cause durable gait deficits in rats similar to the gait abnormalities in children with PIH, as well as persistent signs of brain inflammation. On postnatal day 2 (P2) Sprague-Dawley rat pups of both sexes were randomly allocated to intracerebroventricular injection of lipopolysaccharide or saline. Rats were monitored for weight gain and intra-aural measurements, a surrogate for head circumference. Gait was assessed at P30 using digital analysis of videotaped running on a treadmill (Digigait). Opening pressure (OP) was measured at P60 via cisterna magna puncture. Cerebrospinal fluid and serum cytokines were quantified using multiplex electrochemiluminescence. Data were analyzed for normality and compared using a Mann Whitney test with $p<0.05$ considered significant. Results indicate that rats with PIH (n=12) developed macrocephaly compared to sham controls (n=20, $p<0.05$). Rats with PIH also exhibited more ataxia, a shorter stride length and increased step frequency compared to sham controls (all $p<0.05$). At P60, OP was higher in rats with PIH (n=8) compared to shams (n=6, $p<0.01$). Likewise, at P60, CSF chemokine CXCL1 levels were elevated ($p<0.05$) as were serum IL-6 levels ($p<0.01$). In conclusion, infantile onset PIH caused macrocephaly, elevated OP, and sustained brain inflammation. Functionally, infantile PIH led to gait abnormalities reminiscent of gait deficits observed in humans with PIH. Immune dysregulation and immunomodulation associated with the observed pro-inflammatory cytokine elevation is but one component of the complex multicellular dysfunction that leads to impaired CSF dynamics resulting in hydrocephalus and severe motor deficits. Our results define a reliable preclinical model with similar functional deficits in humans, shedding light on the development of nonsurgical pharmaceutical treatment to treat children with PIH globally.

Disclosures: X. Jia: None. **T. Heck:** None. **A. Odukoya:** None. **L.L. Jantzie:** None. **S. Robinson:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.09/R2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIA Grant 1RF1AG078677-01A1

Title: Uncovering the Role of Integrin α5β1 Co-Receptor in SARS-CoV-2 Infection: Therapeutic Implications of Transcriptomic Analysis @font-face {font-family:"Cambria Math"; panose-1:2 4 5 3 5 4 6 3 2 4; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:variable; mso-font-signature:-536870145 1107305727 0 0 415 0;}@font-face {font-family:Calibri; panose-1:2 15 5 2 2 2 4 3 2 4; mso-font-charset:0; mso-generic-font-family:swiss; mso-font-pitch:variable; mso-font-signature:-469750017 -1073732485 9 0 511 0;}@font-face {font-family:"Yu Mincho"; panose-1:2 2 4 0 0 0 0 0 0 0; mso-font-alt:游明朝; mso-font-charset:128; mso-generic-font-family:roman; mso-font-pitch:variable; mso-font-signature:-2147482905 717749503 18 0 131231 0;}@font-face {font-family:"\.AppleSystemUIFont"; panose-1:2 11 6 4 2 2 2 2 2 4; mso-font-alt:Cambria; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:auto; mso-font-signature:0 0 0 0 0 0;}@font-face {font-family:UICTFontTextStyleBody; panose-1:2 11 6 4 2 2 2 2 2 4; mso-font-alt:Cambria; mso-font-charset:0; mso-generic-font-family:roman; mso-font-pitch:auto; mso-font-signature:0 0 0

Authors: *G. TALKINGTON[1], T. GRESSETT[2], K. PARÉ[3], N. PARKER[4], E. DAMLE[4], S. ISMAEL[4], I. J. BIOSE[4], J. KOLLS[4], R. BARIC[4], G. BIX[4];
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Abstract: The SARS-CoV-2 virus, responsible for the COVID-19 pandemic, is known to bind predominantly to angiotensin converting enzyme 2 (ACE2) receptors in host cells for fusion and cell entry. However, it is unknown whether ACE2 receptors function synergistically with integrin co-receptors in this instance, much like how integrin a4B7 facilitates HIV fusion. Our prior findings demonstrate a notable decrease in SARS-CoV-2 viral load upon blockade of the integrin α5β1 receptor suggesting a possible co-receptor role. We hypothesize that integrin α5β1 plays a significant co-receptor role alongside ACE2 in SARS-CoV-2 fusion and entry. To investigate, we conducted a COVID-19 study utilizing an innovative non-transgenic mouse model with Mouse Adapted 10 (MA-10) strain of SARS-CoV-2. We inoculated 10-week-old Balb/c mice intranasally with 2x10^4 PFU of MA-10, administered 1 mg/kg ATN-161 retro-orbitally to the treatment group, and followed them to 60 days post-infection. Bulk RNA sequencing on brain tissues and analysis with Ingenuity Pathway Analysis (IPA) revealed substantial net alterations to several signaling pathways, providing potentially critical insights into the mechanisms underpinning acute and chronic COVID-19 morbidities. Specifically, analyses revealed net upregulation of serotonergic signaling pathways ($p<4.11*10^{-5}$), serotonin

biosynthesis ($p<1.10*10^{-4}$), and neurovascular coupling signaling pathways ($p<3.77*10^{-3}$) among others. These findings represent mechanisms by which, in addition to rescuing acute changes such as tight junction loss, ATN-161 may also rescue metabolic and neurotransmitter alterations underlying chronic neurological changes characteristic of long COVID. Given our prior work and current findings, we propose that administration of α5β1 integrin inhibitors such as ATN-161 provides a novel therapeutic strategy for attenuating SARS-CoV-2 viral load as well as associated morbidity and mortality in both acute and chronic conditions.

Disclosures: **G. Talkington:** None. **T. Gressett:** None. **K. Paré:** None. **N. Parker:** None. **E. Damle:** None. **S. Ismael:** None. **I.J. Biose:** None. **J. Kolls:** None. **R. Baric:** None. **G. Bix:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.10/R3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: MOST 111-2314-B-039-027-MY3

Title: Electroacupuncture efficacy in preventing 5-FU-induced xerostomia and salivary gland dysfunction in a mouse model

Authors: *T.-H. V. NGUYEN, T.-M. SHIEH, Y.-H. CHEN;
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Abstract: Saliva plays a crucial role in oral health and gastrointestinal system homeostasis. Radiotherapy and chemotherapy can impair salivary glands' function, which reduces the saliva flow rate and results in xerostomia (dry mouth). The prevalence of chemotherapy-induced xerostomia is varied, treatment regimens and underlying conditions may alter the prevalence. Xerostomia can exacerbate other side effects of chemotherapy and predispose patients to candidiasis, dental decay, and other oral infections, thereby increasing the cost of care, reducing quality of life, and impacting overall survival. Although some studies reported that acupuncture is an effective treatment, the evidence was still insufficient. Thus, this animal study aimed to assess the efficacy of electroacupuncture (EA) on preventing xerostomia, salivary gland (SG) hypofunction, and the decrease in SG's acini number induced by 5-fluorouracil (5-FU). A xerostomia mouse model was induced by four tail vein injections of 5-FU (80 mg/kg/dose). LI4 and LI11 were selected for EA, while sham acupoint was at the middle points of deltoid muscles. EA, sham acupuncture, or oral pilocarpine were given for 7 days. The body weight, food and water consumption, pilocarpine-stimulated salivary flow rate (SFR), and salivary glands weight (SGW) were recorded. Salivary IgA (SIgA) levels and lysozyme activity were determined by ELISA. SMG was collected for H&E staining to measure acini number and acinar cell size. Tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and aquaporin 5 (AQP5) mRNA

expressions in SG were quantified by RT-qPCR. We successfully conducted a mouse model of chemotherapy-induced xerostomia, in which 5-FU caused significant decreases in SFR, SGW, number of acini, SIgA, lysozyme activity, and AQP5 expression, while TNF-α and IL-1β expressions, and acinar cell size were significantly increased. EA can diminish these manifestations, but sham acupuncture cannot. Pilocarpine treatment can only elevate SFR and AQP5 expression. These findings indicate EA can diminish 5-FU toxicity on the salivary gland, thus alleviating xerostomia manifestation. Our study is the first to investigate this protective effect of EA against chemotherapy-induced xerostomia in an animal model.

Disclosures: **T.V. Nguyen:** None. **T. Shieh:** None. **Y. Chen:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.11/R4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Cord Blood Association Foundation and the Marcus Foundation
National Institutes of Health (NS123084)

Title: Cns immune surveillance is regulated by pericytes in the dura meninge

Authors: ***H. MIN**[1], S. M. O'NEIL[2], L. XU[4], A. MOSEMAN[5], J. KURTZBERG[3], A. J. FILIANO[2,1];
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Abstract: The central nervous system (CNS) is an immune-privileged tissue that tightly regulates access to peripheral immune cells. Because of its unique anatomical location, antigen-presenting cells in the dura can assess both CNS antigens and circulating T cells. In this study, we demonstrated that trafficking of CNS-antigen-specific T cell to the dura is regulated by the interaction between pericytes and macrophages. We detected decreased pericyte coverage around blood vessels in the dura prior to the onset of clinical symptoms in experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis. When we experimentally depleted pericytes, to similar levels we found in presymptomatic EAE, macrophages in the dura upregulated antigen processing and presentation machinery. Further, partial pericyte depletion increased trafficking of myelin antigen-specific T cells specifically into the dura in a process that was dependent on resident antigen-presenting cells. T cells that trafficked to the dura were preferentially skewed to T_H17 which has been implicated in autoimmune disease. *In vitro*, pericytes reprogrammed meningeal macrophages to suppress a T cell response through direct cell contact. We further determined that through direct cell contact, pericytes transferred cytoplasmic components to macrophages that included processing bodies (P-bodies), a cytoplasmic organelle

that regulates transcription. Depleting P-bodies from pericytes blocked the ability to reprogram macrophages to suppress a T cell response. Overall, our study revealed a novel mechanism by which T cells trafficking to the dura are regulated by pericyte-macrophage interactions in a process that was dependent on P-bodies. This mechanism could be involved in the early steps of CNS autoimmune disease.

Disclosures: H. Min: None. **S.M. O'Neil:** None. **L. Xu:** None. **A. Moseman:** None. **J. Kurtzberg:** None. **A.J. Filiano:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.12/R5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA Intramural Research Program

Title: Nuclear to cytoplasm export of RNA expressed from a modified HIV provirus in a rat model of HIV associated neurocognitive disorder (HAND)

Authors: *S. BLOSSOM[1], R. SVARCBAHS[1], S. N. WALTON[2], J. J. HINKLE[1], L. R. CAMPBELL[2], C. T. RICHIE[1], B. K. HARVEY[1];
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Abstract: HIV associated neurocognitive disorder (HAND) is a neurological condition of mild cognitive impairment that occurs in approximately half of people living with HIV. HAND occurs when HIV enters the brain and infects resident cells, primarily microglia. When the HIV genome is integrated into the microglial genome, the infected microglia begin shedding HIV viral particles and viral proteins such as gp120 and Nef. This protein secretion is thought to contribute to local inflammation and the development of HAND. To model HAND, we created a transgenic rat with a Cre-dependent HIV provirus (iHIV) and crossed the iHIV rat with a microglia-specific, tamoxifen-inducible CreERT2. In our model, the gag/pol genes have been deleted resulting in no productive viral infection but the accessory proteins such as gp120 and Nef are still present and can potentially be expressed. Immunostaining for Nef protein in the striatum of iHIV rats indicated that Nef protein was not present whereas wild-type animals injected with an adeno-associated virus expressing the Nef protein was detected. Digital PCR analysis of iHIV gene expression in the striatum using digital PCR indicated that splicing of the iHIV genome is impaired. When wild-type HIV is transcribed, the expression of different HIV genes is determined by alternative splicing and export. Spliced HIV mRNAs preferentially leave the nucleus to be translated into viral proteins. The export of HIV mRNAs is dependent on host cell protein human CRM1 (hCRM1) and HIV protein Rev to be efficiently exported to the cytoplasm. Using RNAScope analysis, we showed that the HIV transcripts are localized to the

nucleus but not the cytoplasm. These data suggest that the modification we made to the HIV genome impair splicing and alter export of RNA. Other labs have reported increased HIV production when cells were augmented with hCRM1 and Rev. Currently we are investigating the effect of increased levels of hCRM1 and Rev to further refine our rat model of HAND. This work was supported by the Intramural Research Program at NIDA, NIH

Disclosures: **S. Blossom:** None. **R. Svarcbahs:** None. **S.N. Walton:** None. **J.J. Hinkle:** None. **L.R. Campbell:** None. **C.T. Richie:** None. **B.K. Harvey:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.13/R6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Bowling Green State University, Building Strength Grant
National Sea Grant Program, Graduate Student Fellowship

Title: Corn Grown on Dredge-Amended Soils Impacts Neonatal and Adult Behavior and Hippocampal Development

Authors: *K. A. S. FLANIGAN[1,2,3], M. I. CZUBA[1], V. R. RIESGO[1], T. N. VU[1], J. WILLING[1]; [1]Bowling Green State Univ., Bowling Green, OH; [2]Ohio Sea Grant Program, Columbus, OH; [3]Natl. Sea Grant Program, Natl. Oceanic and Atmospheric Admin., Washington, DC

Abstract: Nutrient depletion affects more than 130 million hectares of agricultural land and is exacerbated by severe weather events due to climate change (Silver et al., 2021). As such, agriculturalists add soil amendments containing nutrients to facilitate crop yield, potentially introducing environmental contaminants. In the northwestern basin Lake Erie, a proposed soil amendment is the sediment at the bottom of the lake. While this sediment provides nutrients, it may also introduce known neurotoxins (heavy metals, polychlorinated biphenyls, polyaromatic hydrocarbons, pharmaceuticals) to crops. These toxins are known to be resistant to degradation, bioaccumulate within plants, and biomagnify in tissues of animals consuming these plants, including livestock and humans. Toxins within the dredge sediment are of particular concern due to their negative impacts on brain development and behavior. Preliminary research investigated the impact of corn grown on dredge-amended soil in Long Evans rats. Female rats were exposed to corn grown on dredge-amended soil (or a non-dredge amended source of corn) during gestation and lactation (E0-P25). These studies found that progeny exposed to dredge-amended corn during development exhibited increased anxiety-like behavior in the Open Field Test and decreased exploratory behavior in the Novel Object Recognition Test. Additionally, hippocampal volume was decreased in males exposed to dredge-amended corn. In the present study, we assess both neonatal and adult behavior and hippocampal development. Neonatal subjects were tested on three days (P5, P10, and P15) to assess sensory-motor developmental milestones. Adult

subjects were tested in the Elevated Plus Maze, Open Field Test, Novel Object Placement Test, and Spontaneous Alternation Test. Results suggest that corn grown with dredge-amended soil may be altering neurodevelopmental processes that manifest in changes in cognition later in life.

Disclosures: **K.A.S. Flanigan:** A. Employment/Salary (full or part-time):; Ohio Sea Grant Program. **M.I. Czuba:** None. **V.R. Riesgo:** None. **T.N. Vu:** None. **J. Willing:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.14/R7

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01NS102448
VA I01BX003195
PHS K12 GM111726

Title: Increased neurogenesis in the subventricular zone of $kmo^{-/-}$ mice enhances olfactory habituation

Authors: *M. A. DE LA FLOR[1], M. C. BUCKNOR[1], S. J. HARRISON[1], N. KUHN-SANDOVAL[1], D. LOZANO[1], J. C. O'CONNOR[2], E. KOKOVAY[1];
[1]Cell Systems and Anat., [2]Pharmacol., Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX

Abstract: Inhibition of the rate-limiting enzyme for oxidative metabolism of kynurenine, kynurenine 3-mooxygenase (KMO) increases levels of kynurenine (KYN) and the neuroprotective metabolite kynurenic acid (KYNA), whereas upregulation of KMO produces metabolites associated with inflammation, oxidative stress, and the neurotoxic metabolite quinolinic acid (QA). $KMO^{-/-}$ mice exhibit increased levels of KYN and KYNA and reduced levels of QA in the brain. In this study, we show that $KMO^{-/-}$ mice have increased levels of neural stem cell (NSC) proliferation and neuroblast (NB) production in the subventricular zone (SVZ), a specialized stem cell microenvironment that supports adult neurogenesis, and that these neuronal precursors migrate via the Rostral Migratory Stream to the olfactory bulb (OB). We also show that *in vitro* QA significantly reduces NSC proliferation. We hypothesized that increased proliferation in the OB of $KMO^{-/-}$ mice could improve olfactory function and the plasticity involved in olfactory learning. Results from Buried Food Seeking test showed no significant differences between $KMO^{-/-}$ and wild-type mice, indicating normal foraging and olfaction in both groups. Habituation and dishabituation are forms of non-associative learning and memory that regulate the allocation of neural resources and behavioral responses to environmental stimuli. Findings from the Olfactory Habituation and Dishabituation test show that $KMO^{-/-}$ mice were able to habituate and dishabituate to non-social odors significantly better than wild-type controls. However, no significant differences were detected in habituation and

dishabituation with social odors. Collectively, our findings suggest that the increases in NSC and NB proliferation we detected in the $KMO^{-/-}$ SVZ and OB may result in enhanced olfactory function and associated learning and memory.

Disclosures: M.A. de la Flor: None. **M.C. Bucknor:** None. **S.J. Harrison:** None. **N. Kuhn-Sandoval:** None. **D. Lozano:** None. **J.C. O'Connor:** None. **E. Kokovay:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.15/R8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: RF1NS083704

Title: Generation of a myeloid conditional knockout of interleukin-1 receptor accessory protein (IL-1RAP) mouse model

Authors: *S. DADRAS[1], K. BHASKAR[2];
[1]Mol. Genet. & Microbiology, Univ. of New Mexico, Albuquerque,, NM; [2]Mol. Genet. and Microbiology, Univ. of New Mexico, Albuquerque, NM

Abstract: We and others have shown that interleukin-1β (IL-1β) signaling and single nucleotide polymorphism in *il1rap* gene contributes to Alzheimer's disease (AD) as a major trigger of neuroinflammation. IL-1RAP is a coreceptor which binds to IL-1 receptor1 (IL-1R1) and together they form the IL-1 receptor complex. While many studies have targeted IL-1R1 or its complex as a means to block neuroinflammation, despite the association of *il1rap* to AD, there are no specific genetic models to assess cell specific effects of IL-1RAP. Here, we generated transgenic mice expressing floxed alleles of mouse *il1rap* gene, where two loxP sequences are inserted in both sides of the exon 3 of *il1rap*. We validated the presence of floxed allele of *il1rap* via genotyping, PCR analyses on purified DNA and cDNA from isolated RNAs from $Il1rap^{f/f}$ mice. We then crossed these mice to $Cx3cr1^{Cre}$ (expressing Cre recombinase by the *Cx3cr1* promoter, which are expressed in myeloid cells including monocytes, macrophages, and microglia in the CNS) to delete exon 3 of the *il1rap* gene. A group of $Cx3cr1^{Cre}Il1rap^{f/f}$ mice were used to confirm deletion of exon 3 in DNA of these mice and compared $Il1rap^{f/f}$ mice (5 each). C57B6 mice were used as positive control. For this purpose, after sacrificing mice and brain dissection, percoll isolation of microglia and DNA extraction (using TRIzol Reagent) was carried out. We performed PCR amplification using the extracted DNA and primers specific to *il1rap* exon 3. Gel electrophoresis and fluorescent staining was used to visualize bands. PCR amplification of DNA (from tail snips, as control, and isolated microglia) showed a strong 223bp band in the non-transgenic controls and $Il1rap^{f/f}$ mice but only a weak band in the $Cx3cr1^{Cre}Il1rap^{f/f}$ group. The presence of both Cre and LoxP sites in the $Cx3cr1^{Cre}Il1rap^{f/f}$ mice lead to deletion of *il1rap* exon3 in these mice and lack of the 223bp band, confirming the

knockout. The weak band in *Cx3cr1*^{Cre}*Il1rap*$^{f/f}$ group may be due to the presence of DNA from small numbers of contaminating non-myeloid cells (which still has *il1rap* exon 3) in DNA samples from isolated microglia. RNA-based PCR analyses and functional validation via cytokines (IL-1α, IL-1β, IL-33 and IL-36) for which the IL-1RAP acts as a co-receptor are in progress.

Disclosures: S. Dadras: None. **K. Bhaskar:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.16/S1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NanoString Technologies Grant Award

Title: Spaceflight-induced spatial gene expression profiles are attenuated by treatment with the antioxidant BuOE in the mouse brain

Authors: I. KREMSKY[1,2], S. ALI[1], S. STANBOULY[3], S. JUSTINEN[1], S. ROGERS[4], D. SCOVILLE[4], *E. SCHNEIDER[5], Y. LIANG[4], J. CRAPO[6,7], X. MAO[3];
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Abstract: The physical demands of deep space pose a health risk to the central nervous system and have long been a concern when sending humans to space. While little is known about how spaceflight affects transcription spatially in the brain, understanding this has the potential to guide strategies to mitigate the effects of space-flight on the brain. To characterize the differential expression of genes, we performed GeoMx® Digital Spatial Profiling (DSP) on mouse brains subjected to either spaceflight or grounded controls. Four brain regions were selected for DSP: Cortex, Frontal Cortex, Corunu Ammonis 1 (CA), and Dentate Gyrus (DG). Because spaceflight is associated with increased oxidative stress, treatment with antioxidants has emerged as a potential strategy to attenuate the effects of space flight. We therefore treated a subset of the spaceflight and control mice with the superoxide dismutase mimic, MnTnBuOE-2-PyP 5+ (BuOE) for DSP experiments as well. Our analysis revealed hundreds of differentially expressed genes (DEGs) due to spaceflight in all four regions, with CA having the highest number of DEGs. Each brain region showed a distinct transcriptomic response, while some DEGs were common to two or more regions. A pathway analysis revealed distinct pathways sensitive to oxidative stress, as well as metabolic pathways, to be enriched in each of the brain regions. These data represent the first spatial gene expression profiles taken of rodents in space and may help improve our understanding of brain region-specific susceptibility to spaceflight

conditions. Treatment with BuOE reduced the transcriptomic effects of spaceflight at a large number of DEGs, suggesting that BuOE may attenuate oxidative stress-induced brain damage caused by spaceflight.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.17/S2

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01AG078460

Title: Hypertension-related neuroinflammation in the rhesus monkey: a two-kidney-one clip model

Authors: *A. CAPRIGLIONE[1], Y. ZHOU[1], R. ROBINSON[1], K. NIST[2], N. ARINZE[2], D. L. ROSENE[1], R. D. WAINFORD[2], M. MEDALLA[1], T. L. MOORE[1];
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Abstract: Hypertension (HT) affects 1 in 2 adults in the United States each year. In humans, HT has been found to correlate with cognitive decline and impairments in cerebral blood flow. In experimental HT models, brain pathologies such as disrupted blood brain barrier integrity and neuroinflammation in cardio-regulatory centers of the brain, such as the hypothalamic paraventricular nucleus (PVN), have been observed. However, HT-induced inflammation in the prefrontal cortex (PFC) may be driving impairments in executive function. To address this, we developed a **“two kidney - one clip” model of HT** in monkeys that involves unilateral occlusion of one renal artery. This model is well-established in rodents and results in a slow, progressive onset of HT via activation of the renin-angiotensin system to drive HT and neuroinflammation. We have tested this method in three adult rhesus monkeys and observed sustained, elevated

systolic blood pressure (BP) >140mmHg over a 9 to 12-month period. Immunohistochemical analysis was conducted on brain sections from the PFC and hypothalamus from these HT monkeys and age-matched controls, labeling microglia (Iba1, P2RY12, LN3), astrocytes (GFAP), and basement membrane collagen 4 (COLIV). Analysis of these sections revealed significant between-group differences in microglial and vascular markers, but no differences in GFAP, in PFC area 46. Specifically, a two-way ANOVA comparing microglial markers Iba1 and P2RY12 revealed significant between group and laminar differences ($p<0.01$), with HT animals displaying higher expression of these markers in both gray and white matter. COLIV analysis in this region also revealed significant group and laminar differences ($p<0.01$), with greater expression of COLIV, indicative of altered vascular composition, in grey matter of HT compared to control monkeys ($p<0.01$). Together these results suggest that monkeys in this model of HT have significantly increased cortical neuroinflammation and vascular alterations compared to age-matched control monkeys. Preliminary analyses of sections from the hypothalamus that include the PVN, a region responsible for sustained sympathetic outflow in HT, did not reveal significant differences in these markers, though further analysis is being conducted. However, the PFC findings demonstrate a clear link between systemic hypertension and cerebral inflammation, and further validate this **"two kidney - one clip" model of HT** as a model of HT in the rhesus monkey.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.18/S3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NSERC PDF

Title: Effects of chronic gut inflammation on transcriptional programs in limbic brain networks

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Abstract: Chronic inflammatory diseases are frequently comorbid with generalized anxiety disorder, which often persists during periods of inflammatory remission. This suggests functional changes to neural circuits involved in the contextual regulation of fear. Our recent data indicate that chronic gut inflammation evokes contextual fear generalization in mice, which is a hallmark of generalized anxiety. Here, we test how such chronic gut inflammation affects several brain structures in the limbic system, which are thought to be involved in contextual fear. Male

C57Bl/6 mice were exposed to the colitic agent dextran sodium sulfate (DSS) over a 6 week period to induce a chronic relapsing gut inflammation. Mice then performed several tasks to assess anxiety and threat coping. After euthanasia, four regions of the brain were microdissected for gene expression analysis; the anterior cingulate cortex (ACC), CA1 hippocampus, nucleus accumbens (NuACC), and primary motor cortex (M1). The analyses focused on expression of mRNA transcripts related to mitochondrial (mt) dynamics (drp1, mfn2) and function (mt-co1) because of the links between inflammation, mitochondrial dysregulation, and anxiety. The levels of mt mRNA were altered in DSS-treated animals, but the specific pattern of changes was heterogeneous among brain structures. Expression of mt-co1 was reduced in ACC and NuACC of DSS animals, suggesting lower ATP production. In the ACC, increases in drp1, reflective of mitochondrial fission, were correlated with the severity of gut inflammation. The expression of mt-co1 and drp1 in ACC (and CA1) were negatively correlated with exploratory behaviors. Other relationships between mt gene programs and inflammation and behavior emerged when we considered multivariate changes in gene expression via principal component analysis. This analysis revealed distinct clusters for DSS vs control animals in CA1 and NuACC, and the first principle component (PC) was strongly correlated with the severity of inflammation. The same PC for ACC mt gene expression also correlated with severity of gut inflammation. In sum, a 6-week course of gut inflammation increased markers of mitochondrial dysfunction in CA1, ACC, and NuACC, and this was correlated with changes in exploration and threat coping in a region-specific manner. These relationships were not observed for expression in M1. These data therefore suggest that mitochondrial function in limbic brain structures is vulnerable to chronic peripheral inflammation, which may contribute to altered brain function in chronic inflammatory illnesses.

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Poster

PSTR332. Neuroinflammation: Animal Models

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Harry Weaver Neuroscience scholar award form National MS society to PB

Title: Immunomodulatory and remyelinating role of gut microbiota-derived metabolite, Indole-3-lactate in multiple sclerosis

Authors: *S. SINGH, L. JANK, K. GUPTA, D. JOSHI, M. SMITH, J. LEE, A. DHUKHWA, X. CHAMLING, M. KORNBERG, P. CALABRESI, P. BHARGAVA;
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Abstract: Multiple sclerosis (MS) is a multifactorial autoimmune disorder of the central nervous system (CNS) with demyelination, neuroinflammation, and neurodegenerative factors being the major underlying drivers of this disease. Multi-targeted MS therapy, which can suppress the autoimmune response, repair degenerated myelin, and promote remyelination, would be ideal. Some recent studies have suggested that the gut-brain axis and certain metabolites related to it, such as aromatic amino acid metabolites could play a role in the onset and progression of MS. Metabolomic profiling indicates that the circulating level of Indole-3-lactate (ILA) a tryptophan metabolite is reduced in MS patients. This study was designed to investigate the effects of ILA supplementation on the neuroinflammatory cascade and repair/regeneration of myelin in experimental autoimmune encephalomyelitis (EAE) and a cuprizone-induced demyelination model of MS. For mechanistic insights we also tested ILA *in-vitro* on myeloid cells, T-cells-wild type (WT) and myelin-specific, human iPSC derived and murine glia cells. We observed a significant reduction of clinical EAE symptoms after oral ILA supplementation in MOG_{35-55} induced animals, histological and molecular analysis demonstrated reduced T-cell infiltration, reactive gliosis, and demyelination in the spinal cord. In the cuprizone model ILA supplementation promoted remyelination following demyelination induced by cuprizone in the corpus callosum. Additionally, ILA promoted oligodendrocyte (OL) differentiation both in human iPSC-derived PDGFRα/MBP/PLP-triple reporter cell lines and murine Oligodendrocyte precursor cells (OPC). Mechanistically, ILA inhibited the proliferation of T cells, and Th1 and Th17 polarization. In a nutshell, our findings suggest that ILA reduces neuroinflammation and promote repair *in-vivo* and *in-vitro* models of MS. Based on these data ILA may be a promising molecule for MS treatment.

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Poster

PSTR332. Neuroinflammation: Animal Models

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Program #/Poster #: PSTR332.20/S5

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS116704

Title: Role of protease inhibitor 16 in attenuating paclitaxel-induced neuropathic pain

Authors: *M. HAQUE[1], R. GARRITY[1], R. TRINH[1], A. KAVELAARS[1], C. HEIJNEN[1,2], A. SHEPHERD[1];
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Abstract: Role of protease inhibitor 16 in attenuating paclitaxel-induced neuropathic pain

AuthorsMd. Areeful Haque[a], Rachelle Garrity[a], Ronnie Trinh[a], Annemieke Kavelaars[a], Cobi Heijnen[a,b], Andrew Shepherd[a]
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AbstractChemotherapy-induced peripheral neuropathy is one of the prevalent dose- and therapy-limiting side effects of several anti-cancer agents, including paclitaxel (PTX). Although very effective in blocking tumor growth, PTX induces neuropathic pain with numbness, allodynia, tingling, and burning sensations on hands and feet in 60–70% of cancer patients. Pharmacological treatment options are very limited, as there are currently no recommended treatment strategies for effective prevention of PTX-induced neuropathic pain (PINP). Therefore, this study aimed to investigate the role of protease inhibitor 16 (Pi16) in attenuating PINP. Male and female C57BL/6J mice homozygous for the global deletion of Pi16 (Pi16-/-) were used as experimental animals, and C57BL/6J mice were used as wild-type (WT) controls. PTX of 10 mg/kg b.wt. was administered intraperitoneally (i.p.) every other day for two weeks to induce PINP, and normal saline (NS) was administered as a vehicle control. To measure pain-related behavior, paw withdrawal threshold (PWT) was measured at baseline and over time with calibrated Semmes-Weinstein von Frey filaments. The data were presented as the group mean by averaging the PWT obtained for the left and right hindpaws of each mouse. It was evident that baseline mechanical sensitivity was not affected by the genetic deletion of Pi16, and there were no significant differences in PTX-induced mechanical allodynia observed between WT and Pi16-/- mice until day 7 post-PTX injection. Moreover, mechanical allodynia persisted for ≥3 weeks in both male and female WT mice. Pi16-/- mice, however, began recovering on day 9 and returned to pre-injection levels of mechanical sensitivity by day 14 post-PTX injection, which indicates deletion of Pi16 protects against PTX-induced persistent mechanical allodynia in experimental animals and is prospectively a potential target for managing PINP.

Keywords: Protease inhibitor 16; neuropathic pain; chemotherapy-induced peripheral neuropathy; paclitaxel-induced neuropathic pain; allodynia

Conflict of interest The authors declare that they have no known competing interests

Disclosures: M. Haque: None. **R. Garrity:** None. **R. Trinh:** None. **A. Kavelaars:** None. **C. Heijnen:** None. **A. Shepherd:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Ciencia de Frontera CONACYT2023-133
Proyecto Viep 2023
scholarship from CONACYT-Mexico (1173960)

Title: Memory deficits and gastrointestinal non-motor dysfunction in Parkinson's disease model rats with lipopolysaccharide

Authors: *A. GUTIÉRREZ-HERNÁNDEZ[1], I. PARRA[1], F. LUNA[1], V. ALATRISTE-BUENO[1], I. MARTÍNEZ-GARCÍA[1], V. PALAFOX-SANCHEZ[2], L. MENDIETA[1]; [1]Benemérita Univ. Autónoma de Puebla, Puebla, Mexico; [2]Hda Laureles 6529, Inst. Tecnológico y de Estudios Superiores de Monterrey, Monterrey, Mexico

Abstract: The incidence of Parkinson's disease (PD) has increased worldwide to 6.2 million people diagnosed. Progressive neurodegeneration leads to a motor clinical hallmark, however, in recent years a variety of non-motor symptoms (NMS) such as constipation and memory loss have been associated. Studies of patients and animal models of PD have recently observed a bidirectional gut-brain impairment, related to intestinal and behavioral alterations. Previously, we have characterized that the lipopolysaccharide (LPS) unilateral lesion causes the activation of microglia and the selective and progressive decrease of the dopaminergic (DAergic) neurons at the SNpc. The present project aims to investigate the effects of LPS intrastriatal injection on non-motor symptoms such as memory, intestinal activity, and alterations of the gut in rats. The adult male Wistar rats (n=7 per group) were stereotaxically injected with LPS [32 µg/2µL] or not into the left dorsolateral striatum. The behavioral tests were made before LPS-lesion and between 7-8 days post-lesion. We tested the LPS lesion in the cylinder model, so we found that LPS group has motor asymmetry since the percentage of use of both extremities decreases by 30% with respect to the control group. We used the novel object recognition (NOR) test to evaluate the memory, we found that the administration of LPS causes a deficit in working memory in rats, and the discrimination index was negative ($\bar{x}$= - 0.14 ± 0.3) in comparison to the control group. On the other hand, we evaluated the intestinal activity, the 20-min fecal collection was performed, and each rat was assigned to a clean and transparent plastic cage. The number of stool pellets was recorded every 5 min for 20 min. We found that LPS striatal lesion decreased the number of fecal depositions by 50% between the intact group in each 5-minute interval, as well as the fecal pellet output decreased by 60% pellets per 1 hour in the LPS group. To support the possible damage at the intestinal level, we evaluated the portion of the distal colon by hematoxylin and eosin (H&E) staining. We identified changes in the morphology of the mucosa, the lamina propria, and infiltrated cells present in the crypts from the distal colon, which has been associated with an inflammatory process. These findings provide evidence of the critical role of the gut-brain axis during the inflammatory effect by the unilateral LPS model, which impacts the short-term recognition memory and causes constipation of the gut, both important hallmarks in the early EP.

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR332.22/S8

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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ISCIII-Redes de Investigación Cooperativa Orientadas a Resultados en Salud (RICORS), Red de Investigación en Atención Primaria de Adicciones (RIAPAd; grant RD21/0009/0013)

Title: Neuroinflammation and memory impairment induced by high-fat diet are regulated by pleiotrophin and receptor protein tyrosine phosphatase β/ζ

Authors: H. CAÑEQUE-RUFO, T. FONTÁN-BASELGA, M. VICENTE-RODRÍGUEZ, A. ZUCCARO, M. GALÁN-LLARIO, M. RODRÍGUEZ-ZAPATA, M. SÁNCHEZ-ALONSO, J. SEVILLANO, ***E. GRAMAGE**, J. ZAPICO, B. DE PASCUAL-TERESA, M. RAMOS-ÁLVAREZ, G. HERRADON;
Univ. San Pablo CEU, Madrid, Spain

Abstract: Metabolic disorders, such as obesity, are highly related to neurodegenerative diseases through neuroinflammation. Pleiotrophin (PTN) is a cytokine that is upregulated in various neuroinflammatory disorders. PTN is an endogenous inhibitor of Receptor Protein Tyrosine Phosphatase (RPTP) β/ζ. This study aimed to determine the role of PTN/RPTPβ/ζ in the crosstalk between the central nervous system and the periphery in a high-fat diet (HFD)-induced obesity model. To investigate the role of PTN, 3-month-old C57BL/6J wild-type ($Ptn^{+/+}$) and *Ptn* genetically deficient ($Ptn^{-/-}$) mice were fed with chow (standard diet, STD) or HFD (60 kcal% fat) for 6 months. To investigate the role of RPTPβ/ζ, we administered the selective inhibitor of RPTPβ/ζ, MY10, or vehicle as control, every other day while mice were fed with HFD for 3 months. Male $Ptn^{-/-}$ mice fed with HFD exhibited lower body weight and lower increment of body weight than $Ptn^{+/+}$ mice fed with HFD. Moreover, female $Ptn^{-/-}$ mice fed with HFD were fully protected against HFD-induced obesity. In contrast, we observed that MY10-treated mice fed with HFD exhibited a higher increase in body weight, increased food intake and kcal intake/week compared to control mice. Interestingly, we found that $Ptn^{+/+}$ mice suffered long-term memory loss after three months with HFD and short- and long-term memory loss after six months with this diet, as observed in the novel object recognition (NOR) test. In contrast, $Ptn^{-/-}$ male and female mice fed with HFD did not show any sign of memory loss. The cognitive decline observed in $Ptn^{+/+}$ mice with HFD correlated with increased neuroinflammatory markers compared to $Ptn^{-/-}$ mice with the same diet. Pharmacological inhibition of RPTPβ/ζ with MY10 potentiated HFD-induced short- and long-term memory loss. MY10 administration tended to reverse the HFD-induced decrease of GFAPir independently of sex, whereas it differentially modulated microglial responses in a sex-dependent manner. The data demonstrate that *Ptn* deletion protects against HFD-induced obesity and HFD-induced short and long-term memory loss, whereas pharmacological inhibition of RPTPβ/ζ results in the opposite. The data suggest an important role of the PTN/RPTPβ/ζ signaling pathway in the connection between obesity, cognitive decline, and the development of neuroinflammatory processes.

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Poster

PSTR332. Neuroinflammation: Animal Models

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Program #/Poster #: PSTR332.23/S9

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Development of new human models to study the role of complement in neurodegeneration

Authors: *F. PONTARELLI, A. COMER, G. WIRAK, S. NAIR, V. RAJAGOPAL, D. OFENGEIM, T. HAMMOND;
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Abstract: Complement overactivation has emerged as a key mechanism in neurodegenerative disease, and in mouse models of Alzheimer's disease genetic or therapeutic inhibition of the complement pathway reduces synapse loss, prevents neuron death and improves cognition. Despite this data, the mechanisms driving complement-induced neural dysfunction in the human brain are not fully understood. Complement proteins vary substantially between rodent and humans, so we have developed human *in vitro* iPSC models and humanized *in vivo* mouse models to bridge the gap. We show in a human iPSC-derived brain tri-culture system with motor neurons, microglia and astrocytes that complement deposition on neurons disrupts firing as measured by calcium imaging and MEA recordings, and that this effect is caused by complement sub-lytic activation of membrane attack complex (MAC) pores. In addition, complement activation increased human synaptosome engulfment by iPSC microglia, suggesting a dual role for the pathway in disrupting neural connectivity. We also developed a humanized complement mouse model in which human complement-preserved serum is injected into the mouse striatum. We show that serum injection into the mouse brain leads to human complement activation and deposition on mouse neurons. This model will be critical to test human-specific complement inhibitors in the mouse brain, where additional functional readouts can be measured. Modulating complement components or their regulators holds promise for controlling neuroinflammation and preserving neuronal function in neurodegenerative disease, and these models will help pave the way for novel therapeutic development.

Disclosures: **F. Pontarelli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **A. Comer:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **G. Wirak:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Sanofi. **S. Nair:** E. Ownership Interest (stock, stock

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Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.24/S10

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Persistent cognitive deficit despite prompt recovery of well-being behavior following LPS-related neuroinflammation in mice - sequential and temporal cytokine correlates

Authors: S. WAGNER, C. DUCHEMIN-NEVEU, L. PETER, C. ALBAC, F. LAUGA, E. POIRAUD, B. HUYARD, C. PINAULT, *E. ANDRIAMBELOSON;
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Abstract: Neuroinflammation and the associated exaggerated or prolonged cytokine release is believed to be an underlying mechanism of sickness behaviors and cause neuropsychiatric or neurological conditions. In the present work, neuroinflammation was induced in mice by a single intraperitoneal injection of non-sepsis dose of Lipopolysaccharide (0.25 mg/kg LPS). The time course of IL-1β, TNF α and IL-17 expression was assessed in the brain hippocampus. In addition, sickness behaviors were investigated at different timepoints and includes changes in their 1) burrowing performance (surrogate measure of animal's well-being), 2) motor activity in the open-field and 3) spontaneous alternation in the T-maze (to evaluate the cognitive function). In LPS mice the results showed a hippocampal IL-1β release that sharply peaked at 4h and a returned to baseline level by 24h. By 48h (day 2) a raise in TNF α followed and lasted for only about a week. Increase in IL-17 occurred at later timepoints (day 5 post-LPS) but was sustained up to day 21 (3 weeks). Behavioral measures showed a dramatic reduction in burrowing performance of LPS-mice at 2hr post-LPS, followed by a fully recovery by 48h post-LPS. At this timepoint and up to 3 weeks post-LPS, no significant impairment of motor performance of LPS-mice was observed in the open-field. In contrast, cognitive function as assessed by the spontaneous alternation in the T-maze was significantly impaired in LPS-mice as early as day 1 and the deficit remained up to 3 weeks. The above-mentioned burrowing and cognitive deficits were sensitive to anti-inflammatory treatments. The above results highly suggest a sequentially and temporally orchestrated release of brain hippocampal cytokines following LPS administration in mice. These cytokines appear to play a critical role in the genesis and the perpetuation of sickness behavior in mice.

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Poster

PSTR332. Neuroinflammation: Animal Models

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Program #/Poster #: PSTR332.25/T1

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01NS122830
T32NS073548

Title: Deep Machine Learning Using Long Short-Term Memory Network for Gait Analysis of Pain

Authors: *R. RHOADES, C. CHEN, T. DEAKIN, P. TANG, Y. XU;
Anesthesiol. and Perioperative Med., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Chronic pain is a tremendous burden to public health, affecting more than 116 million people in the USA annually. Treatment options are limited, and new analgesics with no or reduced abuse liability are desperately needed. Most of the conventional pain evaluation methods in pre-clinical animal studies rely on supraspinal reflex in response to noxious stimulations and are often subjective to experimenter's biases, which partially account for the failure of many drug candidates in human clinical trials. Objective and high-throughput pain evaluation methods based on non-provoked animal behaviors will facilitate analgesic drug discovery and development. Here, we report a fully automated gait analysis method using the Long Short-Term Memory (LSTM) networks, a deep machine learning algorithm, to detect and diagnose rodents experiencing differing degrees of inflammatory or neuropathic pain. We used a modern digital touch-recording technology with high spatial and temporal resolutions to collect gait data in an open field using the complete Freud's adjuvant (CFA) model of inflammatory pain and spared nerve injury model of neuropathic pain in mice, with naïve animals serving as the control. Gait features are extracted from the touch data to train an LSTM model to differentiate between painful and non-painful conditions. We extracted several features that demonstrate behavioral dichotomies between naïve and CFA mice. These features include the duration of individual steps, distance and latency of segments of a predefined size, the time and distance among four consecutive touches, the time an animal spends in the center of the field versus the periphery, angulation of steps, and speed of the animals' body movement. The model is accurate at predicting painful states from data not previously used in training but is only marginally accurate at predicting non-painful states. Further refinement of the model is currently underway to achieve >90% predictability and reliability. Additional tests of our algorithm include differentiation between animals with and without pain under different pain treatments using

known analgesics. This approach will set a new platform for assessing pain and treatment efficacy of drug candidates.

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Poster

PSTR332. Neuroinflammation: Animal Models

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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the College of Medicine, John G Kulhavi Professorship, Neuroscience Program, E. Malcolm Field and Gary Leo Dunbar endowed Chair in Neuroscience at Central Michigan University.

Title: Administration of Curcumin using PAMAM dendrimers effectively inhibits the emergence of motor deficits in the GFAP-IL 6 mouse model

Authors: *O. SMITH, J. SWIONTEK, J. E. SMITH, A. POUDEL, B. SRINAGESHWAR, A. UPRETY, N. DAY, A. SHARMA, D. SWANSON, G. DUNBAR, J. ROSSIGNOL;
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Abstract: Various diseases in nervous system are associated with neuroinflammation. A recently developed mouse model called GFAP-IL 6 exhibits innate inflammation in the hippocampus and cerebellum due to the upregulated expression of interleukin 6 (IL-6) in astrocytes. The expression of the IL-6 gene in astrocytes leads to a low level of activation through cytokine induction, negatively affecting astrocytes and microglia. Prior studies have suggested that curcumin, a natural compound extracted from turmeric, shows potential treatment for neuroinflammation. Curcumin has demonstrated its ability to effectively reduce the activation of astrocytes, which play a crucial role in neuroinflammatory processes. However, the efficacy of curcumin as a treatment is limited due to its low bioavailability and solubility. To overcome this hindrance our methods used a nanoparticle poly-amino(amine) (PAMAM) with hydroxyl and amine surface functional groups to deliver the curcumin. Encapsulating curcumin into a Generation 4 (G4) 70/30 PAMAM dendrimer containing a (D-Curc). Delivered by intracranial administration directly into the hippocampus and cerebellum of the GFAP-IL 6 mouse model. The effectiveness of D-Curc was evaluated by assessing motor and cognitive function using the accelerated rotarod (accelerod) and water T-maze (WTM) tests, respectively. The initial testing before treatment showed that there were sex-dependent differences between the heterozygous GFAP-IL6 mice (HET) and the wild-type (WT) mice. Female HET mice demonstrated significantly decreased latency to fall when juxtaposed to WT mice($p=0.028$); contrary male

HET mice did not display a significantly substantial latency to fall than WT mice. Following treatment, it was observed that control (HETs) injected with Hanks Balanced Salt Solution (HBSS) and dendrimer alone developed notable motor deficits, whereas those injected with D-Curc did not. WTM performance did not show an observation significance between the HET and WT mice. To our knowledge, this study represents the first use of the WTM and accelerod tests to characterize the effects of curcumin treatment in the GFAP-IL 6 mouse model of neuroinflammation.

Disclosures: O. Smith: None. **J. Swiontek:** None. **J.E. Smith:** None. **A. Poudel:** None. **B. Srinageshwar:** None. **A. Uprety:** None. **N. Day:** None. **A. Sharma:** None. **D. Swanson:** None. **G. Dunbar:** None. **J. Rossignol:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.27/T3

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Spinal cord-tissue derived extracellular vesicles proteomic signature in experimental autoimmune encephalomyelitis

Authors: D. JOSHI, A. KESHARWANI,, D. LADAKIS, L. JANK, T. RYU, M. D. SMITH, C. H. NA, *P. BHARGAVA;
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Abstract: Experimental autoimmune encephalomyelitis (EAE) is a commonly used mouse model to study the pathophysiology of Multiple Sclerosis (MS). Protein abundances are altered in the spinal cord of EAE mice, but have not been studied in spinal cord-derived EVs and could help better understand the disease and identify potential biomarkers. C57BL6 WT mice were injected with MOG emulsion and their spinal cord was isolated at post-injection days 16 (peak) or 25 (chronic). Size exclusion chromatography and ultracentrifugation was used to isolate spinal cord-derived EVs. Using TMT-labeling mass spectrometry EV proteins were quantified. For each pairwise comparison between the three groups, two-sample t-tests were used to determine differentially expressed proteins. Proteins were then ranked on the basis of the product of -log10(p-value) and log2-fold change and pathway enrichment analysis was performed utilizing the GO database. Normalized enrichment scores (NES) were derived for each pathway. Of the 7187 proteins quantified, 2358 were differentially expressed ($p<0.05$) between peak and naïve mice, 2108 between chronic and naïve, and 2183 between peak and chronic phases. Pathways that were upregulated in both peak and chronic models compared to naïve included antigen processing and presentation (peak: NES=1.5, FDR-adjusted p [q]=1.7E-8; chronic: NES=1.8, q=1.1E-10), adaptive immune response(peak: NES=1.5, q=7.4E-18; chronic: NES=1.7, q=1.2E-19) and leukocyte mediated cytotoxicity (peak: NES=1.5, q=1.2E-6; chronic: NES=1.7, q=5.3E-7). Both peak and chronic mice showed downregulation in electron transport chain pathway

(peak: NES=-1.8, q=6E-4; chronic: NES=-2.2, q= 4.7E-6). Peak mice demonstrated an upregulation of humoral immune response compared to both naïve (NES=1.5; q=5.13E-9) and chronic (NES=1.8; q=1.7E-10), as well as an increase in B cell-mediated immunity pathway compared to chronic (NES=1.8; q=6.2E-9). Chronic mice had cell-cell junction assembly (NES=2.1; q=1E-4) and cytoskeleton-dependent cytokinesis (NES=2.1; q=2E-4) proteins upregulated compared to peak group. These results suggest that tissue-derived EV proteomics can identify important processes underlying disease. A prominent role of immune system activation and the presence of mitochondrial dysfunction was found in both the peak and the chronic phase of the EAE model. Interestingly, B cell-mediated and humoral immunity was more prominent in the peak phase. The upregulation of the cytoskeleton-related proteins in the chronic phase could imply a recovery mechanism.

Disclosures: **D. Joshi:** None. **A. Kesharwani,:** None. **D. Ladakis:** None. **L. Jank:** None. **T. Ryu:** None. **M.D. Smith:** None. **C.H. Na:** None. **P. Bhargava:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.28/T4

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: Preventative effects of dexamethasone in lipopolysaccharide induced neuroinflammation

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Sygnature Discovery, Nottingham, United Kingdom

Abstract: Neuroinflammation is an inflammatory response in the central nervous system, mediated by the production of cytokines, pro-inflammatory enzymes by key immune cells, like microglia. Lipopolysaccharide (LPS) is a potent endotoxin used to model aspects of neuroinflammation. Accumulating evidence indicates that neuroinflammation is a common underlying mechanism behind neurodegenerative disorders (e.g. Parkinson's and Alzheimer's disease) and might be key to disease progression. After characterizing the efficacy of two LPS serotypes (055 and 0111) to increase proinflammatory biomarkers in the brain of male C57BL6/J mice 24h post-dose, we have determined the effects of acute LPS administration (4h) on gene expression with and without pre-administration of Dexamethasone (Dexa: 10, 30 or 50 mg/kg, p.o.) on LPS-induced neuroinflammation (055, 0.3 mg/kg, i.p). Dexamethasone was dosed 2h before LPS injection, and at 4h post-LPS injection mice were terminated by a schedule 1 method, a post-mortem blood sample collected, and plasma prepared by centrifugation. Whole brains were collected on dry ice and homogenized with a pestle and mortar in liquid nitrogen. Total RNA was isolated, and cDNA was prepared. Gene expression was performed using TaqMan assays, with RT-PCR performed using the CFX384 RT-PCR detection system. Levels

of IL-1β, IL-6, TNF-α and IFN-γ mRNA were quantified, and values expressed as mean fold change relative to control±SEM; n=10-12 per group. Compared to vehicle, LPS (0.3 mg/kg, i.p.) significantly increased brain mRNA expression levels of IL-1β, IL-6 and TNF-α (vehicle: 1±0.22, LPS: 45.89±15.28, 15.54±5.57, 14.82±2.99, respectively, all $p<0.001$). Oral administration of Dexa (10-50 mg/kg) significantly and dose-dependently reduced the expression of IL-1β compared to LPS-treated mice (Dexa 10: 19.28±5.41, $p<0.05$; 30: 13.52±4.40, $p<0.01$; 50: 9.25±2.04, $p<0.001$). Expression of TNF-α was significantly reduced at the highest dose of Dexa (LPS: 14.82±2.99, Dexa 50: 7.10±1.77, $p<0.05$), with no significant reduction in TNF-α at other doses. Dexamethasone (10-50 mg/kg, p.o.) had no significant effect on LPS-induced elevated levels of IL-6. There was no significant effect of LPS alone or in combination with Dexa on IFNγ expression (vehicle 1±0.31, LPS: 0.51±0.17, Dexa 10: 0.73±0.12; 30: 0.70±0.23; 50: 0.60±0.22). Our data show that LPS administered i.p. is a potent inducer of neuroinflammation by increasing the gene expression of brain proinflammatory cytokines, which Dexa inhibited. This *in vivo* LPS model represents a useful approach for testing the efficacy of novel anti-inflammatory agents against neuroinflammation.

Disclosures: L. Jagger: None. **M. Pearce:** None. **E. Mokori:** None. **N. Mody:** None. **J. Willis:** None. **J. Davies:** None. **R. Brammer:** None. **W. Pijacka:** None. **B. Young:** None. **D. Laughton:** None. **Z. Turnbull:** None. **N.R. Mirza:** None. **J. Unitt:** None.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.29/T5

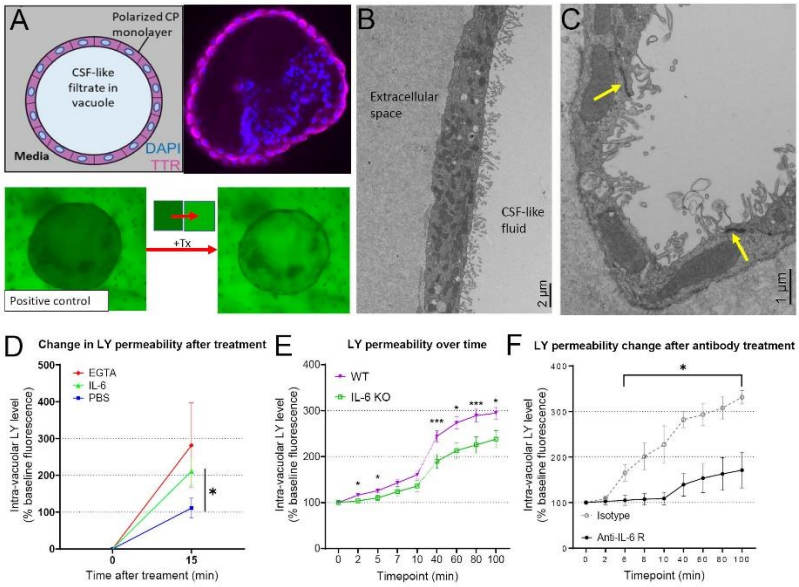
Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: An in-vitro choroid plexus organoid model uncovers the regulation of blood-cerebrospinal fluid barrier permeability by IL-6

Authors: *J. REYNOLDS[1], N. PETERSEN[2], L. TORZ[2], A. BEN-ZVI[3], C. PUTTERMAN[4]; [1]Albert Einstein Col. of Med., Bronx, NY; [2]Global Obesity Res., Novo Nordic, Malov, Denmark; [3]Hebrew Univ. of Jerusalem, Jerusalem, Israel; [4]Azrieli Fac. of Med., Bar-Ilan Univ., Zefat, Israel

Abstract: *In-vivo* study of the blood-CSF barrier (B-CSFB) in disease proves challenging. The choroid plexus (CP) epithelium which forms the barrier resides deep within the ventricular system, and CSF composition, a common readout of barrier leak, also reflects integrity of other brain barriers. *In-vitro* models enable focused interrogation of B-CSFB integrity under inflammatory conditions, such as interleukin-6 (IL-6) exposure. While 2D trans-well formats are widely used, novel 3D organoid approaches can better replicate CP architecture and maximize experimental efficiency. Following a recently published protocol (Petersen et. al, 2020), we generated 2-week-old organoids from CP tissue explanted from adult female IL-6 knockout (KO) and IL-6 competent (WT) MRL/lpr mice. We evaluated IL-6's effect on the accumulation of

lucifer yellow (LY), sodium fluorescein (SF), and 10 kDa dextran (Dex) within their central vacuole (example intra-vacuole color change Fig 1A, bottom). Organoids replicated *in-vivo* CP morphology (Fig 1A-C). IL-6 exposure rapidly increased LY permeability relative to PBS (n=39-40; p=0.014; Fig 1D). Compared to those WT derived, IL-6 KO organoids showed less permeability to LY (n=27-31; p<<0.05; Fig 1E) but no difference in SF or Dex leak (n=19 per genotype; p > 0.5). Anti-IL-6 receptor antibody incubation (n=8) significantly reduced WT organoid LY permeability relative to isotype controls (n=3; Fig 1F). Expression of a*bcg2*, a component of BCRP, was higher in the CP of IL-6 KO mice *in-vivo* compared to WT (n=6 per genotype; p=0.026; Fig 1G). Our study demonstrated that this novel CP organoid platform enables efficient testing of B-CSFB integrity under inflammatory conditions. We found that IL-6 signaling increases CP permeability to LY by potentially altering BCRP function. Thus, neuroinflammatory conditions characterized by high IL-6 expression, including multiple sclerosis and neuropsychiatric lupus, could involve under-explored BCRP dysregulation which hinders the B-CSFB's ability to clear neurotoxic metabolites, contributing to pathologic progression.



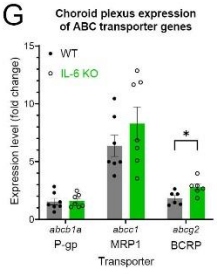


Figure 1. Explant organoid model of mouse choroid plexus (CP) reveals the potential of IL-6 to disrupt the blood-CSF barrier (B-CSFB). 3D spherical organoids were chosen to replicate in-vivo CP physiology in a format easily assayed for differential permeability. For each experiment, CP tissue from 2-5 MRL/lpr mice was pooled then mechanically digested, embedded in surrogate matrix, and cultured in epithelia-selective media for 2 weeks. A) Top: schematic and representative immunofluorescent image of an actual organoid (top). TTR = transthyretin, a canonical CP marker; DAPI = 4′,6-diamidino-2-phenylindole, a nuclear stain. Bottom: A representative permeability experiment wherein fluorescence intensity within the organoid's central vacuole rapidly increases following administration of a permeabilizing agent. B) Electron micrograph demonstrating organoid barrier polarity complete with microvilli and numerous mitochondria. C) Higher resolution electron micrograph showing intact tight junctions (yellow arrows) between neighboring epithelial cells of an organoid. D) Change in permeability to lucifer yellow (LY) tracer over 15 minutes following organoid treatment with positive control EGTA (egtazic acid; tight-junction disruption; n=20), 10 ng/mL IL-6 (n=39), or PBS (n=40; IL-6 vs PBS p= 0.0141). E) To determine if IL-6 is necessary for increased permeability, organoids were generated from either IL-6 knockout (KO) or wildtype (WT) MRL/lpr mice. Permeability to LY was significantly lower in IL-6 KO organoids (n= 31) compared to WT ones (n= 27) at almost all timepoints. F) To determine if IL-6 signaling is responsible for the observed knockout effect on LY permeability, organoids were treated with anti-IL-6 receptor blocking (1 ug/mL; n= 8) or isotype control antibodies (n= 3). All timepoints after 2 minutes showed reduced permeability in the anti-IL-6 receptor antibody treated group. H) Real-time qPCR measurement of ATP-binding cassette (ABC) transporter gene expression within the CP of IL-6 KO or WT MRL/lpr mice to elucidate possible mediators of the IL-6 effect on LY permeability. BCRP expression was significantly increased in IL-6 KO mice (p=0.0259). * p < 0.05, *** p < 0.01.

Disclosures: **J. Reynolds:** None. **N. Petersen:** None. **L. Torz:** None. **A. Ben-Zvi:** None. **C. Putterman:** 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.; Equillium. F. Consulting Fees (e.g., advisory boards); Equillium, Kidneycure, Progentec.

Poster

PSTR332. Neuroinflammation: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR332.30/T6

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R21 AG072327

Title: Characterizing the accumulation of senescent myeloid cells in an experimental model of multiple sclerosis

Authors: *Z. MANAVI[1], P. GROSS[3], J. HU[2], M. COZART[1], G. S. MELCHOR, Jr.[1], M. BAYDYUK[2], J. K. HUANG[2];
[2]Georgetown Univ., [1]Georgetown Univ., Washington, DC; [3]Georgetown Univ. Med. Ctr. Interdisciplinary Program In Neurosci., Georgetown Univ. Interdisciplinary Program In Neurosci., Washington, DC

Abstract: Characterizing the accumulation of senescent myeloid cells in an experimental model of multiple sclerosisAuthors: Manavi Z [1], Gross PS [2], Baydyuk M [1], Hu J, Cozart M [1], Melchor Jr. G [2], Huang JK [1].1 Department of Biology, Laboratory of Neuroinflammation and Glia Biology, Georgetown University, Washington,DC 2 Interdisciplinary Program in Neuroscience, Georgetown University, Washington, DC**Abstract**Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system characterized by a loss of myelinand axonal degeneration. The pathological outcome of MS is mainly known to be driven by both adaptive and innateimmune cells. Early-stage MS is mediated by autoreactive T and B cells, while the later stage of the disease is mediated byactivated microglia and macrophages resulting in chronic demyelination and neurodegeneration. However, the existingdisease-modifying treatments that are effective early in MS have limited efficacy for late-stage MS. Therefore, there is anunmet need for therapies targeting late-stage MS. It has been suggested that the chronic low-grade inflammation in late-stage MS may result in inflammaging and the accumulation of the senescent cells. Cellular senescence, a hallmark of aging,is defined by a permanent cell cycle arrest induced by various stressors such as inflammation and are shown to be damagingto the tissue microenvironment through a senescent-associated secretory phenotype (SASP). However, to date, there havebeen limited studies providing evidence of cellular senescence and their potential role in MS pathogenesis. Here, weperformed MOG35-55-induced experimental autoimmune encephalomyelitis (EAE) in mice, a preclinical model of MS, andobserved a significant increase in senescence markers in EAE spinal cord compared to naïve mice. Using

immunostaining,we further show that while some of these cells are present in the lesion, the majority are localized to the leptomeningesadjacent to the ventral spinal cord demyelinating lesions. Moreover, we found that cells exhibiting senescence markersexpressed myeloid (CD11b) lineage markers in the spinal cord of mice with EAE. Together, our findings demonstrate thatsenescent-like immune cells in EAE accumulate in the meningeal compartment and may contribute to neuroinflammationand demyelination. This further suggests that pharmacological clearance of senescent cells using anti-inflammaging orsenolytic therapy may be beneficial in preventing or delaying disease progression.

Disclosures: Z. Manavi: None. **P. Gross:** None. **J. Hu:** None. **M. Cozart:** None. **G.S. Melchor:** None. **M. Baydyuk:** None. **J.K. Huang:** None.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR333.01/T7

Topic: C.08. Ischemia

Support: FAPERJ Grant E26.0003/015529/2021
CAPES/Fellowship to GCM
FAPERJ/Fellowship to DLBS
PR2-UERJ/Fellowship to ACSL
CNPq/Fellowship to ACR

Title: Physical conditioning during pregnancy reduces oxidative stress caused by prenatal hypoxia ischemia in rats.

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[1]Pharmacol. and Psychobiology, IBRAG/UERJ, Rio de Janeiro, Brazil; [2]Inst. Federal de Educação, Ciência e Tecnologia - Rio de Janeiro, Rio de Janeiro, Brazil

Abstract: Prenatal hypoxia-ischemia (HI) is a major cause of death and disabilities. HI events result in an inflammatory response and imbalance of the antioxidant system, generating oxidative stress (OxS), both harmful to the brain. We evaluated whether maternal physical conditioning (PC) can reduce OxS in the cerebellum of offspring submitted to a rat model of HI. All procedures were approved (CEUA UERJ 027/2021). A progressive swimming training was applied as PC protocol to pregnant rats (TR group). At 18 days of gestation (E18), the pregnant rats (TR or sedentary - SD) underwent HI surgical procedure (HI group) or not (SHAM group - SH). The study comprises 4 experimental groups (SDSH; SDHI; TRSH; TRHI; n=4 litters/group). The effects of HI and PC were evaluated through Thiobarbituric Acid Reactive Species (TBARs) levels and enzymatic assays for the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in the offspring at E19 and at 2 postnatal days (P2). Data were

expressed as means ± standard error of the means, in nMol/mg. Significant differences in TBARS measures were restricted to E19. HI induced a higher TBARS index (SDHI=0.00305 ± 0.00020 vs SDSH=0.00247 ± 0.00024, $p<0.005$) whereas the remaining groups did not show differences in relation to the SDSH. CAT activity had an increase at E19 in the HI groups (SDSH=0.00009 ± 0.00001, SDHI=0.00015 ± 0.00001 and TRHI=0.00015 ± 0.00002, $p<0.005$). In P2, HI reduced CAT activity (SDHI=0.00003 ± 0.000002 vs. SDSH=0.00016 ± 0.00002), which was attenuated by PC (TRHI=0.00007 ± 0.00001, $p<0.005$). SOD activity was reduced at E19 in the SDHI group (SDSH=5017 ± 155.7 vs SDHI=2605 ± 357.4, $p<0.05$], and PC increased it (TRSH=6598 ± 1122; TRHI=7189 ± 744.9, $p<0.001$). SOD activity remained reduced at P2 in the SDHI group (SDSH=5443 ± 533.4 vs SDHI=3748 nMol/mg ± 279.4, $p<0.05$) and PC restored SOD activity since there was no significant difference between TRHI (5885 nMol/mg ± 482.1) and SDSH groups. Immunohistochemistry for isoprostane and oligodendrocytes (CNP) was performed at P9. SDHI showed a huge increase in isoprostane staining (SDSH=5.36 ± 2.449 vs SDHI=19.41 ± 9.913, $p<0.0001$). In TRHI (10.16 ± 3.517), it was possible to observe a reduction ($p<0.002$) of isoprostane. Immunostaining revealed cell bodies double labeled with isoprostane and CNP, indicating that oligodendroglia suffered OxS. Our results showed that PC reduces OxS, improving the post-HI antioxidant response and reinforce the importance of this model in the study of injuries triggered by HI.

Disclosures: **A.C.S. Lopes:** None. **G.M. de Oliveira:** None. **D.L.B. Silva:** None. **A.C. Resende:** None. **V.Y. Rapozo:** None. **P.C. Barradas:** None.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR333.02/T8

Topic: C.08. Ischemia

Support: NIH/NINDS R01 NS111021
NIH/NINDS K08 NS088563
NIH Waisman Core Grant P50HD105353.

Title: Role of membrane estrogen receptor alpha in TrkB mediated female specific neuroprotection following neonatal hypoxia ischemia

Authors: N. CAGATAY[1], **O. TAPARLI**[2], T. SHEIKH[1], E. BICKI[1], S. YAPICI[1], A. SKRAPITS[1], C. LAGOA-MIGUEL[1], F. CAMCI[1], F. CETIN[1], P. FERRAZZANO[2], J. E. LEVINE[3], *P. CENGIZ[1];
[2]Pediatrics, [3]Neurosci., [1]Univ. of Wisconsin-Madison, Madison, WI

Abstract: Background: Neonatal hypoxia ischemia (HI)-related brain injury leads to learning and memory deficits in children. We have previously shown that tyrosine kinase B (TrkB)-mediated neuroprotection is estrogen receptor alpha (ERa)-dependent and female-biased in the

hippocampus following neonatal HI. In this study, we hypothesized that ERa-dependent and TrkB-mediated female biased long-term neuroprotection is regulated by membrane ERa. In order to test this hypothesis we utilized ERa wild type (WT), ERa complete knockout (ERaKO), and nuclear-only ERa (NOERa) mice to determine downstream signaling pathways of TrkB and long-term learning/memory following sham and HI surgery.**Methods**: Postnatal day (P) 9 male and female WT, ERKO, and NOERa mice were exposed to either sham or HI surgery. Starting from 10 min of HI or sham surgery, mice received either vehicle control or the TrkB agonist, 7,8-dihydroxyflavone (7,8-DHF), daily for three days or seven days for subsequent behavioral testing and imaging. At P12, hippocampal AKT1/3, ERK1/2 mRNA and protein expressions were determined as well as total TrkB (f-TrkB, t-TrkB) and actin. At P90+, EPM testing was performed followed by T2 weighted brain MRI (4.7T). Percent hippocampal and hemispheric volume losses were measured using ITK-SNAP. ANOVA was used to analyze multi-group comparisons.**Results**: Our results shows that; 1) HI increases hippocampal AKT1 mRNA in both WT male and females compared to sham 3 days post HI. 7,8-DHF increases the AKT1 mRNA expression further in the female hippocampi post-HI ($p=0.0003$). This upregulation of hippocampal AKT1 mRNA in females is eliminated in ERaKO and NOERa mice, 2) HI decreases AKT3 mRNA in both WT male and female hippocampi compared to sham 3 days post HI. 7,8-DHF further decreases the hippocampal AKT3 mRNA expression only in the female hippocampi post-HI. This downregulation of hippocampal AKT3 mRNA is eliminated in ERaKO and NOERa mice, 3) HI increases hippocampal truncated TrkB (t-TrkB) protein in both WT male and female mice. 7,8-DHF decreases t-TrkB/actin protein expression only in the female hippocampus post-HI ($p=0.01$). 4) WT female mice spend statistically significantly more time in the open arm at 5-10 min segment of the test post-HI ($p=0.0009$) that is recovered by the 7,8-DHF therapy in an ERa dependent way ($p=0.05$). In addition, male mice demonstrate the opposite of female behavior post-HI and post-therapy. 5) The % time spent in the open arm positively correlates with the hemispheric and hippocampal injury ($P<0.001$).**Conclusion**: Our results show that female-biased ERa-dependent and TrkB-mediated neuroprotection is dependent upon activation of membrane associated ERa.

Disclosures: **N. Cagatay:** None. **O. Taparli:** None. **T. Sheikh:** None. **E. Bicki:** None. **S. Yapici:** None. **A. Skrapits:** None. **C. Lagoa-Miguel:** None. **F. Camci:** None. **F. Cetin:** None. **P. Ferrazzano:** None. **J.E. Levine:** None. **P. Cengiz:** None.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR333.03/T9

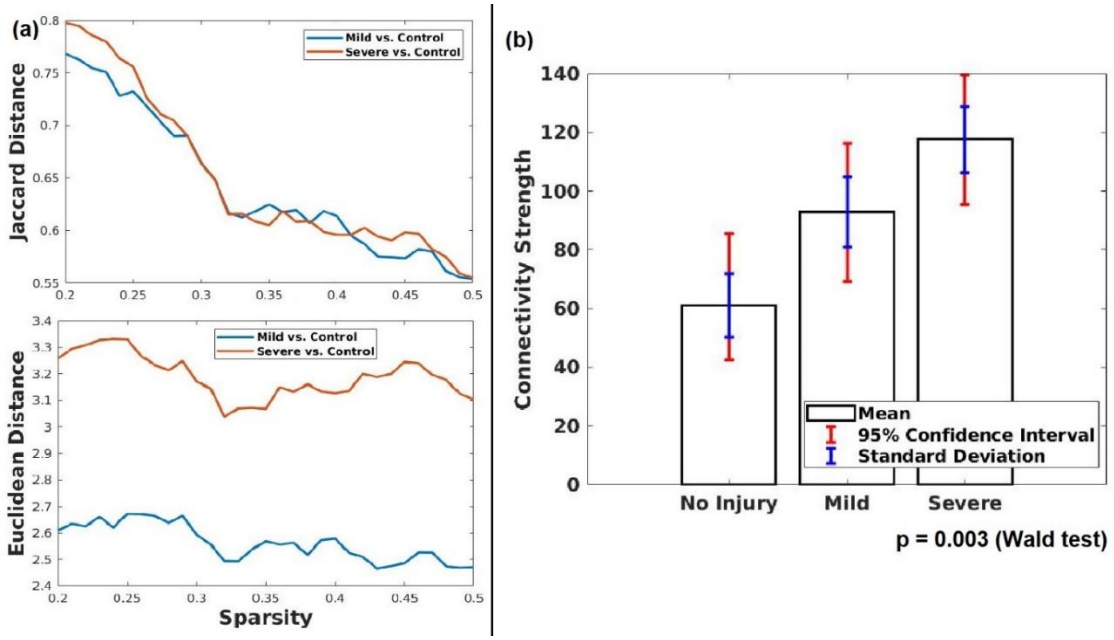
Topic: C.08. Ischemia

Support: CIHR

Title: Altered resting-state functional connectivity in neonates with intraventricular hemorrhage.

Authors: *L. TANG[1], L. M. N. KEBAYA[2], T. ALTAMIMI[2], A. KOWALCZYK[2], M. S. MUSABI[2], S. ROYCHAUDHURI[2], H. VAHIDI[3], P. MEYERINK[2], P. C. MAYORGA[2], S. DE RIBAUPIERRE[4], S. BHATTACHARYA[2], K. ST. LAWRENCE[5], E. G. DUERDEN[6];
[1]Sch. of Biomed. Engin., [2]Neonatal - Perinatal Med., [3]Neurosci., [4]Clin. Neurolog. Sci., [5]Med. Biophysics, [6]Applied Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Intraventricular hemorrhage (IVH) is a major complication following preterm birth. IVH can lead to mortality or long-term motor and cognitive deficiencies. Functional near-infrared spectroscopy (fNIRS) is a brain imaging modality that measures cerebral hemodynamics and has great accessibility. fNIRS can be a new bedside monitoring tool to image brain function in neonates with brain injury, through resting-state functional connectivity (RSFC) derived from fNIRS data. In this study, we compared RSFC maps between neonates with IVH and healthy controls. And we associated the severity of injury with RSFC patterns.fNIRS system was set up with 20 channels, covering the whole head. All neonates, IVH or healthy control, had a 6-min resting-state scan. IVH group included 16 preterm born neonates (gestational age [GA] at birth < 32 weeks, GA at scan = 37.04±0.96 weeks) who were categorized into mild or severe group based on severity of their injuries. 15 healthy term-born neonates (GA at birth = 38.92±1.30 weeks) were also recruited. Cerebral hemodynamics was derived from fNIRS data from each channel then correlated with each other to create a connectivity map for each neonate. We measured the similarity of RSFC maps with mild against no injury, and severe against no injury groups, respectively, by calculating the Euclidean and Jaccard distances. We then assessed the severity of IVH with connectivity strength using generalized linear models. Results showed that mild IVH group overlapped more with healthy control than severe group at different levels of sparsity (Fig 1a). This indicates that functional organization of brain is less disrupted within mild-injury group. Connectivity strength increased with severity of injury (Fig 1b). This indicates that RSFC derived from fNIRS can be a potential marker for severity of IVH.In conclusion, fNIRS revealed differential RSFC patterns between groups of neonates with IVH and healthy controls. We showed that fNIRS can potentially be a new tool for assessing early brain injury and monitoring cerebral hemodynamics of newborns.



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Poster

PSTR333. Mechanisms of Perinatal Ischemia

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR333.04/T10

Topic: C.08. Ischemia

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Title: Neuroprotective mechanisms of hypothermia by maintaining astrocytic erythropoietin production.

Authors: *K. TORIUCHI[1], H. KAKITA[1,2], H. AOKI[1], T. TAMURA[3], S. TAKESHITA[1,2], Y. YAMADA[2], M. AOYAMA[1];
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Abstract: Hypoxic-ischemic encephalopathy (HIE) involves severe neurological deficits, including cerebral palsy. Therapeutic hypothermia has been shown to provide neuroprotection in infants with HIE; however, the cellular mechanisms underlying the neuroprotective effect of therapeutic hypothermia have not been fully elucidated. Astrocytic erythropoietin (EPO) is a key neuroprotective mediator under hypoxic conditions. In the present study, we investigated whether hypothermia attenuates neuronal damage via the release of astrocytic EPO. Hypoxic-ischemic brain injury (HI) was induced in 7-day-old Wistar rats by left common carotid artery occlusion followed by 1 h of hypoxia (8% O2). Hypothermic treatment (33.5°C) significantly reduced the infarct volume. In HI brains treated with hypothermia, EPO mRNA expression was higher than that in the normothermic group. Rat cortical astrocytes were cultured under oxygen/glucose deprivation (OGD) conditions as a model of severe HIE. After OGD, astrocytes were cultured under normothermic (37°C) or hypothermic (33.5°C) conditions. OGD induced EPO expression, although at lower levels than hypoxia alone. Hypoxia inducible factor (HIF)-2α

protein expression was lower under OGD than hypoxia alone. EPO expression after OGD was significantly higher under hypothermia. Moreover, the expression of HIF-1α and HIF-2α proteins was enhanced under hypothermia. In the presence of astrocyte conditioned medium (ACM) derived from hypothermic astrocytes following OGD, neuronal apoptosis was suppressed. Finally, blockade of EPO signaling with an anti-EPO neutralizing antibody attenuated the anti-apoptotic effect of ACM derived from hypothermic astrocytes following OGD. In conclusion, hypothermia after ischemic insult stabilized HIF signaling in astrocytes, and upregulated EPO expression could suppress neuronal apoptosis. Investigating the neuroprotective effects of EPO secreted by astrocytes under hypothermic conditions could contribute to the development of novel neuroprotective therapies for HIE.

Disclosures: K. Toriuchi: None. **H. Kakita:** None. **H. Aoki:** None. **T. Tamura:** None. **S. Takeshita:** None. **Y. Yamada:** None. **M. Aoyama:** None.

Poster

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Topic: C.08. Ischemia

Support: PS1 UAI-
COFECYT
FONCYT #3457

Title: Neuroactive steroids as neuroprotective agents against the neurodegenerative effects induced by hypoxia-ischemic brain injury

Authors: N. TORO-URREGO[1], J. P. LUCAES[2], T. KOBIEC[3], S. BORDET[4], C. MELONI[2], M. V. DAMBROSIO ANDRADE[5], C. MARDARAZ[2], C. KUSNIER[2], *F. CAPANI[1]; [1]CAECHIS-CONICET, Buenos Aires, Argentina; [2]CAECHIS-CONICET, CABA, Argentina; [3]CIPP_UCA, CABA, Argentina; [4]CIPP_UCA, CABA, Armenia; [5]Facultad de Medicina, Fundación H. A. Barceló. Buenos Aires, Argentina, CABA, Argentina

Abstract: Hypoxic-ischemic brain injury is one major cause of long-term neurologic disability, morbidity, and death worldwide in adults and children. The following decrease in tissue blood flow and oxygen concentration results in insufficient nutrient supply to the brain, energy depletion, increased free radical generation, and inflammation. In different pathological scenarios, selective estrogen receptor modulators (SERMs) exert several neuroprotective effects. These include a decrease in reactive oxygen species and mitochondrial survival, posing these neuroactive steroids as promising molecules for improving brain response to injury. In this study, T98G cells were seeded on 24-well plates, 10000 cells/well, in a DMEM culture medium containing 10% FBS and incubated for 2-3 days. When cell confluence was reached, the medium was changed to glucose-free DMEM, and the cells were incubated in 1% O_2 in a hypoxia

incubator for 9 hours. Then, reperfusion was driven by changing the media to high-glucose DMEM supplemented with 10% FBS and transferring the cells to 37°C in 95% O_2/5% CO_2. For drug treatments, cell cultures were incubated in DMEM serum-free medium containing 10 and 100 nM Raloxifene and Tibolone, as cotreatment of OGD and reperfusion. Mitochondrial mass and membrane potential were determined using Nonyl acridine orange (NAO) and Tetramethyl Rhodamine Methyl Ester (TMRM) respectively, DIC and fluorescence images were acquired using a NIKON - Eclipse Ti-E PFS microscope, and cellular fluorescence was analyzed with Fiji. Cotreatment with 100 and 10 nM raloxifene and 100 nM tibolone increased cell viability by 65.34% (p= 0.0021), 70.56% (p <0.0001), and 66.49% (p= 0.0013). Mitochondrial mass was preserved by cotreatment with Raloxifene 100 nM (p= 0,0178) and 10 nM (p=0.0014) related to the effect on lipid peroxidation. Tibolone 10 nM (p= 0.0137); 100 nM (p=0,0007) and raloxifene 10 nM (p=0,0180) cotreatment preserved mitochondrial membrane potential in cells exposed to OGD/reperfusion Our results suggest that raloxifene and tibolone exert protective effects in human T98G glial cells exposed to OGD/reperfusion. These effects likely involve decreased lipid peroxidation with preserved mitochondrial membrane potential and cell viability acting in concert to neutralize cell damage in our model.

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Poster

PSTR333. Mechanisms of Perinatal Ischemia

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Topic: C.08. Ischemia

Support: NIH Grant K12EY015398 (JLD)
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Title: Activation of cellular iron sequestration response in microglia/macrophages in a mouse model of neonatal hypoxic ischemic brain injury.

Authors: *J. VITHAYATHIL[1], F. E. JENSEN[2], D. M. TALOS[2], J. L. DUNAIEF[3];
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Abstract: *Background:* Iron deposition has previously been identified in areas of perinatal focal ischemic brain injury, but it is unclear which cell types take up iron in the brain following injury. Recent data has shown that ferroptosis may play a role in neonatal hypoxic ischemic brain injury (HIBI). Evaluating the mechanisms of cellular iron sequestration in the brain will help identify which cell types may be more susceptible to iron mediated forms of oxidative stress and cell

death.
Methods: Postnatal day (PND) 9 pups underwent unilateral hypoxic-ischemic brain injury (HIBI) using a modified Vannucci model with right common carotid ligation followed by 40 minutes of hypoxia in 8% oxygen (control animals underwent sham surgery and no hypoxia). Animals were euthanized at PND10, PND 12 and PND90 and brain dissected for histology and immunofluorescence. Tissue sections were co-labelled with L-ferritin and cell specific markers for microglia/macrophages (Iba1), neurons (NeuN), astrocytes (GFAP) and oligodendrocytes (Olig2). CD11b+ microglia/macrophages from injured and sham hippocampi were isolated by fluorescent activated cell sorting and genes that regulate iron import (transferrin receptor), storage (L-ferritin) and export (Ferroportin1) were analyzed by real-time PCR.
Results: Immunofluorescent labeling for L-ferritin, a surrogate marker for intracellular iron content, showed elevated L-ferritin immunoreactivity 24h and 72h post-injury compared to sham control animals with preferential expression in Iba1+ cells compared to other cell types (n=6 per group). At 72h post injury, evaluation of iron regulatory proteins via RT-PCR showed that microglia/macrophages from the injured hippocampus upregulate L-ferritin (38% increase, $p<0.05$), downregulate transferrin receptor (78% decrease, $p<0.05$) and downregulate Ferroportin1 (44% decrease, $p<0.05$) when compared to microglia/macrophages from the control hippocampus (n=3-5 per group).
Conclusions: Following neonatal hypoxic-ischemic injury, Iba1+ microglia/macrophages activate a cellular iron sequestration response characterized by upregulation of L-ferritin and down-regulation of transferrin receptor and ferroportin.

Disclosures: J. Vithayathil: None. **F.E. Jensen:** None. **D.M. Talos:** None. **J.L. Dunaief:** None.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

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Program #/Poster #: PSTR333.07/U2

Topic: C.08. Ischemia

Support: NIH DSPAN Grant: 5K00NS105220-04
NIH: Medical University of South Carolina COBRE in Stroke Recovery Research Pilot Project Program

Title: Intranasal Administration of BDNF Improves Cognitive Recovery and Promotes Neuroplasticity in a Neonatal Mouse Model of Hypoxic Ischemia

Authors: *S.-K. SIMS[1], M. SADDOW[1], L. MCGONEGAL[1], C. S. ROBINSON[2];
[1]Biol., Col. of Charleston, Charleston, SC; [2]Med. Univ. of South Carolina, Med. Univ. of South Carolina, Charleston, SC

Abstract: INTRODUCTION: Neonatal stroke, which occurs as frequently as 1 in every 2,500 live births is caused by oxygen deprivation to the brain. Previous studies reported that intravenous administration of brain derived neurotrophic factor (BDNF) in the acute post-stroke period reduces cell death and infarct volume in preclinical stroke model in rats. Hence, while neurotrophic factors induce neuroprotection, the large neurotrophic protein molecules do not efficiently cross the blood brain barrier. Here, there is a need to evaluate potential strategies to increase neurotrophic factors in the brain. Hence, there is a need to evaluate potential strategies to increase neurotrophic factors in the brain. Intranasal delivery is attractive in that it is non-invasive and bypasses the blood brain barrier with minimal side effects. Intranasal administration of BDNF lead to widespread distribution of BDNF within both the brain and spinal cord. Our overall hypothesis is that intranasal BDNF will improve functional recovery including overall brain health and development following neonatal stroke. We explored the benefit of intranasal BDNF treatment on functional recovery in a neonatal P7 hypoxic mouse model.METHODS: For our model of hypoxic ischemic, a ligation of the right carotid artery was induced which is followed by a two-hour exposure to an 8% oxygen/ 92% nitrogen in an enclosed chamber. Male and female Black 6 (B6) mouse pups were subjected to a 2 h hypothermia in a temperature-controlled chamber as a standard of care. A solution of saline (control) or recombinant human BDNF (Harlan Laboratories .1uM in saline) was administered with a Gilson pipette at the same time each day for 7 days into each nasal cavity in awake mice. We evaluated cognitive recovery using novel tactile recognition (NTR) and novel object recognition (NOR) and western analysis to analyze neuro-markers and brain health such as synaptophysin and microtubule associated protein -2 (MAP2).RESULTS: The objective of these studies is to address these gaps in knowledge and evaluate the role and therapeutic potential of BDNF in neonatal stroke recovery. Our results suggest a differential impact of intranasal BDNF on pro and mature BDNF in cortical and hippocampal brain regions, which correlate with cognitive and motor outcomes. Our results suggest that higher levels of mature BDNF are predictive of better improvements at day 42 on cognitive and motor assessments. Our results also suggest that greater cognitive improvement correlated with higher levels of synaptophysin and MAP2 which suggests greater neuroplasticity after injury.

Disclosures: **S. Sims:** None. **M. Saddow:** None. **L. McGonegal:** None. **C.S. Robinson:** None.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

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Program #/Poster #: PSTR333.08/U3

Topic: C.08. Ischemia

Support: NIH 1R21NS123814
NIH 1RO1HD110091

Title: Cholinergic Interneuron Denervation in the Entorhinal Cortex Occurs with Neonatal Hypoxic-Ischemic Injury but not with APP/PS1 Mutation

Authors: B. SOLLINGER[1], *O. HATCHER[1], A. CAVANAGH[1], L. DOUCETTE[1], N. KUTER[1], K. CARLIN[6], V. TURNBILL[2], D. FLOCK[1], S. ROBINSON[3], L. L. JANTZIE[7], R. CHAVEZ-VALDEZ[4], L. J. MARTIN[5], F. J. NORTHINGTON[8];
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Abstract: ***Background*** Genotype may contribute to variable clinical outcomes of neonatal hypoxic-ischemic (nHI) brain injury. nHI injury survivors and adults with Alzheimer's disease (AD) experience learning and memory deficits. Transgenic (Tg) mouse models with mutant genotypes seen in AD, including human amyloid precursor protein (APP) and presenilin (PS1) mutations, exhibit cortical cholinergic denervation, accumulation of neuritic clusters (NCs), Aβ proteinopathy, and adult cognitive impairment. Neural networks within the cholinergic septal-Hippocampus (Hip) and entorhinal cortex (EC) -Hip systems are necessary for learning and memory and are damaged in AD and nHI injury. We hypothesized that nHI exacerbates cholinergic degenerative pathology in the EC and Hip of Tg APP/PS1 mice.
Objective To determine the effect of nHI injury in Tg APP/PS1 mice on the presence of pathologic markers in the EC and Hip including cholinergic interneuron (IN) abnormalities, NC accumulation and Aβ proteinopathy.
Methods C57Bl6 mice, with or without Tg APP/PS1 (n=28), were subjected to the modified Rice Vannucci model of HI at postnatal day 10; sham Tg and non-Tg littermates exposed to anesthesia. Mice were perfused at 6-9 months. Immunohistochemistry was performed on 50μm, sagittal sections to visualize Aβ plaques (6E10 antibody) and cholinergic acetyl-transferase positive NC and IN in EC-Hip. Counts were collected and IN analyzed to quantify degree of injury. Soma size was determined using ImageJ software. Abnormal INs were defined by loss of processes and abnormal somas. Normal IN have normal soma and >2 processes.
Results In Tg and non-Tg groups, nHI injury resulted in more abnormal INs in the EC ($p=0.039$, $p=0.031$) while genotype had no effect. IN soma size was decreased in both groups after HI ($p=0.006$, $p=0.031$). Only Tg mice had Aβ deposition and NCs. There was no increased Aβ plaque or NC burden in Tg mice with nHI in the EC-Hip, however there was an association between % abnormal IN and NC ($r^2 = 0.439$, $p=0.038$) in the EC. There was no relationship between plaque burden and either NC or IN counts in the EC and Hip.
Conclusion Early life HI results in cholinergic pathology in the EC with a decrease in soma size and loss of cholinergic IN. The formation of NCs suggest presynaptic pathology, and axonal attrition suggests postsynaptic pathology. APP/PS1 transgenes do not worsen this pathology. In this model, Hip does not display significant cholinergic neurodegeneration after HI. HI appears to drive cholinergic pathology in the EC. The effects of combined pre and post synaptic cholinergic pathology in the EC of APP/PS1 mice after nHI require further testing to determine functional significance.

Disclosures: **B. Sollinger:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **O. Hatcher:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **A. Cavanagh:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **L. Doucette:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **N. Kuter:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **K. Carlin:** None. **V. Turnbill:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **D. Flock:** A.

Employment/Salary (full or part-time):; Johns Hopkins University. **S. Robinson:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **L.L. Jantzie:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **R. Chavez-Valdez:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **L.J. Martin:** A. Employment/Salary (full or part-time):; Johns Hopkins University. **F.J. Northington:** A. Employment/Salary (full or part-time):; Johns Hopkins University.

Poster

PSTR333. Mechanisms of Perinatal Ischemia

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Centro 2020 Regional Operational Programme (CENTRO-01-0145-FEDER-000008: BRAINHEALTH2020)

Title: Euterpe oleracea Mart. (açaí) seed hydroalcoholic extract treatment in a model of prenatal hypoxia-ischemia in rats: different effects on retinal ganglion cells and microglia of male and female offspring

Authors: L. O. C. BOTELHO[1], L. S. FONSECA[1], ***M. C. CUNHA-RODRIGUES**[1], J. MARTINS[2,3,6], R. BOIA[2,3,6], A. F. AMBRÓSIO[2,3,6,4], A. R. SANTIAGO[2,3,6,4,5], A. C. RESENDE[1], R. S. DE MOURA[1], P. C. BARRADAS[1];
[1]Dept. of Psychobiology and Pharmacol., State Univ. of Rio De Janeiro, Rio De Janeiro, Brazil; [2]Coimbra Inst. for Clin. and Biomed. Res. (iCBR), Fac. of Med., [3]Ctr. for Innovative Biomedicine and Biotech. (CIBB), [4]Assn. for Innovation and Biomed. Res. on Light and Image, [5]Inst. of Immunology, Fac. of Med., Univ. of Coimbra, Coimbra, Portugal; [6]Clin. Academic Ctr. of Coimbra (CACC), Coimbra, Portugal

Abstract: Prenatal hypoxia-ischemia (HI) is associated with a high neonatal mortality rate and long-term neurological sequelae. Due to its high demand for oxygen, the retina is mainly affected by HI. The extract of Euterpe oleracea seed (ASE) has been studied as a potential antioxidant and anti-inflammatory agent. We evaluated the effects of ASE on retinal microglia, astrocytes, and ganglion cells (RGCs) in a prenatal rat model of HI. All the procedures and treatments were approved (CEUA UERJ 025/2021). Wistar rats were administered ASE (200 mg/Kg in drinking water) or vehicle (VE) for 10 days after the 17th gestation day (G17). At G18, the uterine arteries were clamped for 45 min (HI group). Sham-operated (SH) subjects were also generated. This

work comprised 5 experimental groups: SHVE, HIVE, SHASE, HIASE, and non-handled animals (NM). Data were analyzed using univariate ANOVA. Procedure (NM/SH/HI) and treatment (VE/ASE) were set as fixed factors and interaction differences were further tested through one-way ANOVA. Tukey posthoc test was applied for pairwise comparisons. At P30, Brn3a immunostaining revealed that HI significantly decreased the number of RGCs in male animals in relation to SH and NM groups (NM: 47.53 ± 2.85; SHVE: 40.96 ± 2.58; SHASE: 44.26 ± 4.71; HIVE: 31.18 ± 5.28; HIASE: 33.94 ± 3.81; in cell bodies/1000µm; $p<0.05$; n = 5-6/group), without alterations in females. ASE treatment had no significant effect in relation to the number of RGCs. Microglia and astrocytes were identified with anti-Iba1 and anti-GFAP antibodies, respectively. The pattern of Iba1+ cells distribution and morphology was different according to sex and surgical procedure. Also, the number of microglial cells in females was higher in the untreated HI group compared to the untreated SH and NM (NM: 20.23 ± 1.34; SHVE: 17.41 ± 1.38; HIVE: 26.17 ± 3.58; in cell bodies/1000µm; $p<0.05$; n= 4-5/group). In males, no statistical difference was found (NM: 19.99 ± 0.80; SHVE: 20.21 ± 0.79; SHASE: 19.83 ± 1.72; HIVE: 21.14 ± 1.99; HIASE: 21.50 ± 2.17; in cell bodies/1000um; n = 5-7/group). After HI, it was possible to observe that both sexes presented microglia with amoeboid morphology, characteristic of microglial activation. No difference was found in the density of GFAP immunoreactivity (Males: NM: 1.41 ± 0.23; SHVE: 1.89 ± 0.32; SHASE: 1.59 ± 0.38; HIVE: 1.74 ± 0.27; HIASE: 1.67 ± 0.33; Females: NM: 1.66 ± 0.24; SHVE: 1.29 ± 0.27; SHASE: 1.59 ± 0.22; HIVE: 2.06 ± 0.43; HIASE: 1.42 ± 0.25; in optical density %; n= 3-6/group). Our results demonstrate that HI differently affects the retina of male and female subjects and that ASE did not prevent the alterations in microglial cell number and RGCs survival.

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Poster

PSTR333. Mechanisms of Perinatal Ischemia

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR333.10/U5

Topic: C.08. Ischemia

Support: NIH Grant R01 NS113921

Title: Identification of a Novel Brain Injury Response Involving Cholecystokinin (CCK) Degranulation in Cerebral Cortex of a Translationally-Relevant Neonatal Porcine Model of Hypoxic-ischemia

Authors: *D. PARK, J. K. LEE, L. J. MARTIN;
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Abstract: Hypoxic-ischemic encephalopathy (HIE) caused by inadequate brain blood flow and oxygenation is a serious condition that can affect peripartum babies. Tragically, HIE has a high mortality rate, and surviving children can develop neurological disorders such as cerebral palsy, epilepsy, and intellectual/developmental disabilities (I/DD). Some countries use neonatal targeted temperature management as a therapeutic; however, molecular targets for mechanism-based therapies need to be identified for better acute, subacute, and long-term treatments because infants with HIE and hypothermia treatments still have, as older children, significant deficits in cognitive and executive functions and language skills compared to peers. These children could have cerebral cortical laminar dysmorphia, neuronal death, and possibly abnormal development of synaptic circuits. We have discovered prominent neuropathological abnormalities in the anterior temporal lobe, including the piriform cortex and contiguous perirhinal cortex (PRC), in a neonatal porcine model of HIE. Pathological changes are seen in CCK expression and localization, microglia distribution, and cortical layering. In sham newborn piglets, CCK is discretely localized as intracellular particles in the neuronal cytoplasm in these cortical regions; in contrast, in HIE piglets, increased CCK is diffusely localized and appears dispersed from the neurons coincident with neuroinflammation seen as microglia. In the PRC, possible laminar dysmorphia is found with increased blood-borne monocyte infiltration. We conclude that we have identified in a translationally-relevant large animal model a novel vulnerability of the multi-sensory, memory-related PRC with neuropathology, involving a classic gastrointestinal peptide, and potentially new therapeutically targetable brain region and injury mechanisms in neonatal HIE and subsequent childhood.

Disclosures: D. Park: None. **J.K. Lee:** None. **L.J. Martin:** None.

Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Grants-in-Aid for Scientific Research: C 22K09236
Mochida Memorial Foundation for Medical and Pharmaceutical Research
SENSHIN Medical Research Foundation
Smoking Research Foundation

Title: Macrophage infiltration is required for pericyte recruitment in poststroke tissue repair

Authors: *M. HIDAKA, K. NAKAMURA, F. YOSHINO, M. TAKASHIMA, T. KIYOHARA, Y. WAKISAKA, T. KITAZONO, T. AGO;
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Abstract: To promote functional recovery after ischemic stroke, we need to elucidate the endogenous mechanisms underlying tissue repair. To achieve this process appropriately, cerebral blood flow recovery, clearance of dead/dying cells and myelin, and fibrotic scarring in the ischemic lesions are necessary. Intrainfarct fibrosis is mediated by accumulation of pericyte-derived fibroblast-like cells secreting extracellular matrix proteins such as fibronectin and collagen type 1. We have recently demonstrated that PDGFRβ-positive pericytes are responsible for infiltration of monocytes/macrophages and their phagocytic activity via CCL2 and CSF1. However, the function of macrophages on pericytes is still poorly understood. Here, we examined the role of macrophages in the poststroke repair process by eliminating macrophages after permanent middle cerebral artery occlusion (pMCAO). Bisphosphonate clodronate encapsulated into liposomes is established as an effective agent for selective depletion of macrophages. We performed pMCAO to the male C57B6/J mice aged 10-12 weeks, followed by intraperitoneal administration of clodronate liposome (CLD) or the same amount of liposome (control mice) every 3 days until postoperative day 14, and evaluated neurological and histological differences between these 2 groups. Mice treated with CLD showed 80% reduction of the F4/80-positive macrophages infiltration in the ischemic lesions at day 14. CLD-treated mice exhibited worse neurological function, assessed by a balance beam test and modified neurological severity score, and larger infarct size than control mice. Interestingly, PDGFRβ-positive pericytes and fibroblast-like cells accumulated within infarct areas were significantly decreased in the CLD-treated mice. Moreover, intrainfarct deposition of fibronectin and collagen type 1 was attenuated by macrophage depletion. Taken together, these results suggest that macrophage infiltration is required for pericytes recruitment and fibrotic scarring in poststroke tissue repair. Macrophages may play an indispensable role for recruitment of PDGFRβ-positive pericytes into infarct areas leading to fibrotic repair and functional recovery.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

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Title: Nox4 plays crucial role in angiogenic responses and in recruitment of pericytes and macrophages in poststroke tissue repair

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Abstract: Ischemic stroke is a leading cause of mortality and disability worldwide. Recent evidence suggests that pericyte recruitment around ischemic lesion and its involvement in cerebral blood flow (CBF) recovery and in macrophage-mediated clearance of myelin debris promote the tissue repair following acute ischemic stroke. Reactive oxygen species are generally thought as a detrimental factor in cardiovascular diseases. However, the roles of Nox4, a NADPH oxidase highly expressed in vascular cells, may depend upon disease models; Nox4 induces breakdown of the blood-brain barrier through MMP9 and oxidative stress in acute phase of ischemic stroke, while also promoting angiogenesis in hindlimb ischemia. Here we examined the roles of Nox4 in tissue repair after acute brain ischemia, using a permanent middle cerebral artery occlusion (pMCAO) model in conventional *Nox4* knockout (Nox4 KO) mice. The expression of Nox4 was upregulated in vascular cells in ischemic areas of wild-type (WT) mice in the acute phase after pMCAO. Nox4 KO mice demonstrated significantly larger infarct volumes than WT mice at 28 days after pMCAO (WT 24.3 ± 5.6% /contralateral hemisphere vs. Nox4 KO 32.9 ± 4.9%). CBF recovery, as assessed by high-resolution 2D laser speckle flowmetry, was significantly suppressed in Nox4 KO mice than WT mice at day 28. Although WT mice showed increased numbers of PDGFRβ-positive pericytes in the ischemic lesion at day 28, accompanied by F4/80-positive macrophage infiltration, the increase in both cell types was inhibited in Nox4 KO mice (PDGFRβ: WT 82.9 ± 11.8% /ischemic lesion vs. Nox4 KO 65.3 ± 15.0%, F4/80: WT 24.3 ± 14.3% /ischemic lesion vs. Nox4 KO 11.8 ± 11.1%). Moreover, GKT137831, a specific Nox4 inhibitor, suppressed not only the pericyte proliferation but also the expression of CCL2 in cultured cerebrovascular pericytes. Combined, these results suggest that Nox4 may be involved in poststroke angiogenesis and in recruitment of pericytes and macrophages, and thus contributing to the tissue repair in a subacute phase after ischemic stroke. Nox4 can be a potential therapeutic target for poststroke functional recovery.

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Poster

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Title: Exploring the interaction of residual corticospinal connections and spinal circuitry during stimulation of the cervical spinal cord post stroke

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Abstract: We have recently demonstrated that epidural spinal cord stimulation (SCS) of the cervical spinal cord enables recovery of upper limb motor control in post-stroke hemiparesis. However, the corticospinal mechanisms involved in SCS-based motor improvement are poorly defined for stroke. Here, we have implanted two chronic stroke subjects with epidural leads along the dorsal roots of the cervical spinal cord to investigate mechanisms of interaction between SCS and residual corticospinal connections. Interestingly, there have been no reports of improved muscle-evoked potentials (MEPs) in SCS study participants; suggesting that none of the clinical improvements detected in the absence of SCS are mediated by strengthening of the corticospinal tract's (CST) monosynaptic connections to the motoneuron. Rather, we posit that the CST primarily engages spinal circuitry pre-synaptically which, in turn, conditions the motor response to SCS. As such, we initiated a paired-pulse protocol wherein we assessed the effect of cortical conditioning (via single pulses of transcranial magnetic stimulation (TMS) over the impaired half of the motor cortex) on single pulses of SCS-evoked spinal reflex potentials (SRPs) of the arm and hand. Inter-pulse-intervals (IPIs) ranged from 5 to 400ms in length. We found that TMS conditions SRPs at both short and long latencies, even in an MEP negative subject. Surprisingly, we further found evidence that the greatest potentiation occurs with an IPI of approximately 50-150ms, which is incompatible with latencies corresponding to monosynaptic coincidence on the motoneuron. Rather, this timing suggests activation of interneurons via residual CST projections that serve to excite or disinhibit the sensory-motor reflex arc. Furthermore, we investigated the effect of residual descending motor commands to SCS-mediated spinal dynamics in passive and active motion. Subjects were configured in a robotic dynamometer and guided through 60 degrees of elbow flexion and extension while we delivered test pulses of SCS to determine innate SRPs. We then delivered 40Hz continuous stimulation and discovered that residual supraspinal connections could overwrite spinal dynamics during active motion, indeed completely reversing them during functional SCS; suggesting that SCS enables the post-stroke brain to actively modulate spinal reflexes as a form of motor control. Overall, this evidence suggests that residual CST projections interact pre-synaptically with spinal circuits and SCS to modulate muscle responses and illuminates the potential for complex mechanisms of interaction between residual, but seemingly nonfunctional, CST fibers and SCS.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

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Topic: C.09.Stroke

Support: UG3NS12313501A1

Title: Epidural spinal cord stimulation reduces agonist-antagonist co-contraction facilitating strength production

Authors: *L. BORDA[1], E. SORENSEN[4], E. CARRANZA[5], R. DE FREITAS[6], N. VERMA[2], A. BOOS[7], G. F. WITTENBERG[7], P. C. GERSZTEN[8], L. E. FISHER[9], E. PIRONDINI[7], M. CAPOGROSSO[7], D. J. WEBER[3];
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Abstract: Stroke is the leading cause of long-term disability in the United States. Our group has recently demonstrated that cervical spinal cord stimulation (SCS) targeting the primary afferents lead to immediate improvements in muscle strength, kinematics and functional movements in people with post-stroke chronic hemiparesis. Although SCS is known to preferentially recruit large-diameter sensory afferents in the dorsal column and dorsal roots, the mechanisms underlying the immediate improvements in motor function observed with tonic stimulation remain unclear. Here, we tested the hypothesis that SCS reduces co-activation of antagonistic muscles by reciprocal inhibition. We hypothesized that the gain of the monosynaptic reflex measured in the antagonist muscles would be suppressed with SCS targeting the agonist muscle. Moreover, we hypothesized that these effects may exist even when SCS was delivered during passive movements (e.g, does not produce voluntary movements) indicating the direct, inhibitory effects of SCS on reflex gains in antagonistic muscles. Two 8-contact SCS leads were implanted percutaneously in the dorsal epidural space of the cervical spinal cord ipsilateral to the paretic arm of an individual with a unilateral subcortical stroke. Myoelectric activity was recorded from an antagonistic muscle pair, specifically biceps brachii (BB) and triceps brachii (TB). We then measured the amplitude of reflex responses evoked by SCS under active and passive conditions. In the active condition, the subject performed an elbow extension movement using a robotic exoskeleton. Tonic SCS was delivered at 40, 60 or 80 Hz on an electrode targeting facilitation of TB. A second electrode was used to elicit posterior root muscle reflexes (PRM) in the BB by delivering SCS concurrently at 2 Hz. In the passive condition, the experiment was repeated with the subject resting passively with the elbow extended at 180 degrees. In both conditions, the amplitude of the 2 Hz stimulation was sufficient to elicit the muscle response (PRM-reflex). Our results show suppression of the PRM reflex elicited in BB when continuous SCS was delivered to facilitate TB in both the active and passive conditions. The inhibitory effects were evident

across all SCS frequencies that were tested. Importantly, the inhibitory effects diminished when SCS was delivered through a contact that was not targeting the agonist muscle. Our results support the hypothesis that SCS can actively inhibit monosynaptic reflex gains in antagonistic muscles. The direct suppression of reflex responses in opposing muscles could reduce agonist-antagonist co-contraction and increase the net torque produced at the joint

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.05/U10

Topic: C.09.Stroke

Title: Mechanisms of Action Related To Motor Cortex and Corticospinal Output Potentiation Through Deep Brain Stimulation of the Motor Thalamus

Authors: *J. HO[1,2], L. TANG[1,2], E. GRIGSBY[1,2], A. DAMIANI[1,2,3], L. LIANG[1,2,3], J. BALAGUER[1,2,3], P. GERSZTEN[1,4], M. CAPOGROSSO[4,2,3], J. GONZÁLEZ-MARTÍNEZ[1,4], E. PIRONDINI[2,5,3];
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Abstract: Stroke is a leading cause of disability. Despite physical therapy being the gold standard for recovery, many patients still suffer from post-stroke motor deficits. Neuromodulation, such as spinal cord stimulation, transcranial magnetic stimulation, and deep brain stimulation (DBS) has been used to help people with motor recovery after stroke. In our previous work, we demonstrated that DBS of the motor thalamus (mThal) potentiates the motor cortex (M1) and motor output in both non-human primates (NHP) and human subjects with white matter lesions. Furthermore, we demonstrated that potentiation occurs orthodromically along thalamocortical projections from the mThal to M1 and that there is a frequency dependent effect where we observed potentiation from 50-100 Hz and suppression above 100 Hz. However, the mechanisms by which this frequency dependence occurs are unclear. We hypothesize that mThal DBS frequency dependent potentiation and suppression occur because of the interplay of polysynaptic thalamocortical and corticothalamic networks.In acute preparations in NHP (N=2),

we implanted depth electrodes into the mThal and two other deep brain electrodes into the hand region of the corticospinal tract (CST) within the internal capsule (IC), and into the motor thalamocortical tract (MTC) within the posterior portion of the IC. Intracortical arrays were implanted in the hand area of M1. To explore thalamocortical temporal interaction in a simple case, we sent single pulses of stimulation within the mThal followed by single pulses in the CST at varying delays. Next, we wanted to understand the contribution of the thalamocortical and corticothalamic networks to the frequency dependent effects. We sent single stimulation pulses within the MTC, M1, and CST at varying delays before and after stimulation pulses in the mThal and CST to selectively activate or block each pathway. We analyzed the resulting motor evoked potentials in upper arm muscles, spinal motor evoked potentials, and M1 cortical evoked potentials in response to the combination of stimulation paradigms.These results suggest that frequency dependent potentiation and suppression from mThal DBS is the result of the interplay of thalamocortical and corticothalamic feedback networks. We believe that this work provides a better understanding as to how mThal DBS potentiates motor output. These results should guide new methods such as closed-loop mThal DBS and further work to improve the clinical efficacy of mThal DBS in patients experiencing motor deficits after stroke.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Program #/Poster #: PSTR334.06/V1

Topic: C.09.Stroke

Title: The role of GABA in corticospinal control of spinal motoneurons during spinal cord stimulation

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Abstract: Spinal cord stimulation (SCS) is an emerging therapy that enables the recovery of voluntary control in patients with motor paralysis, yet its mechanisms of action are not completely understood. It is established that SCS activates sensory afferents in the spine exciting spinal motoneurons. This excitation assists residual supraspinal fibers conveying voluntary commands to produce voluntary movement. Studying the interaction between supraspinal fibers and SCS would seemingly suffice to decipher the underlying mechanisms of SCS, thus

accelerating its translation for widespread clinical use. However, one recent study showed that these sensory afferents recruited by SCS are regulated by presynaptic mechanisms mediated by GABA interneurons. Without these interneurons, spike transmission fails through sensory afferents to spinal motoneurons. Here we examined the role of GABA interneurons in the voluntary activation of spinal motoneurons during SCS.
We conducted a terminal experiment in an anesthetized Macaca Fascicularis (female, 6 yrs, 6 kg). We delivered suprathreshold SCS to the C6 level as single pulses at 2 Hz as well as stimulation bursts 1 s at 10-100 Hz. Motor evoked electromyographic (EMG) responses were recorded via intra-muscular needles in arm and hand muscles. In addition, we used subthreshold SCS bursts for 100 ms at 200 Hz to condition single pulse of corticospinal stimulation via deep brain stimulation of the internal capsule at 5-50 ms delays. Finally, we performed a causal manipulation by blocking GABAA receptors through the intrathecal administration of the GABAA antagonist drug bicuculline (60 μg/dose) and replicated the same stimulation protocols. We analyzed the reflex mediated responses induced by SCS before and after the administration of bicuculline. The blockage of GABAA receptors suppressed EMG responses during 2-100 Hz SCS. We measured the peak-to-peak (P2P) amplitudes for each stimulation pulse for all frequencies. Mean P2P amplitudes significantly decreased after the delivery of bicuculline, suggesting that GABA ensures the production of sustained SCS-evoked EMG motor responses. We then explored the role of GABA during SCS when conditioning a corticospinal pulse at different delays. Again, we quantified the P2P amplitudes of the corticospinal response. SCS modulated the corticospinal input for all delays and all muscles. This modulation was absent after bicuculline and the P2P amplitudes during SCS conditioning were similar to those from the corticospinal pulse alone. In conclusion, our results imply that GABA is critical to modulate voluntary input from corticospinal fibers during SCS for the recovery of motor control.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Program #/Poster #: PSTR334.07/V2

Topic: C.09.Stroke

Title: Hand or Face? Redefining the Map of the Motor Thalamus to Restore Facial and Upper Limb Motor Deficits with Targeted Deep Brain Stimulation

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Abstract: Around one million of Americans suffer from facial and speech motor deficits, as dysarthria and facial paresis. For these patients, speech therapy is the only significant intervention, with limited results on severe cases. Neuromodulation interventions could significantly impact the field by improving the effects of rehabilitation. In this regard, we previously showed in monkeys that deep brain stimulation (DBS) of the motor thalamus increases excitability of the motor cortex (M1) facilitating upper limb and face motor output. However, we posit that current inaccuracies in the understanding of motor thalamocortical interactions limit the effect of DBS and other neuromodulation approaches for face motor deficits. Here, we propose a novel hodological map of the motor thalamus along a rostral to caudal gradient, with face related regions located in more rostral nucleus (ventral oralis anterior, VOA) and hand/arm regions in caudal nuclei (ventral oralis posterior, VOP/ ventral intermedium, VIM). We performed intraoperative experiments in n=2 human patients undergoing DBS implantation of the motor thalamus. Through the procedure, patients were kept awake to monitor brain electrophysiology and perform stimulation testing of three microelectrode trajectories passing through the VOA, VOP, and VIM. Such trajectories were mapped with single unit activity to optimize target localization. Patients performed facial or upper limb movements while we simultaneously recorded thalamic spiking activity from the microelectrodes and muscle activity through EMG needles. We observed that face and tongue movements correlated with increases in VOA single unit spiking activity, whereas arm movements correlated with increases in VOP/VIM firing. In the same intervention, we proved that this thalamic organization resulted in a somatotopic facilitation of motor output from M1. For this, we placed subdural electrocorticography (ECoG) electrodes over the M1 arm/hand representation as well as the M1 face representation. We first stimulated the VOA, VOP and VIM at 1Hz and recorded cortical evoked potentials (CEPs). We then applied cortical stimulation from the subdural strips and compared MEP amplitudes without and with paired stimulation of the different nuclei. With VOA stimulation, the CEPs over the face representation and the MEPs of facial muscles were significantly increased, while VIM stimulation led to motor enhancement of upper limb muscles but not in the face motor area. Our results eluciate the hodological interactions between the thalamus and motor-related neocortical areas and justify the potential use of targeted DBS to treat facial and hand motor deficits.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.08/V3

Topic: C.09.Stroke

Support: Genentech-Roche

Title: Changes in Spinal Motor Neurons Functions After Spinal Cord Stimulation Therapy: An fMRI Study in Spinal Muscular Atrophy (SMA)

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Abstract: Spinal Muscular Atrophy (SMA) is a genetic disease that causes progressive dysfunction and death of spinal motor neurons, which leads to motor deficits ranging from limb weakness (type 4) to severe muscle weakness with respiratory failure (type 1). Currently, there are three pharmaceutical therapies that have been proven to slow disease progression, however SMA patients still exhibit severe motor deficits urging for the development of effective combinatorial approaches. Here, we aim to develop a targeted neurostimulation therapy to improve residual motor neuron function in Type 3 SMA patients to supplement existing treatments. Recent experiments in mice have shown that SMA motor deficits are due to a combination of motor neuron death and decreased firing rates in surviving motor neurons as a consequence of a maladaptive response to a loss in the excitatory Ia sensory synapses. Epidural spinal cord stimulation (SCS) can selectively activate Ia sensory fibers; thus we hypothesize that targeted stimulation of Ia afferents via epidural SCS would increase inputs to the motor neurons, resulting in increased firing ability and improved leg functions through immediate and long-term stimulation effects. To demonstrate our hypothesis, we are conducting a 30-day pilot clinical trial using off-label epidural SCS implants in two SMA type 3 patients. We quantified long-term changes in motor neuron functions by performing functional magnetic resonance imaging (fMRI) of the lumbar spinal cord during passive and active mobilization of the knee joint pre- and post- SCS therapy. The first participant has shown that SCS treatment significantly increased their knee joint force and mobility. These improvements correlate with fMRI results, on both the passive and active tasks, showing an increased number of active voxels and higher voxel z-scores post SCS therapy. The second, more severely affected, participant did not exhibit the same level of improvement in the knee and therefore failed to show significant results in the fMRI knee tasks, suggesting a need for either longer term SCS therapy or earlier interventions. While pharmacological treatments can slow progression of the disease, they appear not to provide a consistent benefit in motor neuron function. Our data shows that SCS is contributing to long term changes by increasing the firing rate of vulnerable motor neurons in patients with type 3 SMA resulting in improved leg motor functions. Our results raise the possibility that SCS can provide a permanent treatment in conjunction with pharmaceutical interventions for people living with SMA.

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Poster

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Topic: C.09.Stroke

Support: UG3NS123135

Title: Assistive and therapeutic effects of cervical spinal cord stimulation in patients with chronic post-stroke hemiparesis

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Abstract: We have previously shown that cervical spinal cord stimulation (SCS) improves the upper limb motor function of individuals with chronic hemiparesis after stroke. Specifically, dexterity and strength were both significantly improved (*i*) immediately while stimulation was delivered (i.e., assistive effects), as well as (*ii*) in the absence of stimulation (i.e., therapeutic effects). In this study, we examined the relationship between assistive and therapeutic effects of SCS on upper limb motor recovery in 4 participants with chronic post-stroke hemiparesis. Cervical SCS was applied using two lead electrodes implanted in the epidural space spanning from the C3 to T1 spinal segments at the contralesional side. Stimulation parameters were chosen to selectively activate dorsal root afferent fibers innervating different arm and hand muscles. Cervical SCS was administered 5 times per week for 4 weeks, while strength and dexterity tasks were performed. To quantify assistive and therapeutic effects enabled by SCS, we assessed sensory and motor functions using Fugl-Meyer Assessment (FMA) across different timepoints. Specifically, FMA scores were compared between stimulation ON and OFF conditions across *baseline* (a week prior implantation of SCS electrodes), *mid-study* (2nd week after implantation) and *end-study* (4th week after implantation). Moreover, we used multivariate analysis of arm kinematics during planar reach and pull movements to compare fine-grained improvements in motor functions across the 4 weeks. We found that all subjects had significant assistive effects. Notably, arm kinematics were gradually improved over time for stimulation ON and OFF conditions, indicating that *both* assistive and therapeutic effects can be potentiated over the course of motor practice. We also found that increase of FMA motor scores across time were linearly correlated with FMA sensory scores at *baseline*, indicating that residual *afferent sensory tracts* are critical for promoting therapeutic and assistive motor recovery using SCS. To further examine the contribution of residual *efferent motor tracts* (e.g., corticospinal tract), we further tested weather transcranial magnetic stimulation evoked potentials (MEP) on upper limb

muscles. We found no correlation between the presence, or absence, of MEPs and increase of FMA motor scores at *end-study*, supporting evidence that individuals with *less* sensory impairments have *more* potential for motor recovery irrespective to residual motor function after stroke. In future studies, these results will help with stratification of stroke patients and customization of effective rehabilitation protocols using cervical SCS.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.10/V5

Topic: C.09.Stroke

Support: NIH UG3NS12313501A1

Title: Closed-loop control strategies for improving the effects of spinal cord stimulation on motor recovery after stroke

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Abstract: Stroke damages the corticospinal tract, causing motor impairments that are often permanent. Recently, we demonstrated that tonic epidural stimulation of the cervical spinal cord improves upper limb motor function, specifically strength and dexterity in the arm and hand of people with chronic hemiparesis post-stroke. While tonic stimulation enhances the recruitment of targeted muscles, manual tasks of daily life involve coordinated and sequential activation of muscles throughout the limb. Previous studies of SCS in animals have shown that phase-specific patterns of SCS were required to enable compound movements comprising reaching, grasping, and retraction phases. Four participants with chronic post-stroke hemiparesis were implanted with two 8-contact percutaneous SCS leads in the epidural space of the cervical spinal cord, ipsilateral to the paretic arm, for a duration of 30 days. SCS was delivered using a custom-built stimulation system that allowed for real-time control of the amplitude and frequency of stimulation applied toeach contact. Participants performed planar and 3D reaching and grasping movements with their affected limb with and without SCS. Tonic and phasic stimulation was applied during the reaching tasks. During tonic SCS, the stimulation parameters were fixed and

delivered continuously throughout the different phases of movement. During phasic SCS, stimulation wasapplied to different groups of electrodes during the extension and flexion phases. Furthermore, stimulation amplitude and frequency were modulated in real-time based on arm position relative to the target. The participants were able to perform the task faster and with smoother trajectories with tonicSCS than without. Phasic SCS further improved reaching kinematics by increasing maximum hand speed, reducing movement duration, and smoothing hand trajectories. An up to 150% increase in muscle activations for phasic SCS was measured compared to the tonic and the no SCS conditions. These results show that although tonic SCS was effective in promotingsignificant gains in motor function, phasic SCS produced even stronger effects.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.11/V6

Topic: C.09.Stroke

Support: Genetech Roche

Title: Spinal cord stimulation in spinal muscular atrophy

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Abstract: Spinal Muscular Atrophy (SMA) is a rare genetic disease occurring from 1 to 6-10K newborns. SMA manifests with different degrees of severity in infants (Type 1), children (Type 2), and young adults (Type 3) and is caused by deletion of the Survival Motor Neuron 1 (SMN1) gene, resulting in ubiquitous reduction of SMN protein. The hallmarks of SMA are dysfunction and death of select spinal motor neurons, muscle atrophy and severe motor deficits. Surviving motor neurons exhibit maladaptive responses to loss of excitatory Ia afferent synapses resulting in lower firing rates. Pharmacological increase of excitation in Ia afferents improves motor neuron function in SMA mice, but this approach results in nonspecific undesirable toxic effects in the long term. To overcome this, we propose to use epidural spinal cord stimulation (SCS) targeted to stimulate directly only the SMA-affected Ia afferents. To study the potential effects of SCS in a spinal cord affected by SMA, we built a biophysical model. First, we developed a Hodgkin-Huxley model that reproduced the electrical and firing rate properties of SMA-affected motor neurons. Then, we connected a SMA-affected pool of motor neurons with the Ia afferents recruited by SCS and a pool of supraspinal neurons. Using the biophysical model, we found that 1) SCS can immediately potentiate motor neuron function by increasing the activity in Ia afferents and 2) SCS could revert the maladaptive response on motor neuron properties producing long-term improvements in motor function. To verify these, we are running a pilot clinical trial using temporary off-label epidural implants (NCT05430113) for 1 month. Three adults living with SMA Type 3 were enrolled in a series of experiments to detect immediate (same day stimulation ON vs OFF) and long term (pre-implant vs post-implant, stimulation OFF) improvements in motor function. Preliminary results show that SCS can increase immediately, as well as long-term, the maximum voluntary contraction of the leg muscles producing higher torques and gait quality variables (e.g. step height and length). We used High-Density-EMG to decompose the EMG activity in single units action potentials during muscle contraction and found that the motor neuron firing rate increased, indicating an improvement in motor neuron function. Moreover, using TMS, we found that the motor-evoked potentials were reduced after the period of SCS suggesting that SCS could revert the dysfunction of SMA motor neurons. These results show that SCS could improve motor neuron function and may serve as a disease modifying treatment in SMA patients that could act in combination with existing treatments.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.12/V7

Topic: C.09.Stroke

Title: Direct Motor Thalamus Stimulation Facilitates Voluntary Control of Facial Muscles

Authors: *L. W. TANG[1,2], E. M. GRIGSBY[3], A. DAMIANI[4], J. HO[2], S. KALLAKURI[2], D. J. CRAMMOND[8], T. CONSTANTINE[5], M. CAPOGROSSO[6], J. GONZÁLEZ-MARTÍNEZ[5], E. PIRONDINI[7];
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Abstract: Neuromodulation using deep brain stimulation (DBS) has been established as a safe and effective intervention for patients with motor dysfunctions. In recent work, we showed that DBS of the motor thalamus potentiates the cortical activity of the primary motor cortex (M1), facilitating activation of cortico-spinal tracts, and ultimately producing enhanced upper-limb motor output. Given that M1 also has projections through the corticobulbar tract that innervate facial muscles, we posit that motor thalamus stimulation can also potentiate facial motor output. Here, we test whether targeted DBS of the motor thalamus will potentiate i) facial motor-evoked potentials (MEPs) and ii) voluntary face movements. To demonstrate that motor thalamus DBS increases facial MEPs, we performed intraoperative neurophysiological experiments in human subjects (n=2) undergoing DBS implantation for the treatment of Essential Tremor (ET). During the surgery, a 6-channel subdural strip electrode was inserted over the face representation of M1. We then applied direct cortical stimulation (DCS) from one of the 6 contacts and recorded facial MEPs with and without paired stimulation of the motor thalamus at 50 and 100 Hz. We found that stimulation of the motor thalamus significantly increased evoked MEPs at 50 and 100 Hz. To test if the increased MEPs improved volitional control of face motor actions, we then performed acute stimulation studies in participants (n=4) completing voluntary facial motor tasks, rapidly moving between instructed and neutral facial expressions with and without motor thalamus stimulation at 50 Hz and 100 Hz. Two subjects had no facial muscle paresis and the other two had chronic motor symptoms from traumatic brain injury (TBI), resulting in mild (n=1) and severe (n=1) reductions in oral motor strength, speed, and range of motion. Videos and surface electromyography (EMG) from jaw, cheek, and neck muscles were recorded to quantify subjects' facial muscle strength. Both EMG and kinematic recordings showed a significant increase in velocity and amplitude of voluntary movements with motor thalamus stimulation as compared to no stimulation. This potentiation of facial motor output was consistent even in subjects with post-TBI facial muscle impairments, demonstrating the restorative effect of thalamic stimulation to improve facial muscle control. The results confirm that motor thalamus stimulation increases M1 excitability and potentiates facial movements. Application of DBS stimulation of the motor thalamus can serve as a potential therapeutic approach to recover facial motor control for patients with facial paresis, speech, and swallowing impairments.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.13/V8

Topic: C.09.Stroke

Title: Direct electrical stimulation of the motor thalamus improves speech motor deficits following traumatic brain injury

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Abstract: Traumatic brain injury (TBI) is a leading cause of permanent motor disability in the United States, often resulting in partial or complete loss of speech production. Studies have estimated that up to 65% of TBI patients suffer from dysarthria, but treatment options for motor speech deficits are limited. Despite having only a limited impact in more severe cases, speech therapy remains the gold standard. We hypothesize that thalamic stimulation could improve speech motor-deficits by driving excitatory inputs to M1 and augmenting facial muscle activity. Notably, we recently observed increased facial muscle responses in non-human primates with motor thalamic stimulation, suggesting enhanced excitation of the corticobulbar tract, a crucial structure in the coordination of facial movements and speech-motor functions. Here, we demonstrate the facilitation of speech production in two chronic TBI patients through motor thalamus stimulation. We conducted multiple sessions of acute stimulation in n = 2 participants with chronic motor symptoms induced by TBI, including mild and severe speech impairments. The mildly impaired subject was implanted with temporary stereoelectro-encephalography electrodes (SEEG) in the motor thalamus for seizure monitoring. The severely impaired patient was implanted bilaterally with chronic deep brain stimulation (DBS) electrodes in the motor thalamus for tremor treatment. Both participants completed speech-therapy exercises to measure their articulation and speech fluency. The mildly impaired subject recited two-word “tongue-twister” phrases as quickly and clearly as possible over a 20-second time period while the severely impaired subject recited single words five times as clearly as possible. We tested words composed of commonly impaired phonemes (i.e. /k/, /p/, /t/) and recorded video and audio to assess performance. All tasks were repeated with and without thalamic stimulation. In both subjects, stimulation produced immediate effects, including increased duration of the speech envelope plateau, decreased pitch variability, and increased frequency resonance. This resulted in improved sustained sound, clearer speech, and cleaner phoneme separation (i.e. refined articulation). Furthermore, the mildly impaired subject exhibited a significant decrease in articulation errors during the “tongue-twister” task. These preliminary results provide promising evidence that some aspects (i.e. articulation, fluency, voice/pitch, and muscle coordination) of speech production may be improved with stimulation of the motor thalamus.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.14/V9

Topic: C.09.Stroke

Support: UG3NS123135-01A1

Title: Spinal cord stimulation and proprioception: kinesthesia versus position sense

Authors: *E. CARRANZA[1], R. DE FREITAS[1], E. SORENSEN[1], N. VERMA[5], M. POWELL[1], A. BOOS[1], L. FISHER[1], P. GERSZTEN[1], D. J. WEBER[6], J. W. KRAKAUER[7], G. F. WITTENBERG[2], M. CAPOGROSSO[3], E. PIRONDINI[4];
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Abstract: The loss of proprioception after stroke can substantially affect motor behaviors, which relies on accurate information of the position (position sense) and movement (kinesthesia) of the limbs. We recently demonstrated that continuous cervical spinal cord stimulation (SCS) improves voluntary motor control in post-stroke upper limb hemiparesis, although the effects of SCS on sensory feedback, especially on proprioception, remains unclear. It has been previously shown that continuous SCS applied to lower spinal cord segments can cancel kinesthesia of lower limbs in individuals with spinal cord injury, whereas bursts of stimulation were necessary to mitigate these undesired effects as well as enable robust control over motor neuron activity. Based on these previous findings, we first questioned herein whether this cancellation was also present during upper limb movements. For this, we assessed kinesthesia on the upper limbs of two stroke patients with chronic hemiparesis after stroke, who were implanted with two percutaneous spinal leads spanning from C3 to T1 spinal segments. Specifically, kinesthesia was assessed by using the threshold to detection of passive movement (TTDPM) in the elbow joint of the paretic arm during stimulation ON and OFF conditions. Starting from a 45° angle, a robotic platform (Humac Norm) passively produced elbow flexion and extension at 1°/s with a 15° displacement limit. Participants reported the movement direction, i.e., flexion or extension, when it was perceived. Successful trials correctly identified movement direction, while unsuccessful trials lacked perception or misclassified the movement. Consistent with previous results, we found that participants were not able to detect movement direction when continuous SCS was delivered. We then tested the effects of SCS on position sense. For this, we used a robotic exoskeleton (KINARM) to measure hand position accuracy and variability. Specifically, participants were asked to mirror match the position of the paretic arm using the contralateral, and intact, arm, while the robot passively moved the paretic arm to a determined position. Interestingly, we found that, contrary to kinesthesia, position sense was not disrupted by continuous SCS. Indeed, the position sense task showed no significant differences in measured variables between stimulation ON and OFF conditions. In conclusion, our results show that SCS

differently affects position and kinesthetic proprioceptive information. Future work involving computational modeling are now necessary to clarify the mechanisms underlying these contrasting results.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: C.09.Stroke

Title: Mapping sensorimotor circuitries via epidural spinal stimulator in a porcine model

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Abstract: Preclinical models are essential for identifying changes after neurologic injury and assessing therapeutic interventions, such as spinal cord stimulation for neuromotor recovery following spinal cord injury (SCI). While small animal models, such as rodents, have lower associated costs, large animal models like the Yucatan miniature pig (minipig) may be more successful for translating SCI research to improve outcomes in humans due to the similar dimensions of the brain and spinal cord. Unfortunately, little work has been done to map minipig sensorimotor distribution similarities and differences with the human spinal cord. To characterize the spatiotemporal differences in efferent and afferent spinal signaling we implanted a 32 contact four-column array into the dorsal epidural space over the lumbosacral and cervicothoracic spinal cord in two Yucatan minipigs. Spinally evoked motor potentials (SEMP's) were recorded bilaterally in four hindlimb muscles during stimulation from different portions of the array, muscle selectivity was calculated and mapped back to the array. In addition, somatosensory evoked potentials (SSEP's) were recorded via the epidural array by stimulating the right and left tibial nerves. Using epidural spinal stimulation, we achieved selective ipsilateral proximal and distal activation in the hindlimb and forelimb muscles, which confirmed correct positioning of the array overlaying the targeted sensorimotor networks. During SEMP caudal stimulation resulted in the largest ipsilateral distal muscle activation. During peripheral nerve stimulation, peak-to-peak SSEP amplitude was largest at the ipsilateral rostral portion of the array. The variation in spatial location was then quantified using the correlation between SEMP's and SSEP's. Our findings demonstrate the spatial differences between efferent and afferent signals,

help better characterize the spinal sensorimotor networks, and demonstrate the feasibility of using epidural spinal stimulation arrays for mapping spatiotemporal spinal activity.

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Poster

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Topic: C.09.Stroke

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Title: Task-specific training using robotic exoskeleton in combination with cervical transcutaneous electrical stimulation improves upper limb motor output in individuals with tetraplegia

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Abstract: Task-specific motor training has been shown to be effective in improving motor output in individuals with tetraplegia due to spinal cord injury (SCI). By engaging in task-specific motor training, individuals with tetraplegia can target the specific neural pathways and muscles involved in the desired movement. Non-invasive, transcutaneous spinal cord stimulation (TSS) is a novel electrical neuromodulation strategy that has the potential to increase the excitability of spinal circuits and facilitate functional recovery after SCI. We hypothesized that task-specific training using robotic exoskeleton, combined with TSS, improves volitional upper limb (UL) function in individuals with severely impaired UL function by inducing changes at spinal and cortical levels. Four participants with tetraplegia due to SCI were recruited in this study. Participants underwent two 4-week interventions consisting of sessions performed three times per week, which included four UL task-specific movements: elbow flexion/extension, forearm pronation/supination, wrist flexion/extension, and wrist radial/ulnar deviation. Additionally, task-specific hand grip training was performed, and maximum grip strength was measured using a handgrip dynamometer. The interventions also included either sham stimulation or TSS, counterbalanced across the participants. TSS waveforms consisted of biphasic 0.5 ms pulses, at a frequency range between 10 to 90 Hz, applied to C3-C4 and C7-T1 vertebrae. During the sessions, visual feedback from the robot and electromyography (EMG) activity from UL muscles, were presented to the participants. To assess changes in the spinal and corticospinal excitability after intervention, recruitment curves of spinal (SMEP) and corticospinal motor evoked potentials (MEP) were obtained. We observed that TSS combined

with task-specific robotic UL training resulted in improved desired muscle activation and quality of movements in the trained UL. This was demonstrated by an increase in the number of repetitions in the joints of the trained UL and a reduction in co-contraction of non-targeted muscles in three out of four participants. In addition, task-specific hand grip training combined with TSS improved maximum hand grip strength. Our findings suggest that a task-specific UL training protocol using robotic exoskeleton, combined with TSS can result in significant improvements and facilitate the restoration of UL motor function in individuals with tetraplegia.

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Poster

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Title: Targeted reactive transcutaneous spinal stimulation promotes best stepping patterns during locomotion in robotic exoskeleton

Authors: *C. A. MARTIN[1], A. G. STEELE[1], S. W. TURNER[1], J. W. WENZLAWSH[1], D. G. SAYENKO[2];
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Abstract: Background Transcutaneous Spinal Stimulation (TSS) is a neuromodulation technique that alters excitability of spinal sensorimotor network and facilitate neural circuits rhythm/pattern generation. TSS has been utilized in research studies with individuals with chronic spinal cord injury (SCI) demonstrating improvements in sitting posture, self-assisted standing, and in stepping movements. We studied three TSS approaches, (1) continuous, (2) interleaved, and (3) step-pattern (SP) stimulation in supine and step-triggered (ST) stimulation in standing to determine the optimal setting for gait training. **Methods** Data was collected on 4 individuals with SCI (ASIA Impairment Scale A, neurological level of injury T2-7, 5-20 years post-SCI) enrolled in our sham-controlled study investigating the effects of TSS with exoskeleton (EksoGT) locomotion. Each participant completed 3 sessions weekly for 8 weeks (4 weeks Sham and 4 weeks Active) with up to 60 minutes of intervention per visit. Electrophysiological assessments were completed at baseline, mid-point, and post study; stimulation was applied in a supine position with the participant fitted in a custom-built device

(Exolab) designed to measure isometric forces generated by voluntary or stimulation-induced plantarflexion/dorsiflexion and knee extension/flexion. Stimulation applied between the T10 and L2 vertebral levels with muscle responses measured with surface EMG and force production measured with Exolab. During sham and active interventions in EksoGT, EMG recordings of lower extremity muscles and foot pressure sensor data were collected during individual cycles of gait. **Results** SP stimulation was advantageous as compared with continuous stimulation for generating step-like movement patterns in supine position. Step force magnitude improved with continuous stimulation plus volitional movement as compared to stimulation alone. However, SP stimulation with/without volitional movement generated the best force production compared to continuous stimulation environments. Interleaved stimulation and ST stimulation demonstrated ability to promote different phases of the step cycle during locomotion. **Conclusion** TSS can be individually tailored and delivered to promote specific motor tasks but requires systematic exploration of responses based on location- and task-specific stimulation delivery. Continuous stimulation is the standard TSS technique reported previously and demonstrated modest specificity during standing, but the same parameters have inferior effects on motor responses during stepping limiting potential functional outcomes during dynamic movements.

Disclosures: **C.A. Martin:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); ekso BIONICS - clinician training. **A.G. Steele:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **S.W. Turner:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **J.W. Wenzlawsh:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **D.G. Sayenko:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute, Weill Cornell Faculty Appointment.

Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.18/V14

Topic: C.09.Stroke

Support: NeuroSpark Seed Funding Program (20130009)
Craig H. Neilsen Foundation (733278)

Title: Functional reorganization of brain-spinal connectivity after stroke using non-invasive spinal neuromodulation and robotic upper limb exoskeleton

Authors: *M. SCHEFFLER[1], J. OH[1], E. MAHAN[3], S. KING[3], C. MARTIN[1], J. DINH[1], M. O'MALLEY[3], D. SAYENKO[2];
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Abstract: Background: Stroke is a leading cause of long-term disability. A large proportion of cerebral strokes disrupt descending commands from motor cortical areas to the spinal cord and peripheral muscles, which can result in motor deficits of the arm and hand. **Hypothesis:** We hypothesized that critical and functional reorganization of the brain and spinal cord interface following stroke can be enhanced by salient, task based, functional movements, in combination with transcutaneous spinal stimulation (TSS). The goal of the study was to assess the effect of the combinatorial approach of task specific robotics training and TSS on upper limb (UL) function in individuals post stroke, using clinical outcomes and high quality electrophysiological data. **Methods/Results:** Three stroke survivors with unilateral UL hemiparesis (ranging from 1-7 years post stroke) were enrolled as part of an ongoing study. The subjects were assessed at baseline for their electrophysiological and functional status and completed the intervention comprising task specific UL robotic training in combination with sham stimulation (4 weeks) or TSS (4 weeks). The training was focused on movements of the hemiparetic elbow, forearm, and wrist of the affected UL. Subjects were assessed again after sham and TSS phases, with a two-week period of washout/rest between the phases. Collected data included motor threshold (MT) and maximum responses (MaxR) of key UL muscles trained (biceps, triceps, wrist flexors, wrist extensors) as well as EMG signals of trained UL muscles. Conventional clinical UL outcome measures were also completed. We found that following intervention with TSS, MT was lowered and MaxR was higher in various UL muscles on the affected/trained UL, compared to the unaffected UL. EMG data indicated improved muscle activation and decreased compensatory movement patterns with training. Participants also demonstrated improvements in the Fugl Meyer Assessment of Upper Extremity Function clinical measure during the TSS phase, and overall improvements in chronic UL hypertonicity. **Implications:** These preliminary findings provide insight into the effects of TSS in combination with task specific UL robotics training on post-stroke motor recovery. When this intervention paradigm is undertaken based on high quality electrophysiological assessment data, it will allow for researchers to make informed decisions in planning for larger studies. These larger studies would ideally employ a targeted approach which would incorporate progressive, task specific upper limb training paired with individualized TSS paradigms, to maximize individuals' desired UL recovery post spinal cord injury.

Disclosures: M. Scheffler: A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **J. Oh:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **E. Mahan:** A. Employment/Salary (full or part-time):; Rice University. **S. King:** A. Employment/Salary (full or part-time):; Rice University. **C. Martin:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **J. Dinh:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute. **M. O'Malley:** A. Employment/Salary (full or part-time):; Rice University. **D. Sayenko:** A. Employment/Salary (full or part-time):; Houston Methodist Research Institute, Weill Cornell Faculty Appointment. 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.; NeuroSpark Seed Funding Program (20130009) and Craig H. Neilsen Foundation (733278).

Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.19/V15

Topic: C.09.Stroke

Support: NIH NHLBI T32 HL125242
F31NA129326

Title: Evaluation of Mitochondrial Cardiolipin Modification in Neonatal Hypoxic Ischemic Encephalopathy

Authors: *K. EMAUS[1,2], J. M. WIDER[1,3], E. GRULEY[1], J. MATHIEU[1], G. FOGO[1,2], F. TORRES TORRES[1,2], T. SANDERSON[1,2,4,3,5];
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Abstract: Neonatal hypoxic/ischemic encephalopathy as the result of perinatal disruption of oxygen delivery to the brain and is the cause of approximately 1 million deaths per year. Surviving infants suffer irreversible neurological damage contributing to developmental abnormalities such as cognitive and motor impairments including cerebral palsy. While reinstating blood flow and oxygen delivery is pertinent for survival, it induces a secondary form of injury termed reperfusion injury. Reperfusion injury produces excessive amounts of reactive oxygen species (ROS) and creates a highly oxidative environment within the mitochondria. When exposed to oxidants, such as ROS, lipids undergo peroxidation which can disrupt their structural integrity. There are several lipids that when oxidized are thought to contribute to reperfusion injury, specifically cardiolipin (CL). CL is found on the inner mitochondrial membrane where it plays integral roles in cellular respiration and cristae structure. Following oxidative stress, CL is externalized to the outer mitochondrial membrane where it is suggested to play a pivotal role in mitophagic flux and cell death signaling cascades. Additionally, CL biosynthesis produces a premature form of CL before converting it to its final, mature form. During this remodeling process, monolysocardiolipin (MLCL) is produced as an intermediate. The purpose of this study is to investigate CL biosynthesis and remodeling pathway as a potential therapeutic target for neonatal HIE. In our clinically relevant piglet model, we have observed an accumulation of MLCL following HIE. Additionally, our rodent model demonstrates that CL remodeling and MLCL accumulation to occur in the early phases of reperfusion. Taken together, our data suggests this remodeling pathway to play a role in injury progression in large and small animal models of HIE. We hypothesize MLCL accumulation contributes to mitochondrial dysfunction and inhibition of this accumulation could provide neuroprotection. Further studies will utilize novel transgenic rodent lines to target MLCL accumulation, visualize its impact on mitochondrial morphology and evaluate its role in white mater injury and behavioral outcomes. Together, these studies demonstrate CL remodeling and MLCL accumulation to be translationally relevant to neonatal HIE injury and pose as a potential therapeutic target.

Disclosures: K. Emaus: None. **J.M. Wider:** None. **E. Gruley:** None. **J. Mathieu:** None. **G. Fogo:** None. **F. Torres Torres:** None. **T. Sanderson:** None.

Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.20/Web Only

Topic: C.09.Stroke

Support: EU Horizon 2020 MSCA Grant 101032054

Title: Alterations of the sleep-wake cycle architecture on an animal model of stroke

Authors: *V. R. COTA[1], M. CARÈ[2], G. FEDERICI[2], F. BARBAN[2], L. DE MICHIELI[1], M. CHIAPPALONE[2];
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Abstract: Stroke and traumatic brain injury are major causes of adulthood impairment having a major impact on the patient's quality of life. They can cause cognitive and motor deficits which jeopardize the execution of daily life activities imposing a burden not only on the individual but also on the family and the society. On the other hand, it is currently well established that sleep is a key factor not only for overall health but particularly for brain function. A better understanding of the still unclear relationship between sleep and the neuropathology of brain injury may yield novel insights that can spur novel therapeutic strategies. Here, we submitted Long-Evans rats to a chronic stroke model to investigate the impact on the sleep-wake cycle architecture by means of electrophysiology and behavioral assessment of motor function. After one week of handling, arena habituation, and training on the motor tests, a control group (CTRL; N = 6) was submitted to a surgical procedure for chronic implantation of two 32 channels electrode arrays (somatosensory cortex S1 and pre-motor cortex RFA; left hemisphere). Another group of brain injury animals (LESION; N = 6) underwent the same procedure but with also induction of stroke (CFA; left hemisphere) by controlled microinjection of Endothelin-1 (ET-1). After a 7-days recovery period, all animals were submitted to a 6-hour long electrophysiological recording session in which they were allowed to freely sleep. Afterward, on the same day, animals were tested for gross and fine motor functions by means of the Grid-walking test (5 minutes), the Mirror test (5 minutes), and the Pasta Matrix test (20 minutes). Animals were then euthanized, and the brain was removed for histological processing. Animals in the LESION group showed clear signs of motor impairment, including an increased number of the right hind pawn faults in the Grid-walking test, an increased number of touching with the left forepaw in the cylinder walls of the Mirror test, and a decreased number of successfully grasped pasta sticks in the Pasta-Matrix test. Preliminary analysis suggests (still without statistical significance) alterations of the electrophysiological activity of sleep, including spectral content of specific sleep-wake cycle stages, coordination of important sleep oscillations, the frequency of relevant electrographic signatures (e.g., thalamocortical spindles), and the regularity of neuronal firing. Further investigations and completion of the analysis (currently ongoing) are mandatory for a better understanding of current results.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.21/V16

Topic: C.09.Stroke

Support: NIH R01NS058784

Title: Food restriction enhances stem cell-induced recovery in ischemic mice

Authors: X. LIANG, ***V. GUPTA**, T. M. BLISS, G. K. STEINBERG;
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Abstract: Food restriction enhances stem cell-induced recovery in ischemic mice
Keywords Stroke; Stem cell; Food restriction; Functional recovery
Abstract
Preclinical studies investigating the effectiveness of stem cell transplantation for stroke therapy utilize various behavior testing paradigms. Some behavior tests require a period of food restriction during baseline training (prior to stroke) and during the testing period. However, the potential impact of food restriction on treatment outcome is rarely considered. Here we investigate the effect of food restriction on stem cell efficacy. Human neural stem cells or vehicle were transplanted into the ipsilesional hemisphere of C57BL/6 mice one week after inducing a 35-minute transient middle cerebral artery occlusion. For 3 weeks prior to the stroke, mice were either given normal access to food (normal diet) or subjected to restricted food access (food restricted: 1g/10g body weight). Long-term functional recovery was evaluated every 2 weeks using a rotating beam paradigm; food was restricted for 2 days prior to behavior testing in the food-restricted group. Results showed that mice with restricted food access had a slightly lower body weight than those with ad libitum access. Food-restricted mice exhibited a faster rate of functional recovery with stem cells than normal diet mice, with a marked improvement in stem cell-induced recovery observed at 8 weeks post-transplantation. There was no difference in recovery between vehicle-treated mice in each group. These data suggests that food restriction makes the ischemic brain environment more receptive to stem cell-induced modulation and recovery.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

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Program #/Poster #: PSTR334.22/V17

Topic: C.09.Stroke

Support: Penn State Clinical and Translational Science Institute (2UL1TR002014-05A1)

Title: The H67D HFE Mutation Modifies the Antioxidant Response, Cellular Damage, and Recovery After Intracerebral Hemorrhage

Authors: *T. B. HELMUTH[1], R. KUMARI[2], K. PALSA[3], E. NEELY[3], B. SLAGLE-WEBB[3], S. SIMON[3], J. R. CONNOR[3];
[2]Neural- Behavioral sciences, [3]Neurosurg., [1]Penn State Col. of Med., Hershey, PA

Abstract: Intracerebral hemorrhage (ICH) is characterized by bleeding into the brain parenchyma. In the setting of ICH, iron released from the breakdown of hemoglobin creates a cytotoxic environment in the brain through increased oxidative stress. Interestingly, the loss of iron homeostasis is associated with the pathological process of other neurological diseases. We have previously shown that a commonly found H63D mutation in the homeostatic iron regulatory (HFE) gene acts as a disease modifier that limits oxidative stress. The following study aims to examine the effects of the murine homolog, H67D HFE, on ICH. An autologous blood infusion model was utilized to create an ICH in the right caudate of H67D and WT mice. The motor recovery of each animal was assessed by rotarod. Neurodegeneration was measured using Fluorojade-B and mitochondrial damage was assessed by immunofluorescent numbers of CytC+ neurons and CytC+ astrocytes. Finally, the molecular antioxidant response to ICH was quantified by measuring Nrf2, GPX4, and FTH1 levels in the ICH-affected and non-affected hemispheres via immunoblotting. At 3-days post-ICH, H67D mice demonstrated enhanced performance on rotarod compared to WT animals despite no differences in lesion size. Additionally, H67D mice displayed higher levels of Nrf2, GPX4, and FTH1 in the ICH-affected hemisphere, however, these levels were not different in the contralateral, non-ICH-affected hemisphere. Furthermore, H67D mice showed decreased numbers of degenerated neurons, CytC+ Neurons, and CytC+ astrocytes in the perihematomal area. Our data suggest that the H67D mutation induces a more robust antioxidant response 3-days following ICH through Nrf2, GPX4, and FTH1 activation. Activation of this pathway could explain the decrease in degenerated neurons, CytC+ neurons, and CytC+ astrocytes in the perihematomal region, leading to the improved motor recovery seen in the H67D animals. Investigation into the mechanisms of this response and the effects of the H63D HFE mutation (human homolog) in an ICH patient population is warranted.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.23/V18

Topic: C.09.Stroke

Support: National Institute on Aging of the National Institutes of Health under Award Number RF1AG058603

Title: Arogen mediates PBM cerebrovascular protection

Authors: *Y. FENG[1], Z. HUANG[1], X. MA[1], X. ZONG[2], Q. ZHANG[1];
[1]Dept. of Neurol., Louisiana State Univ. Hlth. Sci., Shreveport, LA; [2]Dept. of Neurol., Louisiana State Univ. Hlth. Sci. Ctr., Shreveport, LA

Abstract: BACKGROUND: Numerous epidemiological studies have reported a link between low testosterone levels and an increased risk of cerebrovascular disease in men. However, there is ongoing controversy surrounding testosterone replacement therapy due to potential side effects. PBMT has been demonstrated to improve cerebrovascular function and promote testosterone synthesis in peripheral tissues. Despite this, the molecular mechanisms that could connect PBMT with testosterone and vascular function in the brain of photothrombotic (PT)-induced stroke rats remain largely unknown. **METHOD:** We measured behavioral performance, cerebral blood flow (CBF), vascular permeability, and the expression of vascular-associated and apoptotic proteins in PT-induced stroke rats treated with flutamide and seven consecutive days of PBM treatment (350 mW, 808 nM, 2 min/d). **RESULTS:** We showed that PT stroke caused a decrease in cerebrovascular testosterone concentration, which was significantly increased by 7-day PBMT (808 nm, 350 mW/cm^2, 42 J/cm^2). Furthermore, PBMT significantly increased cerebral blood flow (CBF) and the expression of vascular-associated proteins, while inhibiting vascular permeability and reducing endothelial cell apoptosis. This ultimately mitigated behavioral deficits in PT stroke rats. Notably, treatment with the androgen receptor antagonist flutamide reversed the beneficial effects of PBMT. Cellular experiments confirmed that PBMT inhibited cell apoptosis and increased vascular-associated protein expression in brain endothelial cell line (bEnd.3) subjected to oxygen-glucose deprivation (OGD). However, these effects were inhibited by flutamide. **CONCLUSIONS:** Our study provides evidence that PBMT attenuates cerebrovascular injury and behavioral deficits following ischemic stroke. Our findings suggest that PBMT may be a promising alternative approach for managing cerebrovascular diseases.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.24/V19

Topic: C.09.Stroke

Support: CIHR (MOP-427791)

Title: Enhancing motor cortex excitability in mice through optogenetic intermittent theta burst stimulation

Authors: *S. ALGHARBI[1], G. SILASI[1,2];
[1]Cell. and Mol. Med., [2]Brain and Mind Res. Inst., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: Animal models of non-invasive brain stimulation paradigms could help optimize stimulation parameters and identify effective stimulation targets for therapeutic stimulation following disease or injury. Intermittent theta burst stimulation (iTBS) is a non-invasive form of brain stimulation that transiently enhances cortical excitability in humans and could be an effective post-stroke therapy when combined with rehabilitation.
We established an iTBS protocol in mice using transcranial optogenetic stimulation. Using blue light stimulation in ketamine/xylazine anesthetized Thy1-ChR2 mice we measured the time course of enhanced cortical excitability based on the amplitude of evoked forelimb movements as well as EEG recordings from the motor cortex. Stimulation was performed through a transcranial chronic window that provides optical access to the motor cortex in both hemispheres, thus allowing repeated assessments of iTBS effects. Laser doppler imaging was used to record blue light-evoked hemodynamic changes in the motor cortex before, during and after iTBS. Our group data show significant potentiation of the amplitude of evoked forelimb movements after iTBS. Preliminary EEG analyses suggest increased overall evoked electrical activity in the motor cortex following iTBS.
Our non-invasive optogenetic stimulation approach enables longitudinal experiments that enable multi-target assessment, control of stimulation parameters, and within-subject measures of treatment effects. By elucidating optimal parameters and targets for iTBS, this research may identify therapeutic interventions to improve function following motor cortex stroke.

Disclosures: S. Algharbi: None. **G. Silasi:** None.

Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR334.25/V20

Topic: C.09.Stroke

Support: NIH Grant R01HD082216

Title: Enhanced phasic calf muscle activation with swing resistance enhances propulsion of the paretic leg in people post-stroke

Authors: *S. PARK[1,2], S. YAN[2], W. DEE[2], R. KEEFER[2], E. ROTH[2], W. Z. RYMER[2], M. WU[2];
[1]Hlth. and Human Performance, Univ. of Houston, Houston, TX; [2]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Reduced propulsion of the paretic leg contributes to impaired walking in people post-stroke. The goal of this study was to determine whether phasic electrical stimulation to the paretic gastrocnemius muscle combined with non-paretic leg swing resistance during walking would enhance muscle activation of the paretic gastrocnemius and propulsive force of the paretic leg. Fifteen individuals who had a stroke visited the lab once to complete two experimental sessions (i.e., cross-over design; session order randomized). Each session consisted of 1) treadmill walking with either "motor stimulation and swing resistance" or "swing resistance only" (10-min walking: 1-min baseline, 7-min adaptation to intervention, & 2-min post-adaptation) and 2) split-belt treadmill walking before and after treadmill walking. Participants showed enhanced muscle activation of the paretic gastrocnemius (P=0.03) and improved anteroposterior ground reaction force of the paretic leg (P=0.01) immediately after the treadmill walking with "motor stimulation and swing resistance", whereas no improvements after the walking with "swing resistance only". These enhanced gastrocnemius muscle activation (P=0.02) and improved ground reaction force (P=0.01) were retained until the late post-adaptation period and 10 min after treadmill walking, respectively. In conclusion, walking with "motor stimulation and swing resistance" may enhance forced use of the paretic leg and improve propulsive force of the paretic leg. Applying phasic electrical stimulation to the paretic gastrocnemius muscle and swing resistance to the non-paretic leg during walking can be used as a novel intervention strategy to improve motor control of the paretic leg and walking in people post-stroke.

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Poster

PSTR334. Stroke Recovery: Non-Pharmacological Approaches to Therapy II

Location: WCC Halls A-C

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Topic: C.09.Stroke

Support: National Natural Science Foundation of China (82101375, 82071338)
Natural Science Foundation of Chongqing (2022NSCQ-MSX1183)
Young Outstanding Talents Program of the First Affiliated Hospital of Chongqing Medical University

Title: Microglial-membrane camouflaged ultrasound and magnetic- responsive nanoparticles loaded with anti-RGMa: Integrated treatment of vascular recanalization and neuroprotection of ischemic stroke

Authors: R. CHENG[1], ***R. ZHANG**[2], X. LUO[1], Y. CAO[3], X. QIN[2];
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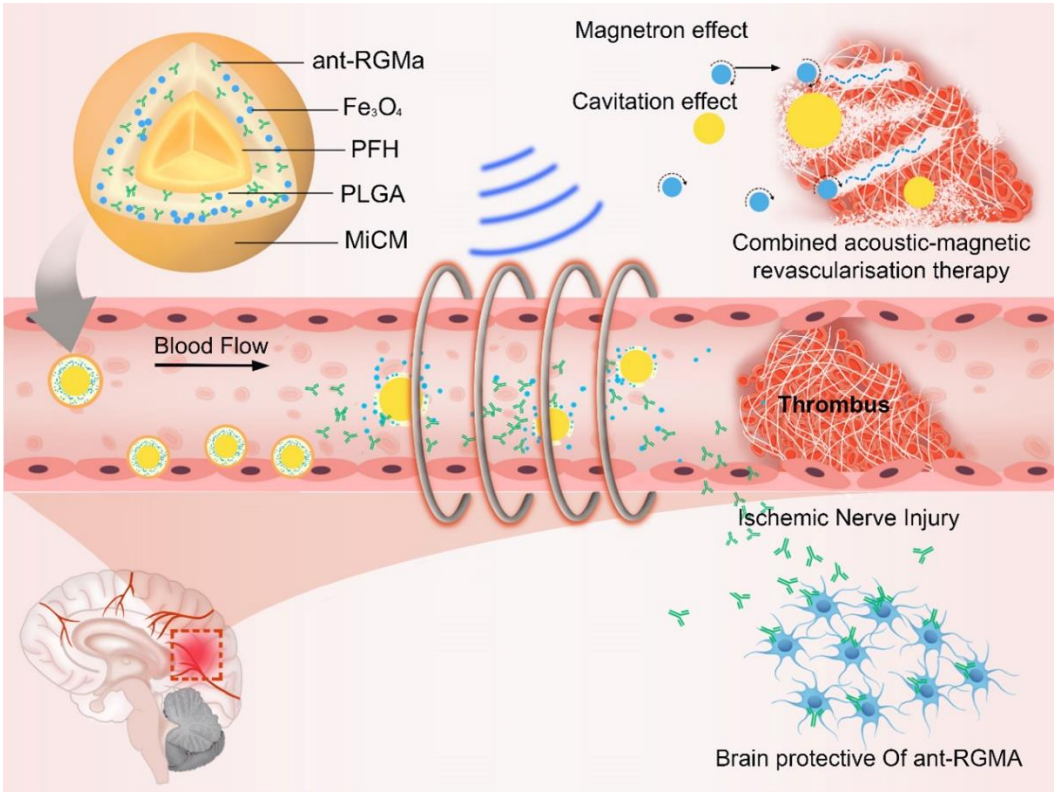
Abstract: The currently widely confirmed treatment of ischemic stroke is to recanalize the blood flow in acute ischemic stroke is intravenous administration of tissue plasminogen activator (tPA) and mechanical thrombectomy (MT). However, tPA has a low recanalization rate and MT has a high risk of intraoperative complications. Furthermore, reperfusion injury occurs after recanalization. Emerging preclinical evidence suggests that neuroprotection treatment adjunctive to reperfusion therapy reduces infarct volume and increases neurological function by protecting ischemic region from reperfusion injury. Thus, it is desirable to develop a novel targeted-therapeutic strategy of ischemic stroke which combines a new method of destroying thrombus with effective neuroprotection drug. In the current study, we developed a new strategy of vascular recanalization and neuroprotection of ischemic stroke by formulating microglial-membrane camouflaged ultrasound and magnetic- responsive nanoparticles (MiCM@PLGA/anti-RGMa/Fe_3O_4@PFH). The results showed that MiCM@PLGA/anti-RGMa/Fe3O4@PFH could target the cerebral clot by MiCM. When applied with external low-intensity focused ultrasound (LIFU) and rotating magnetic field, MiCM@PLGA/anti-RGMa/Fe3O4@PFH disrupt the clot under the energy of the phase change of PFH induced by LICU and the movement of Fe3O4 induced by magnetic field. And we also showed that the nanoparticle released anti-RGMa, which protected ischemic tissue from ischemia/reperfusion injury. Therefore, this new strategy departs from routine methods and reveals the potential use of it as an effective and noninvasive alternative to current therapy.



Disclosures: **R. Cheng:** None. **R. Zhang:** None. **X. Luo:** None. **Y. Cao:** None. **X. Qin:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

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Program #/Poster #: PSTR335.01/V22

Topic: C.10. Brain Injury and Trauma

Support: Partially supported by PAPIIT IN228223

Title: Diurnal variation induced an endogenous neuroprotection in a rat model of traumatic brain injury.

Authors: *R. MARTÍNEZ-TAPIA[1], F. ESTRADA-ROJO[1], T. LOPEZ-ACEVES[3], V. RODRÍGUEZ-MATA[2], E. PULIDO-CAMARILLO[2], A. PÉREZ-TORRES[2], R. NORIEGA-NAVARRO[1], L. NAVARRO[1];
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Abstract: Traumatic brain injury (TBI) is a great public health problem that needs to be studied. It has been described that a TBI induces two types of brain damage: primary and secondary. The long-term perpetuation of the pathophysiological processes of secondary damage has deleterious consequences for neuronal function, survival, and the organism's functionality. Previous literature has described the influence that biological rhythms' influence on the outcome of certain diseases. We use male Wistar rats (250-300 g) habituated to environmental conditions 15 d prior to experiments. We divided the rats into the Day Group (DG) and the Night Group (NG). We induced the TBI with an adapted closed head injury model at 13 h for DG and at 01 h for NG; we also considered a Sham group with no induction of TBI. We performed behavioral tests (neurobehavioral scale, cylinder test, and beam walk test) 24 h before TBI and 24 and 72 h after TBI. At each time point, histopathological damage was also evaluated in different central nervous system regions using three different stains. Finally, we evaluated the glia response with the Iba-1 antibody for microglia and the GFAP-antibody for astrocytes. All tests followed the requirements of the University' Ethics Committee of the School of Medicine (project 018/2016). We found that rats subjected to TBI in the NG lost less body weight than those subjected to TBI in the DG, despite no change in food intake. Then we found that rats subjected to trauma in the NG had a better behavioral performance than those injured in the DG in the three different behavioral tests. Also, we observed that rats with less morphological damage in the perilesional zone (primary motor cortex) and specific areas of the hippocampus (CA1 and DG) were less prone to damage than others (CA2/3) in rats of the NG than in the rats of the DG. Finally, we found that microglia showed differences in branching in the Sham DG vs. NG but not in the TBI groups. Our results showed that the morphological damage induced by TBI depends on the phase of the light-dark cycle in which it occurs. Furthermore, we found that the damage in the perilesional zone is correlated with better behavioral performance and less morphological

damage in rats with TBI during the dark hours (NG) compared with those injured during the light hours (DG). It is important to conduct more experiments on the cellular and molecular characteristics of the microenvironment at the time of TBI. The variation in the excitatory and inhibitory neurotransmitters and their receptors, the immunological function of the microglia and astrocytes, and oxidative stress could be the main factors that determining the degree of damage or tissue preservation after TBI.

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Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.02/V23

Topic: C.10. Brain Injury and Trauma

Title: Heme oxygenase 1 upregulation in a model of repetitive mild traumatic brain injury: opportunities for neuroprotection

Authors: *M. HISKENS[1], A. FENNING[2], A. SCHNEIDERS[2];
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Abstract: The cumulative effect of mild traumatic brain injuries (mTBI) can result in neurodegeneration, however further exploration is necessary to understand the molecular mechanisms that influence this progression for the development of effective therapies. Heme oxygenase 1 (HO-1) is an isoform of the stress protein heme oxygenase that is involved in safeguarding against oxidative stress, regulating apoptosis, and modulating inflammation. Under normal conditions HO-1 is present in low concentration in the brain, however it is induced following TBI and contributes to the neuroinflammatory cascade. Additionally, HO-1 colocalizes with tau in senile plaques and neurofibrillary tangles in several neurodegenerative diseases. In animal models of TBI, HO-1 is overexpressed within areas of hemorrhage, and HO-1 has been detected in human tissues at the borders of lesions following TBI. However, much is still unknown regarding the role of HO-1 in mild TBI without gross pathology, and in repetitive injuries. We investigated this concept by allocating 12-week-old male C57BL/6J mice to mTBI groups receiving either one, five, or 15 impacts across 24 days, or a control group without impact. Mild impacts were administered across 24 days using a modified weight drop apparatus previously shown to induce acute neuroinflammation and chronic neurodegeneration in the absence of lesions. The cortex and hippocampus were collected at two days (acute) and 3 months (chronic) following final injury and were examined by qPCR for HO-1, Tau, GFAP, and IBA1 gene expression (n = 4 per group). To enable blinding conditions, samples were coded so that

group names were not accessible to the investigators undertaking analysis. All procedures were approved by the Central Queensland University Animal Ethics Committee. We found that at acute assessment, gene expression in the cortex and hippocampus showed elevated HO-1, Tau, GFAP, and IBA1 in the 15-impact group, with no change in expression in the 1- and 5-impact groups and control. At chronic assessment, only gene expression of Tau and IBA1 remained elevated in the 15-impact group, with HO-1 and GFAP levels not different to control or 1- and 5-impact groups. In previous animal model studies of moderate/severe brain injury involving intracerebral hemorrage, non-specific heme oxygenase inhibition has shown the ability to attenuate injury. Our results suggest that in mild and repetitive injury paradigms, manipulating the expression of HO-1 could offer a potential avenue for therapeutic intervention.

Disclosures: **M. Hiskens:** None. **A. Fenning:** None. **A. Schneiders:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.03/V24

Topic: C.10. Brain Injury and Trauma

Support: Seoul National University Hospital Grant 0720215034

Title: Fingolimod Protects against Inflammation by Inhibiting NF-ĸB Signaling Pathway after Traumatic Brain Injury in Mice

Authors: ***S. SONG**[1], H. LEE[1,2], J. LEE[1], J. JEON[1], B.-M. OH[1,3];
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Abstract: Traumatic brain injury is one of the leading causes of morbidity and mortality throughout the world. Its increasing incidence, and its fundamental role in the development of neurodegenerative disease, proves that TBI is especially concerning disease.Despite extensive preclinical and clinical studies, researchers have yet to identify a safe and effective neuroprotective strategy. FTY720 acts as a functional antagonist of S1P1 receptors. It is FDA-approved first oral treatment for adults and adolescents with RRMS.The beneficial effects of fingolimod in MS therapy are currently thought to be Down-modulation of adaptive immunity in CNS, which down-regulate S1P1 on lymphocytes, slows egress kinetics of pro-inflammatory T(H17) cells from LNs to the CNS. A total of 52 adult mice were subjected to controlled cortical impact, CCI, or sham injury which underwent craniotomy only. And CCI mice were subjected to high dose fingolimod, low dose fingolimod, or normal saline for control. In the behavioral assessments, fingolimod treated mice showed significantly improved motor performances in Neurological severity score, rotarod tests, cylinder tests, and openfield tests, compared with

control mice. Particularly, MSD multiplex immunoassay showed a trend for pro-inflammatory cytokines IL-1B and IL-6 to decrease and anti-inflammatory cytokine IL-10 in high dose fingolimod treated group compared with the control group in the hippocampus.The levels of NF-κB, quantified by WB, was significantly decreased in the high dose fingolimod treated group in the cerebral cortex compared with the control group.In this study, both high and low dose fingolimod exhibited immunomodulatory effects on neuroinflammation in experimental TBI.Since the safety was approved previously by FDA, Exploring new therapeutic uses of fingolimod for TBI treatment could lead to safe, affordable and timely new treatment options And The dose and therapeutic window should be considered before using fingolimod to treat clinical TBI. **Keywords**:Traumatic brain injury(TBI), NF-ĸB Signaling Pathway, Fingolimod, Controlled cortical impact(CCI), Neuro Inflammation

Disclosures: S. Song: None. **H. Lee:** None. **J. Lee:** None. **J. Jeon:** None. **B. Oh:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.04/V25

Topic: C.10. Brain Injury and Trauma

Support: NIGMS NIH Grant R01GM140143

Title: Real time measurement of vancomycin pharmacokinetics in the brain of mice via electrochemical, aptamer-based sensors

Authors: *K. SCIDA[1], M. DEPASQUALE[1], G. V. CARR[1], N. ARROYO-CURRÁS[2]; [1]Drug Develop., Lieber Inst. for Brain Develop., Baltimore, MD; [2]Pharmacol. and Mol. Sci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Electrochemical, aptamer-based (E-AB) sensors enable real-time pharmacokinetic measurements in the body of research animal models. Aptamers are synthetic DNA sequences that are selected in vitro for their ability to bind with high affinity to a specific target. The sequences are typically modified at their 5’ and 3’ ends with a redox reporter and a thiol linker, respectively. The thiol linker aids in the attachment of the aptamer to gold surfaces (i.e., the sensor surface) and the redox reporter is used for signal transduction. The aptamers used in the E-AB sensor platform undergo changes in electron transfer when binding to their target. These changes directly affect the currents measured via voltammetry, providing an instantaneous relationship between signal strength and target concentration. E-AB sensors have been successfully deployed in vivo in the circulatory systems of rats for real time tracking of small molecule drugs, including aminoglycoside (tobramycin, gentamycin, and kanamycin) and glycopeptide (vancomycin) antibiotics, chemotherapeutics (irinotecan, doxorubicin), and metabolites such as adenosine and phenylalanine, amongst others. Within the past 2 years, E-

ABs were deployed in the brain of rodents to measure the passage of drugs, such as procaine and vancomycin, through the blood-brain barrier (BBB). The latter, was the first-ever translation of this technology to tissue measurements, that is, the brain of mice (C57BL/6J males). In such publication, we demonstrated the ability to monitor vancomycin arrival to the cortex immediately following a 75 mg/kg IV bolus. Moreover, the drug reached a plateau after 1 h with no decay the following 3 hours of measurement, an indication of accumulation in the mouse brain. Here, using a model of penetrating brain injury, we expand our study of vancomycin permeation through the BBB and study its distribution and pharmacokinetics across various brain regions. We demonstrate that following an I.V. bolus of 35 mg/kg (to the tail vein), the concentration of vancomycin reaches a C_{max} of approx. 15 µM (t = 1 h) and 9 µM (t = 3.75 h) in the cortex and hippocampus, respectively. In the thalamus, however, a C_{max} is not reached after 4 h of measurements, but the maximum recorded concentration was of 3.5 µM. Current and future experiments in this project will measure vancomycin concentrations in the cortex following an intraventricular injection and validation of the concentration measurements using gold-standard methods.

Disclosures: K. Scida: None. **M. DePasquale:** None. **G.V. Carr:** None. **N. Arroyo-Currás:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.05/W1

Topic: C.10. Brain Injury and Trauma

Support: Grant from Arabian Gulf University Bahrain #: (E006-PI-04/17).

Title: The effects of caffeine on the frontal cortex of type 2 diabetic rats, a histological and immunohistochemical study

Authors: *M. A. M. OTHMAN;
Anat., Arabian Gulf Univ. Col. of Med. and Med. Sci., Manama, Bahrain

Abstract: Background: Type 2 diabetes (T2D) is linked with injury in many organs, including the brain, which can result in cognitive impairment and dementia. This may be due to degenerative changes such as neuronal loss, demyelination, and apoptosis. The psychoactive beverage, caffeine, was reported to reduce the risk of cognitive and memory impairment. The aim of this work was to study the neuroprotective role of caffeine in the frontal cortex of T2D rats. **Materials & methods:** Twenty-four male Wistar rats, divided into 4 groups (6 each) were used; Group 1 (Control), group 2 (Diabetic), group 3 (Diabetic + Caffeine), and group 4 (Caffeine only). Type 2 diabetes was induced in rats by feeding them with a high-fat high-sugar diet and injecting them (intraperitoneally) with a single low dose of streptozotocin. Other

diabetic rats were given caffeine, orally for 5 weeks. After sacrificing the animals, brains were fixed in 10% formalin and processed to obtain paraffin blocks, for general histological evaluation and immunohistochemistry. Markers for neuronal, myelin, and apoptosis were used. **Results:** Histological evaluation of the frontal cortex of diabetic rats revealed cellular degeneration. Additionally, there was a decrease in the immunostaining of neuronal and myelin markers and an increase in the immunostaining of the apoptotic marker in diabetic rats. When caffeine was administered to diabetic rats, there was an improvement in the structural changes and immunomarkers' expression. **Conclusion:** Caffeine resulted in neuroprotection suggesting an ameliorative role in frontal cortical degenerative changes in T2D.

Disclosures: M.A.M. Othman: None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.06/W2

Topic: C.10. Brain Injury and Trauma

Support: The Deputyship for Research & Innovation "Ministry of Education" in Saudi Arabia, grant number "IFKSUDR_H163"

Title: Therapeutic Benefits of Quercetin in Traumatic Brain Injury Model Exposed to Cigarette Smoke

Authors: *F. ALQAHTANI, Sen[1], Y. ALI[2], W. QAMAR[2], M. ALMUTAIRI[2], T. ALBEKAIRI[2], I. IMRAN[3];
[1]Dept. of Pharmacol. and Toxicology, Col. of Pharmacy, King Saud Univ., Riyadh, Saudi Arabia; [2]Dept. of Pharmacol. and Toxicology, King Saud Univ., Riyadh, Saudi Arabia; [3]Dept. of Pharmacology, faculty of Pharm., Bahauddin Zakariya Univ., Multan, Pakistan

Abstract: Therapeutic Benefits of Quercetin in Traumatic Brain Injury Model Exposed to Cigarette Smoke
Scientific evidences reported deleterious effect of cigarette smoking or passive smoking on the brain health particularly cognitive functions, blood brain barrier (BBB) permeability, up-regulation of inflammatory cascades and depletion of efficient antioxidant system. These combined effects become more progressive in the events of stroke, traumatic brain injury (TBI) and many other neurodegenerative diseases. In the current study, we planned to investigate the long-term administered therapeutic potential of quercetin in ameliorating the deleterious neurobiological consequences of chronic tobacco smoke exposure in the TBI mice. After the exposure of 21 days of cigarette smoke and treatment with 50mg/kg of quercetin, C57BL/6 mice were challenged for the induction of TBI by weight drop method. Subsequently, the battery of behavioral tests, immunohistochemical analysis and mRNA expression levels for inflammatory,

apoptosis and BBB integrity markers were assessed. Our results revealed the beneficial effect of quercetin on the locomotive and cognitive function of mice in TBI + smoked group ($P<0.05$ vs control sham). Immunohistchemistry analysis (Nrf2, HO-1, NFkB, Caspase 3) and mRNA levels (Nrf2, HO-1, Bax, NF-kB, occludin, claudin, and ZO-1) demonstrated the marked protection by 21 days of quercetin treatment in chronic tobacco smoked group possibly by up-regulation of antioxidant pathways, decreased apoptosis and preserveness of BBB integrity markers. In conclusion, our findings support the therapeutic effectiveness of quercetin in partly protecting the central neurological functions that become aberrantly severe in combined habitual cigarette smoked individual impacted with TBI.
Keywords: traumatic brain injury; tobacco smoke; quercetin; behavioral; antioxidant

Disclosures: **F. Alqahtani:** 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.; The Deputyship for Research & Innovation "Ministry of Education" in Saudi Arabia. **Y. Ali:** None. **W. Qamar:** None. **M. Almutairi:** None. **T. Albekairi:** None. **I. Imran:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.07/W3

Topic: C.06. Neuromuscular Diseases

Title: Schwann cells respond to axon injury with distinct neuroprotective programs

Authors: *B. BEIROWSKI[1], E. BABETTO[2];
[1]Neurology, Neurosci. Res. Inst., Ohio State Univ., Columbus, OH; [2]Neurology, Neurosci. Res. Inst., The Ohio State Univ., Columbus, OH

Abstract: Schwann cells (SCs) rapidly sense axon injury and mount unique neuroprotective injury responses. We recently reported that, upon axon injury, SCs reprogram their bioenergetic phenotype toward glycolysis to support axon integrity through enhanced axoglial metabolic coupling. This early adaptation to stabilize perturbed axons is promoted by mTORC1 signaling in SCs. In parallel to this bioenergetic remodeling, SCs flanking injured axons are widely known to undergo a transformation to a repair cell phenotype coordinated by the transcription factor c-Jun. An important component of this phenotypic conversion is the activation of the autophagy machinery in SCs. This raises the possibility that the bioenergetic injury response of SCs is an intrinsic feature of the c-Jun-regulated repair program. Surprisingly, despite the key roles of c-Jun and autophagy in the regulation of cellular metabolism, here we demonstrate no impact of glial c-Jun and autophagy inactivation on the bioenergetic reprogramming of SCs and the survival of injured axons. This indicates fundamentally different functions of the bioenergetic versus the c-Jun regulated injury responses in SCs for the biology of axons. We propose that the

c-Jun-independent bioenergetic changes of SCs are aimed at the recovery of compromised axons segments not destined to degeneration. In contrast, the c-Jun-mediated reprogramming of SCs to repair cells is intended to aid the regeneration of new axons following the disintegration of distal axon segments unable to recover. We conclude that SCs respond to axon injury with distinct neuroprotective programs directed at topographically different aspects of axon repair.

Disclosures: B. Beirowski: None. **E. Babetto:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.08/W4

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: W81XWH1810166
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W81XWH2210462

Title: Accelerating Recovery in Post-Traumatic Hydrocephalus: Normalizing Cerebrospinal Fluid Dynamics with Novel Immunomodulatory Compounds

Authors: *A. ODUKOYA[1], *A. ODUKOYA[1], T. HECK[2], Y. KITASE[2], L. L. JANTZIE[2], S. ROBINSON[1];
[1]Neurosurg. (Pediatric), [2]Pediatrics, Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Post-traumatic hydrocephalus (PTH) is a serious complication of traumatic brain injury (TBI). Several weeks often occur between the initial TBI and the development of symptomatic hydrocephalus requiring shunt insertion. This delay between the TBI and PTH provides a window of opportunity for therapeutic intervention to prevent a permanent shunt. We discovered that neuroinflammation broadly impacts CSF dynamics. Ongoing inflammation impacts secretion of trophic and toxic factors into CSF by the choroid plexus and ependyma, and CSF reabsorption by the glymphatic system. Our data confirm widespread neuro-inflammation is propagated by immune cells and responds to immunomodulation. These results inspired us to design a novel, second-generation, neuroreparative cocktail that acts synergistically to restore CSF dynamics. Based on prior work, we chose to test a cocktail therapy with roxadustat (ROX), a member of a new class of oral prolyl hydroxylase domain inhibitors, plus high dose melatonin (MLT). To induce PTH secondary to systemic inflammation and TBI, young adult rats received 3 mg/kg LPS or saline control intraperitoneally on postnatal day 21 (P21) and P23. On P25, rats of both injury groups underwent TBI via impact. Injured rats of both sexes were then randomly allocated to a dosing regimen for ROX (10mg/kg)+MLT(20mg/kg) or saline vehicle. We piloted a 10-day regimen administered from P26-P36. Differences were examined using two-way

ANOVA with Bonferroni correction ($p<0.05$; n=10-15/group). Rats with PTH developed ventriculomegaly and elevated intracranial pressure at P45 consistent with PTH ($p<0.01$). Treatment with ROX+MLT attenuated increases in intracranial pressure, stabilized glymphatic flow, and normalized brain and serum cytokines concomitant with diffusion metrics in PTH animals (all $p<0.05$). Sham and ROX+MLT-treated PTH rats performed better on a touchscreen test of cognitive flexibility and visual discrimination. Specifically, they required fewer correction trials per session, than vehicle treated PTH rats all $p<0.05$. In conclusion, chronic inflammation may suppress spontaneous neurological recovery and accelerate development of PTH. Neuroimmunomodulation with a cocktail after TBI may provide a clinically-viable therapeutic strategy to prevent PTH.

Disclosures: **A. Odukoya:** None. **A. Odukoya:** None. **T. Heck:** None. **Y. Kitase:** None. **L.L. Jantzie:** None. **S. Robinson:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.09/W5

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: Abbvie
Boston Scientific
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Medtronic
MSA coalition
Abbott
American Academy of Neurology
Boston Scientific
Brainlab
Ipsen
Merz
Movement Disorders Society
Sunovion

Title: Concussions and idiopathic normal pressure hydrocephalus: is there a correlation?

Authors: *M. HANCOCK[1], A. FASANO[2], G. SORRENTO[2], D. TANG-WAI[2], C. TARTAGLIA[2];
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Abstract: Title: Concussions and idiopathic normal pressure hydrocephalus: Is there a correlation?Mack J Hancock[4], Gianluca Sorrento, PhD[2], David Tang-Wai, MD[2], Carmela Tartaglia, MD, Alfonso Fasano, MD, PhD[1,2,3]1 Edmond J. Safra Program in Parkinson's Disease, Morton and Gloria Shulman Movement Disorders Clinic, Toronto Western Hospital, UHN, Toronto, Ontario, Canada Division of Neurology, University of Toronto, Toronto, Ontario, Canada2 Krembil Brain Institute, Toronto, Ontario, Canada3 Center for Advancing Neurotechnological Innovation to Application (CRANIA), Toronto, Ontario, Canada4 Dalhousie University **Corresponding author:** Prof. Alfonso Fasano, MD, PhDChair in Neuromodulation, UHN and UoTProfessor of Neurology, University of TorontoMovement Disorders Centre, Toronto Western Hospital399 Bathurst St, 7McL410 - Toronto, ON Canada M5T 2S8e-mail: alfonso.fasano@uhn.ca
INTRODUCTION: Traumatic brain injury (TBI) is a common cause of morbidity and mortality. Some studies have hypothesized a link between TBI and neurodegeneration. Normal Pressure Hydrocephalus (NPH) is a heterogeneous disorder with different etiology, including TBI. Some NPH patients have no apparent cause, thus are labeled as idiopathic (iNPH). iNPH is a condition commonly underdiagnosed and poorly understood. Previous studies have shown that concussion or TBI can increase the risk of neurodegenerative diseases such as AD and PD (Fleminger et al., 2003; Bramlett & Dietrich., 2015). The relationship between TBI and secondary NPH, led us to the question of whether mild TBI plays a role in iNPH, an association unexpectedly not explored before. In this study we gathered data on the association between a history of TBI - even if mild - and later development of iNPH.**METHODS:** The history of head trauma was collected in a consecutive series of 54 iNPH patients by means of a case report form consisting of history of TBI assessment through the Ohio State University TBI-ID (OSU TBI-ID) (Corrigan, J. D., & Bogner, J. (2007) and Brain Injury Screening Questionnaire (BISQ) designed by the Icahn School of Medicine (Dams-O'Connor, K., et al., 2014) and compared with 50 patients diagnosed with Parkinson's, as well as an age- and sex-matched control group of 40 healthy subjects.**RESULTS:** 74% of iNPH patients reported at least one minor head trauma throughout their lifetime, as opposed to 50% and 27.5% of patients diagnosed with Parkinson's and health controls, respectively**CONCLUSIONS:** Our preliminary findings indicate a possible association between TBI and iNPH, association needs to be further explored by future studies.

Disclosures: M. Hancock: None. **A. Fasano:** None. **G. Sorrento:** None. **D. Tang-Wai:** None. **C. Tartaglia:** None.

Poster

PSTR335. Brain Injury: Neuroprotection, Neurodegeneration, and Factors Affecting Recovery

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR335.10/W6

Topic: C.10. Brain Injury and Trauma

Support: Department of Veterans Affairs RR&D IK2-RX003376 (O'Donnell)
Department of Veterans Affairs [BLR&D I01-BX005017 (Cullen)

Department of Neurosurgery, Perelman School of Medicine, University of Pennsylvania (Petrov)

Title: Traumatic brain injury with coma, neurocritical care, and recovery in swine

Authors: *J. C. O'DONNELL[1,2], K. D. BROWNE[1,2], D. HAN[1,2], N. FEDORCZAK[1,2], M. R. GROVOLA[1,2], K. L. WOFFORD[1,2], D. K. CULLEN[1,2,3], D. PETROV[1,2];
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Abstract: Positive outcome following moderate-to-severe traumatic brain injury (TBI) often requires monitoring and responding to secondary injury processes, such as high intracranial pressure (ICP). Recapitulating the injury mechanisms (e.g., rotational loading) and responses (e.g., coma) observed in humans is necessary for translational study of the resultant neurocritical care phase and beyond. Herein we report on our study applying neurocritical care during coma followed by subacute recovery in the swine rotational head acceleration model of TBI. Femoral artery and internal jugular vein catheterizations were performed to allow for sampling, blood pressure monitoring, and drug administration. A lumbar drain was placed to facilitate cerebrospinal fluid (CSF) sampling. Anesthetized subjects were then secured to a pneumatic actuator that provided rapid rotational acceleration of the head in the sagittal plane (sham subjects were secured to the device without activating it). Immediately following TBI or sham injury subjects were transferred to our swine neuroICU, and a quad-lumen bolt was secured 1cm rostral to bregma for placement of a parenchymal ICP probe, PbtO2/temp sensors, depth electrode, and microdialysis probe. After probes were placed and subjects were stable, total intravenous anesthesia with propofol/fentanyl was initiated and isoflurane was shut off. Animals were monitored continuously in the ICU for a minimum of 6 hours, after which they were transferred out of the ICU when neurological assessments indicated emergence from coma. EKG, SpO2, capnography, blood pressure, ICP, PbtO2/temperature, and EEG (depth+scalp) were time-synchronized and continuously recorded with waveform resolution on a Moberg CNS-200. We collected arterial blood, CSF, microdialysate, and urine. We continued to collect blood and CSF following tranfer out of the ICU. Neuroimaging was conducted one day prior to injury and 7 days post injury immediately prior to euthanasia and perfusion. Events mirrored those seen clinically (e.g., apnea, coma, increased ICP, etc.) and were managed according to treatment algorithms adapted from the clinic (e.g. mechanical ventilation, sedation, hypertonic saline, vasopressors, etc.). A large animal model replicating the mechanisms and manifestations of human TBI is essential to bridge the translational gap between rodent studies and clinical trials. This paradigm further increases translational relevance and allows for preclinical study of neurocritical care, while extending the study period for moderate-to-severe TBI with coma.

Disclosures: J.C. O'Donnell: None. **K.D. Browne:** None. **D. Han:** None. **N. Fedorczak:** None. **M.R. Grovola:** None. **K.L. Wofford:** None. **D.K. Cullen:** None. **D. Petrov:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.01/W7

Topic: C.10. Brain Injury and Trauma

Title: Novel closed-head, awake model of repetitive concussion with momentary loss of consciousness in mice

Authors: *E. K. BRENGEL, S. BALAJI, P. P. KULKARNI, C. F. FERRIS;
Ctr. for Translational Neuroimaging, Northeastern Univ., Boston, MA

Abstract: Mild traumatic brain injury, commonly known as concussion, is the most prevalent form of head trauma, with over 2 million injuries documented per year in the U.S. alone. Of particular concern are cases of repetitive concussion, a risk factor for neurodegenerative disease onset later in life. In order to develop treatments and better understand the mechanisms underlying increased neurodegenerative risk, preclinical models must be developed with maximal translational value. In pursuit of this aim, our lab has adapted an existing momentum exchange rat model of concussion for repetitive use in mice, preserving skull integrity and minimizing the experimental confound of anesthesia. Adult female C57BL/6 mice were lightly anesthetized with isoflurane, subcutaneously injected with 0.1 mg/kg buprenorphine to minimize pain, and secured atop a chassis with the head angled downward. A helmet consisting of a firm 0.5 mm 3D-printed resin secured to an interchangeable 2.5 mm polyurethane sheet was used to distribute the impact, preventing skull fracture. Upon arousal to a respiration rate above 200 bpm, a pneumatic 50g rubber-tipped impactor was accelerated to an impact velocity of 3.9 m/s approximating Bregma. This generates an energy input of 0.38 J, consistent with existing mouse concussion models. Head motion during arousal was minimized by use of a bite bar and a neck restraint, however the linear and rotational acceleration responsible for the shearing forces of concussion are preserved as the head depresses a 5 mm soft foam pad and the chassis is pushed along its tracks. Our initial pilot has yielded a 100% survival rate (n=4) with no signs of gross anatomical injury and an average loss of consciousness of approximately 60 seconds, defined by time until resumption of spontaneous ambulation upon return to the home cage. No such delay was observed in uninjured sham controls (n=2). Further model validation with a larger cohort is ongoing, with mice undergoing concussion or sham injury for three consecutive days followed by diffusion and functional connectivity MRI on the first- and fourteenth-days post-injury. Upon validation, this model will be applied to a third cohort to examine the interaction of repetitive concussion with high-fat Western diet consumption as neurodegenerative risk factors longitudinally, from time-of-injury in early adulthood to end-of-life. Three rounds of diffusion MRI will be used to track changes in neuroinflammation and myelination alongside behavioral assays to track changes in memory and aggression. Development of this model will create a reliable foundation towards maximizing the translational value of preclinical concussion research.

Disclosures: **E.K. Brengel:** None. **S. Balaji:** None. **P.P. Kulkarni:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ekam Imaging. **C.F. Ferris:** E. Ownership Interest (stock, stock

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.02/W8

Topic: C.10. Brain Injury and Trauma

Support: CDMRPL-21-0-EP210005

Title: Increase of glial reactivation in brain of rats exposed to repeated blast-induced traumatic brain injury and its possible association with post-traumatic epilepsy.

Authors: *F. ROSSETTI, M. N. FLEETWOOD, D. M. WILDER, J. B. LONG;
Walter Reed Army Inst. of Res., Silver Spring, MD

Abstract: Post-traumatic epilepsy (PTE) is a devastating disorder that can develop at different intervals following traumatic brain injury (TBI) or potentially following repetitive low-level insults to the brain. The interval, incidence, and severity of TBI(s) are crucial variables influencing development of PTE, and several risk factors such as loss of brain tissue, neuroinflammation, loss of consciousness or amnesia are noted among individuals who suffered head injuries and consequently developed PTE. Often, in the absence of symptoms, military personnel resume duties where they are exposed to new or ongoing insults without an adequate period of safe recovery. This repetitive exposure likely increases the risk of developing PTE, and when blast exposure is involved, this risk is poorly defined. The objective of this work was to characterize an animal model that provides insights into the risks of development of PTE caused by repetitive blast exposure in rats. Twenty-four male Sprague Dawley rats were exposed to overpressures 5 times in a week (24 hr intervals), using an Advanced Blast Simulator to replicate repetitive open field blast waves. An additional group of twenty-four animals was anesthetized and handled on the same scheduled without overpressure exposure (Sham). One group of animals (12 blasted and 12 shams) was euthanized 3 days after the last exposure and other group (12 blasted and 12 shams) was euthanized at 14 days. The brains were removed, and 40µm brain slices were collected to evaluate degeneration and neuroinflammatory immunohistochemistry. Quantitative analysis of reactivated glia density (cell/mm^2) was performed in caudate putamen, dentate gyrus, medial thalamus, basolateral and central amygdala, piriform and motor cortex brain structures. The results reveal a statistically significant increase of glial reactivation in hippocampus, amygdala, and piriform cortex at 3 and 14 days post-exposure. These results show that neuroinflammation acutely appeared in important structures related to seizures, and persisted until 14 days after last blast exposure, providing a possible signature indication of conditions evolving into PTE. Further characterization of this model, including EEG assessments revealing PTE, may provide a useful platform for development of therapies and healing strategies.

Disclosures: **F. Rossetti:** None. **M.N. Fleetwood:** None. **D.M. Wilder:** None. **J.B. Long:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.03/W9

Topic: C.10. Brain Injury and Trauma

Title: Development of a long-term high-channel count implant to track hippocampal hyperexcitability in an awake, behaving rat model of post-traumatic epilepsy

Authors: A. GIBSON[1], S. BOEHMAN[2], *P. KOCH[3];
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Abstract: Post-traumatic epilepsy (PTE) is a significant long-term complication of moderate-to-severe traumatic brain injuries (TBI), which are associated with 20% of all seizure disorders. There are currently no effective biomarkers or therapies to prevent PTE, as the changes in the post-TBI brain which lead to the development of late spontaneous seizures are unknown. A well-characterized loss of mossy cells within the dentate gyrus (DG) hilus of the hippocampus occurs in both humans post-TBI, as well as in Sprague Dawley rats which have undergone an established lateral fluid percussion injury (FPI) model of PTE. There is a sizable in vitro and ex vitro literature on these acute effects of traumatic injury on the brain, especially relating to the importance of the gating action of DG hilar mossy cells on perforant path input from the entorhinal cortex as part of the trisynaptic loop. However, these models do not capture the complexity of the resulting network dysfunction in the intact brain, nor how these changes post-injury may evolve over time and contribute to the late seizures which characterize PTE. To address this we have developed a long-term (up to 8 months), high-channel-count recording apparatus in freely behaving, adult male Sprague-Dawley rats undergoing an FPI model of PTE. The assembly hosts a 64-channel probe placed along the dorsal CA1-DG axis, multiple pairs of bipolar electrodes targeting perilesional cortex as well as contralateral EEG targets. Precise probe localization to the CA1-DG axis was achieved using the region's known laminar pattern of sharp wave ripples in the CA1 and discrete local field deflections of the DG known as dentate spikes (DS), creating complementary sinks and sources of DS activity along this axis which are also observed in human laminar recordings of this region. By taking advantage of this characteristic activity, we were able to identify superficial drift of the probe from post-injury day 20 to day 36, which was then correctable to achieve stable probe positioning by post-injury day 50 with no significant loss of Broadband Root Mean Square (RMS) voltage observed over time in serial recordings of freely moving rats.

Disclosures: **A. Gibson:** None. **S. Boehman:** None. **P. Koch:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.04/W10

Topic: C.10. Brain Injury and Trauma

Support: Weston Brain Institute # TR192003

Title: Characterization of EEG epileptiform activity and pathophysiology after repetitive mild traumatic brain injuries (rmTBIs) in APP^{NL-F} and APP/PS1 mouse models of Alzheimer's disease.

Authors: *V. CARRIQUIRIBORDE[1], J. YUE[2], S. TOK[1], T. YILDIRIM[1], M. KELLY[1], J. FAN[3], W. H. CHENG[3], C. L. WELLINGTON[3], D. J. VOCADLO[2], B. A. KENT[1]; [1]Psychology, [2]Mol. Biol. and Biochem., Simon Fraser Univ., Burnaby, BC, Canada; [3]Pathology and Lab. Med., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Seizures occur at higher rates in patients with Alzheimer's disease (AD) than in the general population. This increased risk for seizures has been linked to increased neuronal hyperexcitability, as indicated by more frequent subclinical epileptiform activity (SEA) in AD mouse models. Repetitive mild traumatic brain injuries (rmTBIs) are a known risk factor for AD and previous studies have shown increased AD-related physiological markers after rmTBIs, including neuronal hyperexcitability in mice. This study investigates the effects of rmTBIs on driving the initiation of the pathophysiologies in AD mouse models. Specifically, we aim to characterize intracranial electroencephalographic (EEG) activity, assess neurological injury by measuring neuroinflammatory biomarkers in blood plasma, and quantify soluble and insoluble Aβ42 fragments and Aβ-plaque formation in brain tissue one month after rmTBI. To do this, we used the closed-head impact model of engineered rotational acceleration (CHIMERA) method to deliver rmTBIs to two APP mouse models, APP^{NL-F} (n=18) and APP/PS1 (n=18), at six months of age. We used both female and male mice. One month after the rmTBIs, we recorded 72 continuous hours of EEG, followed by brain and plasma sample collection. Using the Seizure Pro Sirenia software, we searched for seizures and SEA, which was further divided into single spikes and poly-spikes. Only one APP/PS1 mouse showed seizures at one month post rmTBI, and no seizures were found in the 72 h recordings of the other mice. Preliminary results show an increase in SEA (both single spikes and poly-spikes) in the APP/PS1 rmTBI group. Using a Simoa assay, we found increased neurofilament light (NF-L) and glial fibrillary acidic protein (GFAP) in the plasma of the APP/PS1 mouse line, indicating sustained neurological injury one month post-TBI. Immunostaining of Aβ-plaques did not reveal injury-induced amyloidosis in the cortex and hippocampus, suggesting pathology-independent SEA following rmTBI. To further explore the connection between Aβ and the emergence of SEA, we will analyze the deposition of soluble and insoluble Aβ42 fragments, representing an early pathogenic biomarker of AD, in brain tissue using enzyme-linked immunosorbent assay (ELISA). Collectively, our study will

enhance understanding of the pathophysiological mechanisms following rmTBIs in AD, which may help guide post-TBI clinical care designed to minimize long-term risk of neurodegeneration.

Disclosures: V. Carriquiriborde: None. **J. Yue:** None. **S. Tok:** None. **T. Yildirim:** None. **M. Kelly:** None. **J. Fan:** None. **W.H. Cheng:** None. **C.L. Wellington:** None. **D.J. Vocadlo:** None. **B.A. Kent:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.05/W11

Topic: C.10. Brain Injury and Trauma

Support: Rostree Trust Seedcorn2021/100082

Title: Longitudinal changes in brain pathology in the closed head injury model of traumatic brain injury

Authors: *L. E. ABELLEIRA HERVAS, E. MCFAUL, S. LIANG, M. SASTRE;
Brain Sci., Imperial Col. London, London, United Kingdom

Abstract: Introduction: Traumatic brain injury (TBI) is one of the most common forms of neurological damage. TBI has been linked to later neurodegeneration, including Alzheimer's disease, so understanding the neurological mechanisms behind it is imperative. The association between TBI and dementia may involve changes in glial activation, white matter damage and alterations in blood brain barrier (BBB), which can change over time. The aim of our study was to establish and characterize a closed head injury (CHI) mouse model of TBI, without involving skull fractures, and to investigate functional and pathological alterations over time.
Methods: We induced the CHI in adult wild-type C57 Bl6 males and females with an electromagnetic impactor equipped with a 5mm flat steel tip (Leica Impact One). The impact was delivered at bregma (ML=0.0mm, AP=0.0mm) with a velocity of 4.0m/s, a dwell time of 100ms, and an impact displacement of 1mm. During the impact, the head of the animals was not fixed, to replicate the acceleration and deceleration forces experienced during a head impact. Sham animals were exposed to the same procedure but did not receive an impact. We investigated the effect of CHI at two different time points (7 or 28 days post-injury) on locomotion, anxiety and memory using the fear conditioning test, novel object recognition as well as the open field test. In addition, in the same animals we assessed changes in microglia and astrocyte density as well as BBB disruption by immunohistochemistry and immunofluorescence techniques.
Results: This study demonstrates that CHI resulted in impaired learning and memory, as well as alterations in locomotion, particularly at earlier times post-injury. These changes were associated with focal astrocytic and microglia activation observed mainly in the pericontusional cortical regions, with more pronounced effects at 7 days post-injury. Furthermore, BBB leakage and

reduced blood vessel length were also detected at 7 days post-injury. These effects were more noticeable in females compared with males.
Conclusions: Our results show that the close head injury model displays impairments in behaviour and alterations in brain pathology at short times post-injury, with almost complete recovery seen by 28-days post injury. It also highlights the need to consider sexual dimorphism when conducting TBI research.

Disclosures: L.E. Abelleira Hervas: None. **E. McFaul:** None. **S. Liang:** None. **M. Sastre:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.06/W12

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant RO1EY028039

Title: Axonal degeneration and collateral plasticity in the mouse visual system after traumatic brain injury.

Authors: *A. ALEXANDRIS[1], J. YI[1], Y. LEE[1], Z. ALAM[1], D. S. WELSBIE[2], D. J. ZACK[1], V. E. KOLIATSOS[1];
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Abstract: Traumatic axonal injury (TAI) is one of the most prevalent neuropathologies in traumatic brain injury (TBI) and the cause of significant morbidity due to structural and functional disconnection of neuronal networks. In TAI, the axonal segments located distal to the injury site consistently degenerate due to Wallerian degeneration (WD), a highly conserved molecular program of axon-self-destruction. On the other hand, the regenerative capacity of the proximal axonal stumps is limited, and they typically do not successfully reconnect with their intended targets. Therefore, it is thought that functional recovery after TBI, may depend at least in part on the collateral sprouting of surviving and/or uninjured axons. We and others have shown that suppression of WD (e.g. via genetic ablation of Sarm1) may mitigate axonal degeneration after TBI, making it a promising therapeutic target. However, any potential negative effects on collateral plasticity resulting from WD suppression remain unknown. To address this issue, we leverage they highly compartmentalized anatomy of the murine visual system, in order to study injury-induced degeneration and plasticity of the retinocollicular projections in the context of TAI after impact acceleration TBI. Preliminary analysis in adult C57BL/6J mice revealed that IA-TBI results in 40% reduction of both distal ON axons and terminals in the superior colliculus 7 days after injury; which is followed by partial recovery of terminals at 28 days. Characterizing the time-course of axonal and synaptic degeneration and plasticity in this model system will be pivotal not only for understanding the responses of the

CNS to injury but also for assessing the effect of therapeutic interventions that target axonal degeneration and restoration of neurological function.

Disclosures: A. Alexandris: None. **J. Yi:** None. **Y. Lee:** None. **Z. Alam:** None. **D.S. Welsbie:** None. **D.J. Zack:** None. **V.E. Koliatsos:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.07/W13

Topic: C.10. Brain Injury and Trauma

Support: NIH RO1 NS129922

Title: Cortical spreading depolarizations induced days after experimental traumatic brain injury exhibit characteristics consistent with ongoing damage

Authors: *F. BEST, J. HARTINGS, L. NGWENYA;
Neurosurg., Univ. of Cincinnati, Cincinnati, OH

Abstract: More than 60% of patients with moderate-severe traumatic brain injuries (TBIs) experience cortical spreading depolarizations (CSDs), and these CSDs are associated with poor gross disability outcomes. Depending on the injured tissue's metabolic state, CSDs can have a gradient of effects ranging from complete recovery to cell death. We hypothesized that CSDs would develop more pathologic characteristics through time following experimental TBI. Adult Sprague Dawley male rats were subjected to either a moderate lateral fluid percussion injury or a sham injury. CSDs were induced every 15 minutes with 1M potassium chloride application to the cortex either 1 hour (0dpi), 3 days (3dpi), or 7 days post-injury (7dpi). CSDs were successfully induced in sham+0dpi CSD (n=5), TBI+0dpi CSD (n=4), TBI+3dpi CSD (n=6), and TBI+7dpi CSD (n=4) groups. Cerebral blood flow changes were simultaneously recorded using laser Doppler flowmetry (LDF). TBI+0dpi CSD animals had longer depolarization (1-way ANOVA, $p<0.0001$) and depression durations ($p<0.0001$) compared to the other groups. Transient cerebral blood flow responses all showed hyperemic neurovascular coupling to CSDs. TBI+3dpi CSD animals had longer LDF response durations ($p<0.0001$) than the other groups. TBI+7dpi CSD animals had the largest LDF response amplitudes, while TBI+3dpi CSD animals had the smallest amplitudes ($p<0.0001$). Our study revealed electrophysiologic and cerebral blood flow differences in CSDs induced days after TBI that are consistent with ongoing damage. These findings suggest that the timing of CSDs after injury may further exacerbate secondary tissue damage.

Disclosures: F. Best: None. **J. Hartings:** None. **L. Ngwenya:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.08/W14

Topic: C.10. Brain Injury and Trauma

Support: Browning Family Fund
CDMRP TP210230
USU Intramural

Title: Estradiol alone does not play a role in mediating dysregulation of the HPA axis following mild blast-induced traumatic brain injury

Authors: E. SCHREURS[1], M. RUSNAK[2], *T.-Y. WU[3];
[1]Program in Neurosci., Colgate Univ., Hamilton, NY; [2]Henry Jackson Foundation/Uniformed Services Univ., Bethesda, MD; [3]Uniformed Services Univ., Bethesda, MD

Abstract: Traumatic brain injury (TBI) affects an estimated 69 million people worldwide annually. Mild blast-induced TBI (mbTBI), caused by explosive blast devices, is associated with various long-term effects. One such effect—diagnosis with anxiety-related disorders—is likely caused by dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis, and appears to be sex-dependent. We have previously shown a differential effect of mbTBI on restraint-induced corticosterone (CORT) levels in male versus female C57BL/6J mice, with males demonstrating an increase in restraint-induced CORT with mbTBI while females demonstrated a decrease with mbTBI ($p < 0.05$). Although a reason for these sex-dependent effects of mbTBI on restraint-induced CORT remains unknown, sex steroids, particularly estrogen, may mediate these differences. To determine the role of estradiol 17β in mediating dysregulation of the HPA axis following mbTBI, female C57BL/6J mice were divided into three treatment groups: intact, ovariectomized, and ovariectomized with estradiol replacement. 7 days post-surgery, mice were divided into groups exposed to anesthesia only and groups exposed to a mild blast (~18-20 psi) via the Advanced Blast Simulator (ABS) to determine the effect of mbTBI on basal- and restraint-induced HPA axis reactivity. Steroid condition, blast status, and restraint all had a main effect on CORT levels ($p = 0.003$, $p = 0.034$, and $p < 0.001$, respectively). The results demonstrated an effect of steroid condition and blast status on CORT levels ($p = 0.054$). Steroid condition and restraint both had a main effect on ACTH levels ($p < 0.001$, $p < 0.001$), while blast status did not ($p = 0.254$). No interaction of treatments was observed for ACTH. Interestingly, our data varies from previous data in suggesting an increase, rather than a decrease, in restraint-induced CORT in intact females with mbTBI. This may reflect the differences in study design and time between restraint and serum collection, with data representing one specific time-point of HPA axis activation. Overall, the results suggest that ovarian factors, but not estradiol 17β alone, play a significant role in mediating HPA axis dysregulation with mbTBI in females.

Disclosures: E. Schreurs: None. **M. Rusnak:** None. **T. Wu:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.09/W15

Topic: C.10. Brain Injury and Trauma

Support: NIH IRP ZIA-HD008966

Title: Examining the roles of individual stress response pathways following mild traumatic brain injury

Authors: *E. Y. LLOYD, M. R. ALKASLASI, C. E. LE PICHON;
Natl. Inst. of Hlth. (NIH), Bethesda, MD

Abstract: Mild traumatic brain injury (mTBI) results in physical damage to the brain including tissue bruising, axon shearing, and vasculature disruption. Such injury induces the activation of neuronal stress response pathways including the dual leucine zipper kinase (DLK) pathway, Sarm1 axon degeneration pathway, and the integrated stress response (ISR) pathway which have been shown to be essential for neurodegeneration and neuroinflammation in various injury and disease contexts. These pathways are highly interconnected and share a number of downstream effectors including activating transcription factor 3 (Atf3), and C/EBP homologous protein (CHOP). We have recently demonstrated that layer V projection neurons in the cortex selectively activate certain stress responses one week following mTBI. Here, we investigate the role of stress response factors in mTBI-induced neurodegeneration, focusing on cell death, dendrite degeneration, and axon swellings, as well as neuroinflammation characterized by an upregulation of microgliosis in the injured region. We generated conditional knockout mice where key drivers of stress responses are selectively knocked out in layer V neurons. The animals underwent unilateral closed head controlled cortical impact injury and were assessed 7 and 14 days post injury to evaluate degeneration and inflammation. We find that genetic knockout of stress response-related factors in a murine mTBI model differentially affects various elements of injury-induced neurodegeneration and neuroinflammation. The observed selectivity suggests that such factors play collaborative roles in injury response to induce neurodegeneration, indicating the need for a more nuanced understanding of neurodegenerative cascades for future development of therapies.

Disclosures: E.Y. Lloyd: None. **M.R. Alkaslasi:** None. **C.E. Le Pichon:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.10/W16

Topic: C.10. Brain Injury and Trauma

Support: TriService Nursing Research Program (TSNRP)
Uniformed Services University Intramural Grant

Title: Effects of mild traumatic brain injury and ketamine on stress hormones, righting reflex, and synaptic plasticity in rats

Authors: *M. L. BOESE[1], H. SPENCER[2], R. Y. BERMAN[3], K. D. RADFORD[4], K. CHOI[5]; [1]Grad. Sch. of Nursing, Uniformed Services Univ. of the Hlth. Sci., HAGERSTOWN, MD; [3]Ctr. for the Study of Traumatic Stress, [4]Grad. Sch. of Nursing, [5]Dept. of Psychaitry, [2]Uniformed Services Univ., Bethesda, MD

Abstract: Glucocorticoid levels (corticosterone [CORT] and progesterone [PROG]) increase in response to injury and stress and play key roles in immune responses, metabolism, cognition, and behavior. In humans, low cortisol levels may be associated with the development of posttraumatic stress disorder (PTSD). Additionally, stress hormones can alter synaptic plasticity, and PROG may modulate secondary injury pathways following mild traumatic brain injury (mTBI). Latency to righting reflex (LRR) is the amount of time required for a rat to awaken and become ambulatory post-injury and is a proxy measure of TBI severity. We have previously demonstrated increased stress hormone responses following an intravenous ketamine infusion in rats in an uninjured condition. However, the effects of mTBI on stress hormones over time following injury are not well described. Furthermore, little is known regarding the effects of intravenous ketamine, a potent analgesic commonly administered to trauma patients, on stress hormones and synaptic plasticity after mTBI. This study set out to examine the effects of mTBI and ketamine on plasma stress hormone levels and synaptic plasticity in the brain. Adult male Sprague Dawley rats with indwelling jugular venous catheters underwent mTBI using the Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA) (3 impacts x 1.5 J, single session). Sham animals underwent the same procedures without impacts. LRR was measured immediately after injury and blood was sampled at 1, 3, 5, and 24-hours post-injury. A ketamine infusion was given 1-hour post-injury (0, 10 and 20 mg/kg, 2-hour infusion). Brain tissue was collected 4 days after injury. Plasma CORT and PROG were measured using enzyme-linked immunosorbent assay (ELISA). Sections of the medial prefrontal cortex and the CA1 region of the hippocampus were used for immunofluorescent labeling for synapsin-1 and PSD-95 as pre- and postsynaptic markers. Puncta detection, separation analysis, and synaptic density were carried out with confocal microscopy and computer analysis via Imaris software. CHIMERA injury elevated CORT levels at 1-hour post-injury. Additionally, CORT levels were positively correlated with longer LRR in CHIMERA, but not sham, animals. These findings indicate that LRR may correlate to injury severity and identify animals that are more susceptible to adverse mTBI outcomes. Additionally, plasma CORT levels following mTBI may serve as a biomarker due to the correlation with LRR. Immunofluorescent labeling and synaptic density analysis following ketamine infusion are in progress. Overall, these results may translate to improved diagnosis and care for mTBI patients.

Disclosures: M.L. Boese: None. **H. Spencer:** None. **R.Y. Berman:** None. **K.D. Radford:** None. **K. Choi:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.11/W17

Topic: C.10. Brain Injury and Trauma

Support: Richard T. Anderson Endowment, OU College of Pharmacy

Title: Mild closed head traumatic brain injury induced long-term neurological deficits

Authors: Y. ZHANG, K. M. STANDIFER;
Pharmaceut. Sci., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Mild traumatic brain injury (mTBI) from repeated concussions in military combat and sports can result in significant long-term neurological consequences. A variety of clinically relevant preclinical closed head injury (CHI) models of mTBI have been established, however, long-term effects of CHI on neurological function have been poorly studied. Neurological and behavioral performance and biomarker expression was examined using a published mTBI CHI model (Jamnia et al., 2017). Isoflurane-anesthetized male SD rats (n=5-6/group) were placed on a foam bed in a stereotaxic apparatus without being fixed; the impact point was marked on skin over the forelimb sensorimotor cortex (CTX). TBI was produced with a 5-mm flat tip at 6.5 m/sec at a depth of 1 cm from the surface of skin for 0.3 sec. Rats received CHI 3 times, 48 hours apart or anesthesia only (sham). Neurological deficits were observed after the second impact and function remained impaired for at least 51 days post-first impact (PFI) as determined by modified neurological severity scoring. Vestibular function, as determined by time on rotarod as % of each rat's baseline time, was impaired from 7-44 days PFI. Data were analyzed by two-way repeated measures ANOVA with Sidak's multiple comparisons post-hoc test. Memory dysfunction was observed at days 14 and 52 PFI using the novel object recognition test; analyzed by unpaired student's t-test ($p<0.05$). Rats showed anxiety-like behaviors at days 9, 30 and 51 PFI, using elevated plus maze and analyzed by unpaired student's t-test ($p<0.05$). Nociceptive sensitivity to mechanical and thermal stimuli remained unchanged over 30 days PFI, thus no further pain tests were conducted. Serum, cerebrospinal fluid (CSF), tissue from CTX and hippocampus (HIP) under the impact site were collected on day 52 PFI, quantified by RIA and infrared imaging of western blotting normalized to loading control. Data were analyzed by unpaired student's t-test and considered different if $p<0.05$. Nociceptin/Orphanin FQ (N/OFQ) levels increased in tissue from CTX and HIP after CHI, but not in serum and CSF. CHI increased N/OFQ peptide (NOP) receptor, Tau and caspase expression in HIP, whereas the Tau, caspase and ubiquitin C-terminal hydrolase L1 (UCH-L1) levels decreased in CTX after CHI. Elevated neurofilament light (NFL) expression by CHI was observed in CTX. GFAP expression did not differ between CHI and control groups. This study validated the long-term effects of repeated CHI on neurological function and biomarker levels in male rats, which will be useful to further explore the mechanisms underlying the development and maintenance of neurological deficits after concussive mTBI.

Disclosures: Y. Zhang: None. **K.M. Standifer:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.12/W18

Topic: C.10. Brain Injury and Trauma

Title: Implications of the Timing of Juvenile Traumatic Brain Injury on Social Development

Authors: *S. SHONKA, M. J. HYLIN;
Psychology, Southern Illinois Univ., Carbondale, IL

Abstract: Traumatic brain injuries (TBIs) during development lead to significant social impairments, with injuries sustained at younger ages resulting in more significant deficits. Deficits observed are affected by stages of development. Injuries during the synaptic pruning or myelination phase can increase synaptic pruning and decrease myelination, respectively, leading to long-lasting social deficits. The medial prefrontal cortex (mPFC), orbitofrontal cortex (OFC) and amygdala are three regions involved in social behaviors and damage to these regions leads to social deficits. In this study, 80 animals (40 female) received a single bilateral injury to the mPFC on post-natal day (PND) 17 or 28. Following the injury, the animals' social preference and memory was assessed at prepuberty (PNDs 35-41), puberty (PNDs 42-48), and young adulthood (PNDs 56-63). Additionally, social dominance was assessed once in young adulthood. The integrity of myelinated axons between the mPFC, OFC, amygdalofugal pathway (AFP), and basolateral amygdala (BLA) were assessed using Luxol-Fast Blue staining. It was hypothesized that PND 17 TBI animals would display less axonal integrity of myelinated axons, thereby also displaying increased social deficits and dominance. The results showed that there were no injury effects across time in social preference. However, day 28 TBI animals demonstrated deficits in social memory at the prepuberty timepoint compared to day 28 sham ($p<0.05$), and day 17 TBI animals ($p<0.05$) but no such deficit continued across development. Likely this was due to the shorter period of time between injury and testing for the day 28 TBI group. Meanwhile, both TBI groups demonstrated increased dominance levels compared to their respective sham group ($p<0.001$), but there were no differences based on the age at injury. Finally, histological results demonstrated a significant effect of age at injury in all brain regions. Specifically, day 17 TBI animals demonstrated less myelin in the OFC ($p<0.001$, $p<0.001$), mPFC ($p<0.001$, $p<0.001$), and AFP ($p<0.05$, $p<0.01$) compared to day 17 sham and day 28 TBI animals, respectively. Overall, the only deficit due to injury was increased dominance behaviors. In day 17 TBI animals this may be related to decreased myelin levels between the frontal lobe and the amygdala. Likely preventing the inhibitory effect of the frontal lobe on the amygdala, leading to higher dominance levels (Akirav & Maroun, 2007). However, since day 28 TBI animals did not display the same trend synaptic pruning may be responsible for increased aggression. We are working on assessing the dendritic morphology in the frontal lobe.

Disclosures: S. Shonka: None. **M.J. Hylin:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.13/W19

Topic: C.10. Brain Injury and Trauma

Support: I01BX004561-01A2

Title: Effects of mild traumatic brain injury on negative urgency and brainstem function

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Abstract: Negative urgency is a dimension of impulsivity that describes the tendency to experience strong impulses while experiencing negative affect. Because our military personnel and veterans are subjected to extreme or prolonged periods of stress, elucidating how negative urgency may manifest among this population can guide us in developing new measures to maintain their safety while on duty. One circumstance under which negative urgency may be exacerbated is after a mild traumatic brain injury (mTBI). Impulsivity is modulated by dopamine and serotonin, neurotransmitters synthesized in the brainstem. Thus, damage to this region may serve as the neurobiological framework behind impulsivity experienced after mTBI. To assess the expression of negative urgency, adult male and female Sprague-Dawley rats acquired a lever-press avoidance task before sustaining a single mTBI using the lateral fluid percussion model. After a short recovery period, the number of anticipatory responses performed during subsequent acquisition and extinction sessions were measured. These responses are characterized by lever presses exhibited during the habituation state at the beginning of each session, where the animal was anticipating impending noxious stimuli. Our data indicate a significant main effect of injury for anticipatory responses performed acutely after mTBI. This effect of injury is then lost across extinction sessions. Our lab has previously shown that the acoustic startle response, a brainstem reflex, is suppressed in rodents after mTBI. A separate cohort of animals recapitulated our lab's previous findings; the magnitude of the startle response was significantly reduced in animals who received a mTBI. This suppression of motor responsivity was sustained for 3 months post-injury. Additional experiments examining how mTBI affects brainstem regions -- potentially underlying either or both of these behavioral changes -- on a cellular level are currently underway. In all, these studies will provide a better understanding of the mechanisms behind behavioral changes experienced after mTBIs that could greatly affect the quality of life for those who served in the military.

Disclosures: V.A. Stiritz: None. **K.D. Beck:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.14/W20

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant1R21NS120628

Title: Corticohabenular circuit dysfunction in a model of repeated mild traumatic brain injury

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Abstract: Mild traumatic brain injuries (mTBI) are the most common form of TBIs, associated with increased likelihood of long-lasting disabilities including impairments in cognition, mood/emotional regulation and social interactions as well as suicidal and risk-taking behaviors. Recently, the lateral habenula (LHb) has emerged as an anti-reward brain region that is implicated in motivation and reward-risk based decision making, and pathophysiology of stress-related disorders including depression, yet an understanding of LHb circuit mechanisms in mTBI-induced psychopathology remains unknown. Recently, we showed that a repetitive closed head injury mTBI model in male mice induced persistent LHb tonic hyperactivity through a shift in synaptic excitation and inhibition (E/I) balance toward excitation and insertion of calcium-permeable (CP) AMPARs in LHb neurons. We demonstrated that limiting LHb hyperactivity by chemogenetic inhibition of LHb glutamatergic neurons was sufficient to reverse mTBI-induced deficits in self-care grooming behavior in male mice (Flerlage et al., 2023). Here, we used optogenetics and identified medial prefrontal cortex (mPFC) inputs to LHb as one of the major sources for mTBI-induced LHb hyperactivity in male mice. Consistently, female mTBI mice showed decreased motivation to self-grooming behavior and potentiation of mPFC-LHb excitatory synapses. However, mTBI reduced spontaneous activity of LHb neurons in female mice. This suggests that mTBI-induced potentiation of excitatory drive from mPFC-LHb circuit may contribute to motivational deficits in both sexes. In future studies, we are using chemogenetic manipulation to mPFC-recipient LHb neurons to explore the role of these specific input in self-care and pro-social behaviors. Support: NIH Grant: 1R21NS120628Conflict of Interest: None to declare

Disclosures: W.J. Flerlage: None. **S. Simmons:** None. **M.C. Tsuda:** None. **S.S. Gouty:** None. **B.M. Cox:** None. **F. Nugent:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.15/W21

Topic: C.10. Brain Injury and Trauma

Support: UMMC Department of Anesthesiology

Title: Diet-specific changes to microbiota following juvenile traumatic brain injury

Authors: *A. M. SMITH[1], L. CHALLAGUNDLA[2], Z. J. WARFIELD[3], M. R. GARRETT[2], C. DOS SANTOS E SANTOS[4], B. E. GRAYSON[5];
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Abstract: The brain-gut axis is a robust interconnection between the CNS and the gastrointestinal (GI) tract. Commensal bacteria within the gut consume the macronutrients ingested by the host to produce a myriad of metabolites that bidirectionally cross the blood-brain barrier. Diets higher in fat and protein shift the brain's metabolic processes to depend on ketones. Elevations in ketones are known to be neuroprotective by unknown mechanisms. Traumatic brain injury (TBI) produces profound structural and cognitive damage and secondarily affects the brain-gut axis by releasing chemokines that alter gut permeability. The present work aimed to investigate the impact of a standard chow diet (SD) vs. a 60% lard fat diet (HD) on fecal microbiome populations following juvenile TBI. Long Evans post-natal day (PND) 20 male rats were placed on the two diets for 10 days before sustaining either Sham TBI or TBI using the Closed Head Injury Model of Engineered Rotational Acceleration (CHIMERA). Time to righting and walking was recorded after piston deployment. Body weight and body composition were quantified. Fecal samples were collected at 1- and 9-days post-injury. Animals were euthanized 10 days post-injury, and terminal trunk blood was harvested. Fecal microbiota DNA was isolated and sequenced using the Illumina Iseq100 platform. Post-sequencing processing was performed using the Microbiome Analyst software. 20 days of HD-feeding resulted in no difference in terminal body weight or lean body mass. However, body fat mass was doubled in HD compared to SD rats. TBI rats have significantly increased time to righting and walking. Plasma triglycerides and β-hydroxybutyrate, a proxy for ketone bodies, were elevated in HD rats. Blood glucose was significantly reduced in HD compared to SD rats. Repeated measures of 3-way ANOVA were used to investigate the main effects of injury, diet, and time to understand the shifts of each classification level. *Cyanobacteria* was the only phylum significantly impacted by both diet and injury, with elevations by diet and reductions in normalized counts by injury. The normalized counts of the phylum *Firmicutes*, one of the major phyla of the gut microbiome, were significantly elevated by injury at 1- and 9-days post-injury. The phyla *Lentisphaerae* and *Proteobacteria* counts were significantly elevated at 9 days post-injury by diet. Interestingly, there was also a significant increase in the counts of *Lentisphaerae* over time. Taken together, mild TBI in a juvenile brain injury model results in significant shifts in phyla. Further work is necessary to determine if these shifts are connected to cognitive and stress-related deficits previously reported.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.16/W22

Topic: D.06. Vision

Support: USAMRDC/MOMRP

Title: Assessment of Diet and Stress Effects on Ocular Vulnerability in Rodent Models of Traumatic Brain Insults using a Multi-Omics Approach

Authors: *M. PATEL[1], R. YANG[2], N. CHAKRABORTY[2], S. A. MILLER[2], J. DEMAR[2], A. BATUURE[3], D. WILDER[3], J. LONG[3], R. HAMMAMIEH[2], A. GAUTAM[2];
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Abstract: Blast injury has been implicated as the major cause of traumatic brain injury (TBI) and ocular system injury, in combat operations in Iraq and Afghanistan. Soldiers exposed to traumatic stress also have undiagnosed, chronic vision problems. Numerous studies have looked at the effects of traumatic insults on the brain however not much work has been conducted on eye injuries. We determined the effect of TBI and traumatic stress on ocular gene expression and assessed the role of nutritional deficiency on the vulnerability to these injury models. In this study, rats were fed with three different diets having varying composition of ω-3 and ω-6 polyunsaturated fatty acids (PUFAs) for 6 weeks prior to TBI or stress and maintained till 14 days post-insult. After anesthesia, animals were exposed to a simulated blast overpressure wave followed by a weight drop head-concussion to induce TBI. Separate rats were subjected to forced immersion underwater to induce traumatic stress. Shams received anesthesia and/or handling. Our findings in rats showed that TBI exposure, within 14 days, produced significant neurobehavioral impairments (i.e., in balance and coordination). Likewise, underwater trauma (UWT) exposure produced aberrant behaviors (i.e., “anxiety” situation avoidance) in animals at 8 days post-insult particularly in DHA-deficient groups. Furthermore, we showed that TBI exposure significantly dysregulated retinal DEGs (Differentially expressed genes) in all the diet groups, with a highest change observed in the DHA-rich house chow diet (Down-regulated: 903, Up-regulated: 825; $p<0.05$), in comparison to DHA-deficient 8% and 1% LA diets. In comparison to animals fed DHA-rich diet, induction of TBI decreased genes involved in neuronal plasticity in DHA-deficient 8% diet while stimulated genes involved in inflammatory signaling in the 1% group. Exposure to UWT had a greater effect on retinal DEGs, particularly in rats fed with 1% diet (2249 down-regulated and 1256 up-regulated). Traumatic stress differentially regulated retinal endocannabinoid-mediated neuronal excitability and calcium signaling in rodents fed with 1% versus 8% diet. The findings in the retina demonstrated distinct transcript profiles for each of the stressor in rats fed with different diets. Together our data

advocates diets as a strong preemptive measure to combat the physical and psychological trauma. This work was intramurally funded by the USAMRDC/MOMRP.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.17/W23

Topic: C.10. Brain Injury and Trauma

Support: Florida Department of Health # 20K09

Title: Electronic cigarette vaping exacerbates cortical contusion after traumatic brain injury in female rats

Authors: *G. G. PEREZ[1], H. PRADHYUMNAN[1], S. H. PATEL[1], O. F. ALONSO[2], H. M. BRAMLETT[3,2,4], A. P. RAVAL[1,4];
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Abstract: Electronic cigarette (EC) nicotine delivery devices have become increasingly popular in recent years. Our understanding of the effects of EC vaping on the brain is incomplete and the effects of vaping on traumatic brain injury (TBI) has not been studied. We hypothesize that EC exposure will worsen contusion volume after TBI in female rats. Adult female Sprague-Dawley rats were exposed to EC (5% nicotine Juul pods) or air for 16 days. Per day, rats were exposed to 16 episodes of EC vapor. Each episode was comprised of 2 seconds of EC Juul puffs followed by 8 seconds of air rest over 8 minutes. Exposed rats underwent moderate fluid percussion injury or sham surgery. In brief, animals were anesthetized (3% isoflurane for induction, 0.5-2% isoflurane for maintenance with 70% N2O, 30% O2), and received a 4.8 mm craniotomy over the right parietal cortex (-3.8 mm bregma, 2.5 mm lateral), where a beveled 18-gauge syringe hub was secured to the craniotomy site. Animals recovered for 12-16 hours while fasting with water ad libitum and were re-anesthetized and mechanically ventilated. A fluid-percussion pulse (18 ms duration) of moderate (1.8-2.2 atm) pressure was delivered. Sham animals experienced all surgical procedures but were not subjected to the fluid percussion pulse. Rats were perfused seven days after TBI, and tissue was fixed for histopathology. To determine contusion volume, areas of tissue necrosis in coronal sections spanning the entire antero-posterior extent of the injury were traced using Neurolucida. Data of contusion volume quantification demonstrated that EC exposure significantly increased mTBI contusion volume as compared to the air control group, suggesting the toxic effects of EC vaping on TBI pathology in rats. We observed that

there is an increased contusion and selective neuronal loss in the brain of the animal that is exposed to electronic cigarettes. We also observed that cotinine which is the primary metabolite of nicotine in the body is increased in female rats because of EC exposure. Electronic cigarette (EC) vaping is a recent phenomenon and there have been no comprehensive studies evaluating the potential effects of EC vaping on the brain, neurological diseases, or cognitive declines.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.18/W24

Topic: C.10. Brain Injury and Trauma

Support: VA1903r0925

Title: Peripheral and Central Inflammation Induced by Mild Traumatic Brain Injury: A Potential Connection between Immunity and Impulsivity

Authors: *C. W. YOE, G. A. ROZENBLUM, V. A. STIRITZ, T. COMINSKI, K. D. BECK; Dept. of Veteran's Affairs, East Orange, NJ

Abstract: *Peripheral and Central Inflammation Induced by Mild Traumatic Brain Injury: A Potential Connection between Immunity and Impulsivity*
Christine W. Yoe, Gilliana A. Rozenblum, Victoria A. Stiritz, Tara Cominski, Kevin D. Beck
In the United States, an estimated 1.5 million cases of traumatic brain injury (TBI) occur every year, with mild TBI as the majority. More than 450,000 U.S military personnel have been diagnosed with mTBI since 2000. Both impulsivity and suicidality are included in the psychological sequelae of mTBI. Serotonin is a key player in regulating emotions as well as mood. In addition, previous data has associated serotonin with modulating impulsive behavior. These states are most often conceptualized as largely behavioral; however, neurobiological changes that underlie impulsivity and suicidality have the potential to serve as the framework behind modifying these maladaptive states. As such, studying these changes in both the periphery and within the central nervous system after insults, such as mTBI, are of paramount interest. Male and female adult Sprague Dawley rats sustained an mTBI via the lateral fluid percussion model. Whole blood and brain tissue punches were collected for RT-PCR analysis. mRNA expression of proinflammatory cytokines IL1α;, IL1β;, and TNF-α; were examined at 1-, 3-, and 6-months post-injury. Our data from the brain tissue punches shows no notable difference in serotonergic expression in either males or females in brainstem regions. However, there appears to be an increase in several pro-inflammatory cytokines including IL1-β;;;, and IL1-α;;;, as well as the transmembrane protein CD68, in the locus coeruleus (LC) and the nucleus reticularis pontis caudalis (Pnc). Additionally, vimentin was also elevated in the dorsal

hippocampus of injured females. Blood was similarly analyzed to determine if there may be a circulating biomarker of these signs of brain inflammation. In the blood, our data showed elevation in peripheral cytokine mRNA expression between 1 month and 3 months. Although there were no significant differences in cytokine expression between injury groups, data showed trends in injured male rats for elevated cytokine expression compared to male sham rats. This study aims to understand alterations in various systems after mTBI that is thought to contribute to suicidality, specifically in vulnerable populations such as active military and veterans.
This research was supported by funding from the VA Office of Research & Development, Biomedical Laboratory R&D Service I01BX004561-01A2. The expressed conclusions are those of the authors and do not reflect an official position of the U.S. Dept. of Veterans Affairs.

Disclosures: **C.W. Yoe:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs. **G.A. Rozenblum:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs. **V.A. Stiritz:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs. **T. Cominski:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs. **K.D. Beck:** A. Employment/Salary (full or part-time):; Department of Veterans Affairs.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.19/W25

Topic: C.10. Brain Injury and Trauma

Support: LN-H and a travel award, provided by the Departments of Neurology and Psychology, respectively, University of Texas at Austin

Title: Neutrophil depletion and localization strategies after early age traumatic brain injuries in male and female mice

Authors: *K. A. SMITH[1], M. H. DONOVAN[2], L. J. NOBLE-HAEUSSLEIN[2], R. E. VON LEDEN[3];
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Abstract: Traumatic injury to the young brain often results in adverse long-term neurological outcomes. Unlike the adult brain, the young brain is vulnerable to trauma, which is, in part, related to developmentally regulated, low levels of antioxidant reserves. As activated neutrophils release damaging proteins, there is risk that these leukocytes may serve to catalyze early secondary pathogenesis in young brains. Early studies, using neutrophil depletion paradigms, resulted in mixed outcomes which were likely due to lack of specificity of the antibodies. Here we revisit this challenge by validating neutrophil depletion in young, brain injured mice, using advanced tools to immunologically deplete neutrophils and to evaluate their distribution within the brain. We first confirmed a reproducible platform to selectively deplete circulating neutrophils using a specific Ly6G antibody. Traumatic brain injury (TBI) was performed in male

mice (C57BL6) at postnatal day 21. Mice were randomly assigned to receive systemic administration of either Ly6G or IgG control antibody the day prior to TBI (baseline) and day of TBI (N=8/group). Temporal depletion was confirmed by blood smears; percent neutrophils per total white blood cell count was determined at baseline, day of TBI and days 1, 5, 7 and 14 post-injury (PI). Neutrophils were elevated at day 1 PI in the IgG group (25.55% vs baseline 17.65%, p=0.0002), while the Ly6G group showed a significant decrease (9.19% vs. baseline 15.21%, p=0.0002). By day 5 PI, both groups returned to baseline values. We next determined if effectiveness of depletion varied according to sex, using genetically engineered Catchup IVM-Red, which allows for fluorescent imaging-based tracking of neutrophils in the brain. Using an approach that provides a more selective depletion, we compared Ly6G in combination with IgGk secondary to IgG controls in both males and females (N=8-10/group). There was a significantly greater percentage of neutrophils in the control groups compared to Ly6G treated groups at baseline ($p < 0.001$) and day 1 PI ($p < 0.001$). There were no differences in depletion between males and females ($p > .05$), based upon analysis of blood smears and blinded qualitative inspection of brain sections. Neutrophils were limited to the cortical mantle, in proximity to the site of injury. Collectively, these findings validate an immunologic-based method for selective depletion of neutrophils and provide the first preliminary evidence that immunologic depletion does not vary according to sex.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

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Program #/Poster #: PSTR336.20/W26

Topic: C.10. Brain Injury and Trauma

Support: VA Office of Research & Development, Biomedical Laboratory R&D Service I01BX004561-01A2

Title: Effects of Mild Traumatic Brain Injury on the Acoustic Startle Response and the Gut Microbiome

Authors: *T. P. COMINSKI[1], C. E. STAMPER[2,3,4], A. J. HOISINGTON[2,3,4,5], T. TORRES[1], J. STAMOS[1], L. A. BRENNER[2,3,4], K. D. BECK[1];
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Abstract: Traumatic brain injury (TBI) is a growing public health concern, especially affecting those in the U.S. military. Though most TBIs military personnel experience are mild in severity, they can result in profound effects on brain function and the gut microbiome. Suppression of the acoustic startle response (ASR), a brainstem reflex, has been shown following TBI in both humans and animals, suggesting that mild traumatic brain injury (mTBI) can induce brain stem dysfunction and that ASR could potentially serve as a behavioral biomarker for mTBI. Likewise, changes in the gut microbiome have been detected in both humans and animals following brain injury, and, therefore, gut microbiome analysis could potentially serve as a physiological biomarker for mTBI. The current study utilized the fluid percussion model to induce a single mTBI in rats. ASR was assessed through 6 months post-injury to investigate brain stem function. Fecal samples were collected pre-injury and at days 1-4, 7, 10, 15, 20 and 180 post-injury to assess potential parallel changes in the gut microbiome following brain injury. ASR amplitude (Vmax) and sensitivity are suppressed at 102dB in both males and females lasting at least 3 months post-injury. Gut microbiome analysis indicates that alpha diversity is significantly decreased 1-2 days following mTBI; alpha diversity returned to pre -mTBI levels within four days after injury but declined to a new steady state lower than pre-mTBI levels. Analysis of beta diversity showed the microbial community composition was altered by mTBI for several days after injury. Analysis of taxonomy determined that Oscillospiraceae, Muciispirillum, Erysipelotrichaceae, Lachnosiraceae, Frisingicoccus, Tuzzerella, Colidextribacter decreased after mTBI, while *Lactobacillus, Atopobiaceae, Adlercreutzia, Prevotellaceae_Nk3B31* increased after mTBI. The data from the current study indicate that a single mTBI in rats induces long-lasting acoustic startle response suppression, which may be caused by brain stem dysfunction induced by the injury. The data also show robust effects of mTBI on the gut microbiome. It is possible that mTBI influences the gut microbiome via brain stem nucleus changes or vice versa; further investigation of this bi-directional relationship is warranted given the data presented here.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.21/W27

Topic: C.10. Brain Injury and Trauma

Support: 1R01NS127894

Title: P75ntr mediates retrograde degeneration of basal forebrain cholinergic neurons but not in the locus coeruleus after traumatic brain injury

Authors: *M. A. PANDYA, S. DASGUPTA, J. P. ZANIN, M. W. SHIFLETT, W. J. FRIEDMAN;
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Abstract: p75NTR mediates retrograde degeneration of Basal forebrain Cholinergic neurons but not in the locus coeruleus after traumatic brain injuryMansi A. Pandya, Srestha Dasgupta, Juan P. Zanin, Michael W. Shiflett, and Wilma J. FriedmanBasal forebrain cholinergic neurons (BFCNs) are a population of neurons that project to multiple targets in the brain, including the hippocampus and cortex. BFCNs regulate several cognitive functions such as attention, memory, learning, and the sleep-wake cycle. BFCNs are compromised in various neurodegenerative disorders, a consequence of which is cognitive loss. Our previous results showed a retrograde degenerative effect of cortical injury on the projecting BFCNs via p75NTR. Significantly fewer BFCNs ipsilateral to the injury compared to the contralateral side were observed 7 and 14 days after the injury, an effect that is absent in p75 knockout mice. Interestingly, another afferent population of neurons that projects to the cortex, the noradrenergic neurons of the locus coeruleus (LC), showed no change in the number of TH+ neurons after FPI. These results suggest a cell specific retrograde degenerative effect of the cortical injury on the projecting BFCNs through p75NTR. We further hypothesize that as a consequence of injury to the target region of BFCNs, associated cognitive functions regulated by the basal forebrain will be affected. To test for basal forebrain-specific cognitive behavioral deficits after TBI we generated conditional KO mice lacking p75NTR in cholinergic neurons. Preliminary behavioral characterization of $p75NTR^{fl/fl}$:ChAT-cre mice prior to the injury showed similar cognitive patterns in comparison to p75NTR floxed mice. Behavioral studies on $p75NTR^{fl/fl}$:ChAT-Cre mice after FPI, may reveal cognitive functions specifically affected by ablation of BFCNs following TBI. The knowledge of how the loss of BFCNs after TBI subsequently affects cognitive functions can help further our understanding of functional deficits due to TBI and neurodegeneration.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.22/W28

Topic: C.10. Brain Injury and Trauma

Title: Blast injury induces sex-dependent changes in structural connectivity along nociceptive fiber tracts

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Abstract: After Traumatic Brain Injury (TBI), long-lasting neuropathic pain can profoundly impede patient recovery. Studies show a reciprocal relationship between pain and motor recovery in humans and preclinical models, suggesting they may be competitive processes. Findings from our mouse blast injury (BI) model show male and female cohorts with increased neural atrophy in nociceptive areas significantly surpassed the motor performance of controls in the rotarod test. Intriguingly, low performers had milder atrophy in these areas. Thus, we hypothesize that TBI induces adverse changes in nociceptive fiber tracts leading to to disparate motor performance. Diffusion data was collected from male (N=20; 12 BI) and female (N=16, 8 BI) mice. Regions of interest (ROIs) based on known damage sites and fractional anisotropy (FA) maps were obtained via the FMRIB Software Library (FSL). Probabilistic tractography via MRTrix compared fiber tracts related to nociception. Two-tailed t-tests were used for analysis. BI-induced changes showed sex-dependent profiles in ROIs and fiber tracts. BI females had decreased FA in optic tract (P=0.014), insula (P=0.028), corpus callosum (P=0.029), globus pallidus (P=0.037), and Kölliker-Fuse nucleus (P=0.043). Surprisingly, BI females had increased FA in cerebellar lobule III (Cbl3) (P=0.034), with right hemisphere increases in streamline counts, volume, density (P<0.05) in fibers connecting the parabrachial nucleus (PBN) to Cbl3. In contrast, BI males showed decreased FA in the amygdala (P=0.038), periaqueductal grey (P=0.0086), and paraventricular nucleus of the hypothalamus (P=0.033). They also had increased streamline counts and density (P<0.05) in left PBN-Cbl3 fibers, with decreased counts, volume, density (P<0.05) in the right hemisphere. Myelin basic protein (MBP) stains validated the increased Cbl3 myelination in BI females (P=0.036), while BI males had no change in expression (P=0.57). Our findings support sex-dependent outcomes in TBI. The novel increase found in PBN-Cbl3 connectivity, especially in females, may explain varying motor trajectories and implicates a potential compensatory mechanism. We postulate that increased connectivity along PBN-Cbl3 fiber tracts contributes to impaired motor performance and pain-related behavior, as Cbl3 governs proprioception and increased nociception impairs proprioception and motor performance. Anatomical connections between PBN and cerebellar vermis have been identified and confirmed via retrograde tracing. Further studies are needed to elucidate functional changes along this pathway.

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Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

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Topic: C.10. Brain Injury and Trauma

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Title: Sex Peptide increases female vulnerability to neurodegeneration following mild head trauma in Drosophila melanogaster

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Abstract: Repetitive physical insults to the head, including the mild and seemingly innocuous ones, represent one of the greatest risk factors linked to neurodegeneration and chronic brain dysfunction. Sex-related differences have been documented in many medical conditions, including neurodegenerative disorders and neurotrauma of various severities. However, the underlying drivers of sex differences in brain injury responses and neurodegenerative conditions remain to be fully elucidated. Here, we take advantage of the tractable model organism, *Drosophila melanogaster*, to investigate sex differences in the emergence of long-term brain deficits after mild head trauma. Utilizing a novel headfirst impact model (Behnke et al, PMID: 33958652), we delivered a very mild form of repetitive head impacts to awake and unrestrained adult flies at various ages and investigated their brain degeneration and behavioral deficits through their lifespan. We found that young flies (3-day-old) displayed concussive-like behaviors immediately after the mild head impacts but recovered within minutes. Importantly, these flies exhibited no brain degeneration nor behavioral deficits until they reached the old ages. Specifically, these flies exhibited sensorimotor deficits as assessed by AI-powered negative geotaxis assay and brain degeneration by whole brain imaging at 6-weeks-old. Strikingly, both sensorimotor deficits and brain degeneration were much more profound in females than males, indicating increased vulnerability to neurodegeneration trigged by early life exposure to mild head trauma. We further show that the elevated female vulnerability is not a result of the larger body size of females. Instead, it is mediated by Sex Peptide (SP), an accessory protein that accompanies sperm from male flies. Virgin females, females mated with males without SP, or pan-neuronal knockdown of SP receptors abolished late-life neurodegenerative conditions induced by the early life exposure to mild head impacts. Finally, aging further exacerbates neurodegenerative conditions induced by head injury. Together, our findings validate *Drosophila* as a suitable model organism for dissecting sex differences in the response to head trauma and indicate Sex Peptide and post-mating responses as strong mediators of chronic neurodegeneration in females.

Disclosures: C. Ye: None. **K. Moberg:** None. **J.Q. Zheng:** None.

Poster

PSTR336. Traumatic Brain Injury: Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR336.24/X2

Topic: C.10. Brain Injury and Trauma

Support: This work was supported in part by the Richard J Traystman professorship endowment (SK).

Title: Modeling diffuse axonal injury in juvenile rabbits using the CHIMERA traumatic brain injury model

Authors: *J. ALLENDE LABASTIDA[1], P. VYAS[1], N. SAH[1], J. L. SOWERS[1], M. P. AVALOS[1], J. LIU[2], R. C. KOEHLER[1], S. KANNAN[1];
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Abstract: Traumatic brain injury (TBI) is the leading cause of disability and mortality worldwide, with increasing occurrences of emergency room visits among young children. Diffuse axonal injury can be a particularly severe form of TBI associated with high mortality and long-term neurologic deficits and accounts for approximately one-third of hospital admissions in children under two. To better understand neonatal and pediatric brain injury, we have developed a novel juvenile rabbit model using the Closed-Head Impact Model of Engineered Rotational Acceleration (CHIMERA). By adapting this model for neonatal and pediatric rabbits, we aim to replicate the mechanisms of accidental and non-accidental TBI observed in human cases. The rabbit brain closely mirrors the human brain with respect to white matter maturation and postnatal formation, making it an ideal animal model. This pilot study aims to develop and validate a juvenile rabbit TBI-CHIMERA model that can facilitate mechanistic insights and therapeutic strategies for diffuse axonal injuries. Neonatal (PND5) and pediatric kits were selected for single (sTBI) or repetitive injuries (rTBI, two injuries total, 24hrs apart) with increasing force of injury as reflected by increasing pressure (psi). The determined settings were 4.5 and 15 psi (∽4.5 and ∽9.25 m/sec) for neonatal and pediatric rTBIs, respectively, or 7 and 19 psi (∽6.1 and ∽10.5 m/sec) for neonatal and pediatric sTBIs. Similar to patients, periods of apnea were seen in the injured kits. rTBI injured kits showed decreased body weight in both age groups; sTBI showed developmental delays in PND5. Single and rTBIs found significant motor and cognitive deficits two weeks post-injury. Macroscopically, ventriculomegaly and hematoma of the posterior fossa were observed, akin to injured human subjects. In summary, our findings demonstrate physiological and pathological similarities to human TBI, including developmental delays, neurocognitive deficits, ventriculomegaly, and neuroinflammation, as observed in acceleration-deceleration type injuries such as those also seen in non-accidental trauma or "shaken baby syndrome." Further assessments of axonal injury are currently underway, paving the way for a deeper understanding of this condition and the development of potential treatments.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.01/X3

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS076976
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NIH Grant P50HD103557
NIH T34 GM008563
UCLA QCBio Collaboratory Postdoc Fellowship

Title: Single-cell RNA-sequencing reveals that olfactory ensheathing cells are hybrid glial cells

Authors: *P. E. PHELPS, S. M. HA, R. R. KHANKAN, G. JUAREZ, K. L. I. DIXIE, M. A. MEKONNEN, Y.-W. CHEN, X. YANG;
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Abstract: Following a severe spinal cord transection in rats, transplanted olfactory bulb-derived olfactory ensheathing cells (OECs) modify the inhibitory environment of the lesion site; OECs are neuroprotective, enhance axon regeneration, function as phagocytes, and modify the immune response to injury. We used single-cell RNA-sequencing (scRNA-seq) to study the gene expression programs of OECs and determine the diversity of immunopurified OECs transplanted in our four previous spinal cord injury studies. A total of eight, 8-10-week-old Sprague Dawley female rats were used to obtain four immunopurified and four control (cells leftover after immunopurification) preparations submitted for scRNA-seq performed by the 10X Genomics Chromium scRNA-seq system. We clustered the cells based on transcriptome similarity to identify general cell types as well as OEC subtype clusters. We further identified highly expressed marker genes for OECs as well as those unique to each OEC subtype. In addition to the wide-spread characteristic OEC markers (*$Ngfr^{p75}$, S100β, Sox10, Fabp7)*, our scRNA-seq data revealed five distinct OECs subtypes. Each OEC subtype expressed unique marker genes which were confirmed experimentally, and pathways indicative of progenitor, axonal regeneration and repair, or microglia-like characteristics. As expected, we found substantial overlap of OEC genes with those of Schwann cells and astrocytes, but also detected marker genes typical of oligodendrocytes and microglia. These results suggest that adult OECs are a distinct hybrid glial cell with progenitor characteristics. Furthermore, the OEC gene expression patterns indicate diverse functions related to novel targets associated with wound healing, extracellular matrix, growth factor regulation, and enhancement of axonal outgrowth and regeneration. This study provides an unbiased and in-depth view of the heterogeneity of OEC populations and the potential mechanisms that may underlie their regenerative properties.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.02/X4

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Satt-Lutech (#MA00444)
Medjeduse (#LS218227)

Title: Inflammation pattern after rat spinal cord injury with a comparative analysis of contusion vs transection experimental models

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Abstract: Spinal cord injury (SCI) in mammals leads to a series of cellular and molecular changes that will ultimately inhibit axon regeneration and autonomous nerve tissue repair. The breakdown of the blood-spinal cord barrier favors infiltration of a broad range of immune cells secreting various inflammatory cytokines that, together with activated microglia, will spread inflammation and aggravate the neurological deficits. Infiltrated immune cells include neutrophils, monocyte-derived macrophages (pro-inflammatory M1, anti-inflammatory M2), and T-cells. The medullary inflammatory response is thus correlated to the balance in time between pro- and anti-inflammatory cytokines, which is perturbed with regard to lesions of other organs. Multiple therapeutic preclinical studies evaluated the inflammatory response in the two most commonly employed SCI models: contusion vs. transection. However, it seems difficult to draw a clear-cut picture from these studies as they differ concerning lesion model, spinal level, and post-lesion time window of inflammation assessment. Moreover, the impact of the two lesion models on the spinal tissue is distinct: the contusion sparing the dura mater, the developing glial scar is mainly composed of astrocytes, whereas in case of the transection model a fibrotic-scar results from massive fibroblast colonization due to severe meningeal damage. The local inflammatory response may thus, also reflect this difference in cell populations at the injury site. In the present study, we analyzed the time course of local immune cell infiltration and associated cytokine levels (1, 3, 7, 14, 28 days post-SCI) after contusion and transection SCI at the thoracic level by using Western blot, ELISA, and immunohistochemistry. Our Western blot data revealed differential profiles in the level of neutrophiles, M1 and M2 macrophage markers between the two SCI models, during both acute and subacute periods. Compared to a transection, the extent of a contusion lesion is more important and followed by less significant locomotor recovery. However, transection model exhibits a higher pro-inflammatory state. This is in accordance with ELISA panels showing specific differences between SCI models in the profiles of several pro-inflammatory (Il-1b, TNF-α, Il-6) and anti-inflammatory cytokines (Il-13, IL-10). Our study is the first to compare Inflammatory changes following contusion and transection SCI using level-matched and time-matched animal cohort conditions. Our results highlight the importance of investigating the trauma-induced inflammatory state when applying a therapeutic strategy.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

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Program #/Poster #: PSTR337.03/X5

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant 5R01NS118200

Title: Spinal cord injury directly interferes with pressure ulcer healing in mice

Authors: *S. PATEL[1], C. VADALA[2], A. R. FILOUS[3], F. O. NOVAIS[3], J. M. SCHWAB[3]; [1]Ohio State Univ., Columbus, OH; [2]Dept. of Neurol., The Ohio State Univ. Neurosci. Grad. Program, Columbus, OH; [3]The Ohio State Univ. Wexner Med. Ctr., Columbus, OH

Abstract: Spinal cord injury (SCI) disrupts the communication between the Central Nervous and Immune Systems, which may contribute to the body's inability to resolve inflammatory events. Pressure ulcers (PU) after SCI are prevalent complications and affect about one-third of patients. These wounds pose an immunological challenge and are often unhealing or chronically recurring. Therefore, non-healing PU constitute a largely unsolved medical need, as they are a frequent cause of re-hospitalizations, origin for osteomyelitis, and consecutive septic conversions. Histological analysis of post-SCI ischemic PU development over time has not been investigated previously. This study aims to investigate the effect of clinically relevant SCI on PU healing at critical wound healing timepoints. We established a mouse SCI-associated PU (SCI-PU) model which employs ischemia/reperfusion (I/R) cycles to induce PU in denervated dermatomes. Neurogenic [lesion-level dependent] SCI effects on PU healing were accessed by applying high versus low thoracic spinal cord injuries and compared to sham controls (Sham-PU). Three I/R cycles (12h ischemia x 12h reperfusion) were generated with magnets (50 mmHg compression) at the dorsal skin. This created a defined distinct PU lesion caudal to the SCI surgical site. PU induction starts three days after SCI to match the clinical condition of PU onset. PU injury progression and wound healing were analyzed. This includes Masson's trichrome staining which allows for epidermis and dermis thickness measurement. We have detected that PU wound development and healing sequelae are different in SCI-PU compared to sham-PU. Moreover, differences in the thickness of the epidermis and dermis were detected between the SCI-PU and Sham-PU groups. These differences reveal that PU progression was protracted and extended in SCI-PU compared to sham-PU, implicating a direct, contributing role of SCI to impaired wound healing of PU.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.04/X6

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Institute of Neurological Disorders and Stroke (NS104422)

Title: Level Dependent Effects of Pain Input after Cervical Spinal Cord Injury

Authors: T. JOHNSTON[1], ***K. N. COLPITTS**[1], G. GIDDINGS[2], H. L. BORLAND[1], J. GRAU[1];
[1]Psychological and Brain Sci., Texas A&M Univ., College Station, TX; [2]Neurobio. and Anat., Drexel Univ., Philadelphia, PA

Abstract: Prior work has shown that engaging pain fibers after spinal cord injury (SCI) by applying noxious electrical stimulation or the irritant capsaicin increases tissue loss and impairs long-term recovery (Grau et al., 2017, J Neurotrauma, 34, 1873). These adverse effects have been related to a breakdown in the blood-spinal cord barrier and the infiltration of blood (hemorrhage). Our previous work has examined this issue in a T10 spinal level contusion injury model. The current study examined if these detrimental effects were injury-level dependent by applying noxious stimulation following a bilateral cervical injury. Male Sprague-Dawley rats received a contusion injury at the C6-7 spinal level. In experiment 1, rats (n=8) were randomly assigned to receive six-minutes of electrical stimulation to the tail or no shock. Blood pressure measurements were assessed immediately, one, two, and three hours after pain administration. Following experimentation, a 1-cm region of tissue encompassing the injury site was collected and assessed for hemorrhage using spectrophotometry. In experiment 2, rats (n=6) were randomly assigned to receive a capsaicin injection to the left or right hind or forepaw. Mechanical allodynia levels were assessed with the Von Frey filament for the hind and forepaws at baseline and 2 hours after pain administration. Animals were perfused and tissue was collected for histological analysis of hemorrhage. Contrary to our hypothesis, noxious electrical stimulation to the tail following a cervical SCI did not induce hemorrhage at the site of injury. Pain in the form of capsaicin injection was found to increase mechanical allodynia. Current work is exploring capsaicin's effect on hemorrhage levels following a cervical SCI.

Disclosures: T. Johnston: None. **K.N. Colpitts:** None. **G. Giddings:** None. **H.L. Borland:** None. **J. Grau:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.05/X7

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR f18- 01234

Title: Inhibition of remyelination prior to spinal cord injury in older mice causes delayed locomotor recovery and cognitive deficits

Authors: *S. M. WHEELER[1], B. KONDILES[3], S. B. MANESH[2], M. LU[4], J. LIU[2], W. TETZLAFF[5];
[1]Cell and Developmental Biol., [2]Univ. of British Columbia, Vancouver, BC, Canada; [3]Intl. Collaboration on Repair Discoveries, Vancouver, BC, Canada; [4]Icord, Vancouver, BC, Canada; [5]ICORD, Univ. of British Columbia - ICORD, Vancouver, BC, Canada

Abstract: In western nations, the average age of spinal cord injury (SCI) has increased and is equally spread out between 20–65 year-olds, reflecting the aging population. SCI is a debilitating affliction that impacts multiple functions of the body including motor, sensory, autonomic functions, and well as cognition; outcomes at higher ages are less favourable. The initial impact of SCI triggers secondary injury cascades leading to apoptosis of oligodendrocytes and focal demyelination of spared axons near the site of injury which is followed by remyelination. Surprisingly, no differences in locomotor recovery were observed after SCI when remyelination was inhibited in young adult mice (Duncan et al. 2018, Nat. Comm. PMID: 30076300). To assess the importance of remyelination with age we here compare functional recovery in young (3-5 month) versus older (15-18 month) transgenic mice of both sexes following a sham injury (laminectomy) or a 70kDyne thoracic level 9 (T9) contusion. We crossed a PDGFRαCreERT2 driver line with mice carrying a floxed Exon8 in their Myrf gene ($Myrf^{fl/fl}$), a key transcription factor for myelination expressed in oligodendrocyte progenitor cells (OPCs). Through the application of tamoxifen, we can conditionally restrict oligodendrocyte (OL) maturation and inhibit new myelination. These mice were compared to littermate controls, which carry a functional *Myrf* gene. We have found that older, injured, remyelination incompetent mice had delayed locomotor recovery, as assessed through the Basso Mouse Scale and the horizontal ladder. They also displayed cognitive deficits measured through Y-maze, the object relocation task, and novel object replacement task. No differences were seen in anxiety or depression-like behaviors, analyzed through the elevated plus maze, marble burying task, or fecal boli counts. Ongoing histological work will show the impact of age and remyelination inhibition on the amount/quality of myelin near the lesion as well as OPC and OL density. Further work is required to determine how young SCI mice compensate remyelination failure after SCI. The current results suggest that remyelination treatments may have a greater impact in older than in younger individuals with SCI, giving greater insight to personalized therapies post SCI.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.06/X8

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Daniel & Ada Rice Foundation

Title: Inflammation and age-related effects of spinal cord injury on pain and bone loss

Authors: *B. T. DAVID, Q. SHEN, S. OH, N. WROBEL, D. TUCKER, B. AVONTS, Y. LOPEZ-RAMIREZ, K. RAUE, J. MOSS, K. CARPENTER, R. G. FESSLER; Neurosurg., Rush Univ. Med. Ctr., Chicago, IL

Abstract: Spinal cord injury (SCI) causes disruption of motor, sensory, and autonomic functions. An inflammatory response is initiated after injury and, unlike traditional wound healing responses, is maintained indefinitely. This inflammation triggers or exacerbates a host of secondary complications following the initial trauma, including altered sensation and bone loss in the extremities. Further, age has been shown to play a role in the inflammatory, sensory, and bone preservation responses. The current study was undertaken to compare spinal cord inflammation between young and middle-aged rats, as well as to determine the role that differential inflammation might play in determining the sensitivity to mechanical stimuli and predisposition to bone loss. We report that at both 7 and 42 days post-injury there is a significant inflammatory response at the spinal cord injury epicenter in both young and middle-aged rats, specifically with regard to macrophages and microglia. All animals displayed a positive correlation between macrophage levels in the spinal cord and their hindlimb paw sensitivity to mechanical stimuli. Moreover, all rats also demonstrated a negative correlation between femoral cortical bone strength and microglia presence at the spinal cord injury site.

Disclosures: B.T. David: None. **Q. Shen:** None. **S. Oh:** None. **N. Wrobel:** None. **D. Tucker:** None. **B. Avonts:** None. **Y. Lopez-Ramirez:** None. **K. Raue:** None. **J. Moss:** None. **K. Carpenter:** None. **R.G. Fessler:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR337.07/X9

Topic: C.11. Spinal Cord Injury and Plasticity

Support: R01NS106908
R01NS111761
R01NS122371

Title: Role of KCC2 in sympathetic dysfunction following complete high-level spinal cord injury

Authors: *M. WULF[1], V. J. TOM[2];
[1]Drexel Univ. Col. of Med. Neurosci. Program, Philadelphia, PA; [2]Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: Following severe, high-level spinal cord injury (SCI) at or above thoracic level 6 (T6), the development of heightened sympathetic reflexes (i.e., sympathetic hyperreflexia) occurs resulting in severe dysfunction of organs receiving sympathetic input, such as the spleen and vasculature. This leads to cardiovascular disease and immune dysfunction, two leading causes of morbidity and mortality within the SCI population. Sympathetic hyperreflexia is driven by changes within the spinal sympathetic reflex (SSR) circuit below the SCI that contributes to increased excitability of the circuit and development of sympathetic hyperreflexia. Hyperactive refelexes after SCI are not limited to the sympathetic system. Disrupted chloride homeostasis has been implicated in decreased synaptic inhibition underyling heightened motor reflexes following SCI. The K^+-Cl^- cotransporter type 2 (KCC2) helps to maintain low levels of intracellular chloride that is essential for GABAergic and glycinergic inhibtion. Interestingly, after SCI, expression of KCC2 is reduced in lumbar motorneurons. Moreover, increasing KCC2 activity post-SCI has been shown to improve motor function. However, the role of KCC2 in dysregulation of the sympathetic system after SCI is not known. We theorize that loss of KCC2 contributes to hyperexcitability within the SSR circuit after SCI. We have preliminary data to indicate that there is less KCC2 on the membrane of neurons within the SSR circuit after a complete T3 SCI - an injury that reliably results in sympathetic hyperreflexia - than in uninjured animals. Additionally, upon systemic administration of CLP290, a recently developed KCC2 enhancer drug, after SCI, levels of KCC2 in the membrane of sympathetically-associated spinal neurons were similar to those in uninjured animals. We will assess if increasing membrane KCC2 in sympathetic neurons using CLP290 decreases sympathetic hyperreflexia. We will perform radiotelemetric, hemodynamic recordings of blood pressure and heart rate to examine whether treating with CLP290 after a complete T3 SCI diminishes autonomic dysreflexia - a life-threatening, sudden, and severe hypertension in response to a below-injury, noxious sensory stimulus that is a well-established, real-time readout of sympathetic hyperreflexia. We will also examine if enhancing KCC2 post injury attenuates detrimental changes within the spleen and immunity that are driven by sympathetic hyperreflexia. This will give us insight into the role KCC2 is playing within the sympathetic system following SCI and provide a potential therapeutic target to attenuate the life-threatening secondary consequences of SCI.

Disclosures: M. Wulf: None. **V.J. Tom:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.08/X10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: 5R01NS088475-05

Title: Polytraumatic spinal cord injury with peripheral nerve injury impacts spinal cord synaptic plasticity

Authors: *J. DAVIS, J. GUMBEL, C. OMONDI, R. HUIE, E. IORIO, A. R. FERGUSON;
UCSF, San Francisco, CA

Abstract: In addition to loss of sensorimotor and autonomic functions, spinal cord injury (SCI) also often results in maladaptive plasticity represented by spasticity, hyperreflexia, and intractable pain. Prior research has established that maladaptive synaptic plasticity after SCI results in pain that is refractory to therapeutics and inhibited locomotor recovery in part due to increased spasticity and rigidity. However, the mechanisms driving maladaptive plasticity remain poorly understood. Prior research has suggested that intermittent nociceptive stimulation after injury increases AMPAR subunit GluA1 serine 831 phosphorylation and trafficking to synapses with accompanied reduction in synaptic GluA2. This reflects an increase in calcium-permeable AMPARs. Therefore, the goal of this study is to further assess if inhibiting AMPAR through the administration of NASPM (calcium-permeable AMPAR antagonist) after injury with the addition of spared nerve injury (SNI) reverses injury-driven maladaptive plasticity. We have carried out quantitative near-IR western blotting to assess GluA1 and pS831 expression below the level of injury after intrathecal administration of NASPM (AMPAR antagonist). A total of 80 female mice split into 8 groups, either NASPM treated or vehicle: naïve, SCI+SNI, SCI+sham nerve injury, and sham laminectomy+sham nerve injury. Our analysis demonstrates that both NASPM and polytrauma impact spinal cord synaptic plasticity. Therefore, AMPARs are a plausible target to further investigate to help alleviate maladaptive plasticity after polytraumatic spinal cord injury.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.09/X11

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Chronic in vivo 2 photon imaging reveals plasticity of corticospinal tract neurons after partial spinal cord injury

Authors: C. F. O'BRIEN, *W. CAFFERTY;
Yale Univ., New Haven, CT

Abstract: Voluntary fine motor control requires multiple motor pathways, including the corticospinal tract (CST). The CST descends from Layer V neurons in primary motor cortex (M1), innervates several supraspinal motor centers and all segments of the spinal cord, and is therefore susceptible to axotomy after spinal cord injury (SCI). Injured CNS axons do not

regenerate, resulting in chronic functional impairments. However, modest spontaneous functional recovery has been observed after partial SCI which may involve sprouting of spared axons into denervated spinal and potentially supra-spinal locations. To explore the capacity of these *de novo* circuits from intact CSNs to mediate spontaneous recovery after partial SCI we performed chronic *in vivo* 2-photon calcium imaging of the apical dendrites of CSNs and concurrent limb kinematic imaging in adult male Rbp4-*Cre* mice. To assess functional changes in intact CSNs we injected AAV-FLEx-GCaMP6f into M1 and retro-AAV-tdTomato into the cervical spinal cord of Rbp4-*cre* (n=11) mice, and in the same procedure implanted head-fixation bars and a cranial window over M1. For 4 weeks, mice were trained on an involuntary irregular wheel-running task. After baseline imaging and limb kinematic data were acquired mice received contralateral pyramidotomy (PyX, n=5), or sham lesion (n=6). Imaging was repeated at 1, 5, 7, 14, 20, and 28 days post-injury. We returned to the same field of view each session to follow one cohort of CSNs. Guided by previous studies, we classified units into 4 groups daily based on their movement-related activity according to cross-correlation analysis: movement-active, quiescence-active, indiscriminately-active, and inactive. While injured mice showed significant lesion-induced decrement (and then moderate recovery) in forelimb performance, we saw no significant changes in the proportions of unit phenotypes in the intact cortex after injury. These data suggest that the intact CST may not directly support functional recovery despite PyX-induced sprouting of intact CST axons into the denervated cord. An alternative mechanism for recovery could be reorganization of lesioned CST projections to supraspinal motor centers. To explore, we plan to label all forelimb CST axons via retro-AAV-GCaMP8m injection into the cervical spinal cord and independently label CST collaterals innervating supraspinal motor centers with retro-AAV-tdTomato. This approach, along with DeepLapCut-assisted limb kinematics, will allow us to explore the impact of PyX and spontaneous functional recovery on activity in all lesioned CST neurons and those with collateral innervation to specific supraspinal motor centers.

Disclosures: C.F. O'Brien: None. **W. Cafferty:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

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Program #/Poster #: PSTR337.10/X12

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (LAH).

Title: Quantification of Intra-Urothelial Nerve Fiber Density (IUNFD) in Non-Human Primates

Authors: *G. BORTOLANCA CHIAROTTO[1], N. BISCOLA[2], L. HAVTON[2];
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Abstract: Lower urinary tract (LUT) dysfunction represents a major clinical problem for the management of patients suffering from a variety of conditions related to peripheral neuropathy and neurological injuries including spinal cord injury (SCI). One of the limitations to our understanding of mechanisms for LUT disease processes is the relative lack of established morphological biomarkers based on outpatient procedures. We have developed as a model system an approach to obtain bladder biopsy material from the bladder urothelium and developed an analytical approach based on nerve fiber density assessments that mimic those already established for skin biopsies in humans and animal models. The bladder may show different types of sensory input at varied densities based on anatomical locations, including the bladder neck and lateral bladder wall. First, we performed punch biopsies from the bladder neck and lateral wall, procured from neurologically intact rhesus macaques (n=5) after necropsy. Cryosections were prepared for immunofluorescence using anti-PGP9.5 antibody as a small fiber (C fiber) marker. Images of the urothelium and underlying lamina propria were acquired in a confocal microscope and both crossing (vertical), and non-crossing (non-vertical) fibers were readily identified and counted over a minimum 1000 µm urothelium length using ImageJ and Photoshop software. Intra-urothelial nerve fiber density (IUNFD) assessments were made quantitatively. The results showed high density of fibers in the bladder neck. In addition, the number of non-vertical fibers per length was significantly higher in the bladder neck region compared to the vertical fibers. No density differences were observed between vertical and non-vertical fibers in the lateral wall. Second, we are developing an in vivo procedure for transurethral cystoscopy combined with bladder biopsies in rhesus macaques for longitudinal studies. Our preliminary data indicate feasibility of obtaining biopsy material of the urothelium and underlying lamina propria. In conclusion, IUNFD assessments provide a non-biased and quantitative approach for translational studies of autonomic innervation with the potential of being applied to in vivo procedures such as cystoscopy and bladder biopsies for diagnostic purposes.

Disclosures: G. Bortolanca Chiarotto: None. **N. Biscola:** None. **L. Havton:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR337.11/X13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH NINDS T32 NS096050-24
Craig H. Neilson Foundation 642928

Title: Diet-microbiome interactions limit neurogenic bowel dysfunction following spinal cord injury.

Authors: *A. HAMILTON[1], L. BLACKMER-RAYNOLDS[1], Y. LI[1], S. KELLY[1], J. CHANG[1], S. SRINIVASAN[2], S. GARRAWAY[1], T. SAMPSON[1];
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Abstract: Research Objective & Rationale: Spinal cord injury (SCI) results in a plethora of physiological dysfunctions across all body systems, including neurogenic bowel dysfunction (NBD), characterized most frequently by atrophy of the enteric nervous system (ENS), intestinal dysmotility, and constipation. Recent studies have also highlighted significant changes to the composition of the gut microbiome in humans and animals with SCI. The gut microbiome influences numerous aspects of host physiology including inflammation, metabolism, and neurogenesis. We therefore sought to determine if the SCI-induced microbiome could induce NBD in uninjured mice, and if microbiome-based intervention could prevent NBD in mice with SCI. **Methods:** Adult mice were subjected to a severe midthoracic contusive SCI or laminectomy (sham). Total gut transit time was assessed *in vivo* and colonic contractility *ex vivo*. ENS status and inflammation were evaluated. Uninjured germ-free (GF) mice were colonized with injury-derived microbiomes or mono-colonized with SCI-associated bacterial taxa to assess the sufficiency of the microbiome to induce NBD. Various microbiome-based interventions were assessed for their ability to improve NBD in mice with SCI, including prebiotic, probiotic, and post-biotic interventions. **Results:** Mice with SCI had significantly impaired intestinal transit and increased ENS atrophy, relative to sham controls. Colonization of uninjured GF mice with SCI-derived microbiomes altered inflammation and metabolism but did not impact gut transit. Intervention with dietary fiber or its metabolites, but not with live probiotic species, improved intestinal transit and rescued ENS atrophy in mice with SCI. Mice deficient in an anti-inflammatory cytokine signaling pathway were unable to respond to these interventions post-SCI, identifying one component of a neuro-immune circuit in NBD. **Conclusions:** Our data suggest that intervention with a specific dietary fiber and its metabolites reduces symptoms of SCI-induced NBD in mice. These data highlight a diet-microbiome-neuroimmune axis that promotes intestinal resilience following SCI. Overall, we demonstrate that diet and microbially derived signals distinctly impact recovery of the ENS after SCI and represent a tangible avenue of therapeutics for SCI-induced NBD. These findings provide a clear rationale for the continued advancement of targeted dietary or probiotic therapies to ameliorate GI dysfunction in persons with SCI.

Disclosures: A. Hamilton: None. **L. Blackmer-Raynolds:** None. **Y. Li:** None. **S. Kelly:** None. **J. Chang:** None. **S. Srinivasan:** None. **S. Garraway:** None. **T. Sampson:** None.

Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR337.12/X14

Topic: C.11. Spinal Cord Injury and Plasticity

Support: AMED 17dm0107118h0002
KAKENHI 19k22985
AMED JP18dm0307005
KAKENHI 22k15624

Title: Motor recovery and changes in sensory response from the unaffected hand after spinal cord injury

Authors: *T. TOHYAMA[1,2], R. YAMAGUCHI[3], N. GODA[2], T. YAMAMOTO[2], N. SADATO[2], T. ISA[3,4], M. FUKUNAGA[2];
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Abstract: Plasticity of sensory-motor systems may subserve recovery after incomplete spinal cord injury, but little is known about the relevance of post-injury plasticity of sensory systems to central motor reorganization, and its role in recovery of hand dexterity. We examined longitudinal recording of fMRI responses to tactile stimulation of each hand, and changes in resting-state connectivity, combined with observations of hand movements in monkeys after spinal cord injury. Five macaque monkeys were trained to perform a food retrieval task, and then underwent C5/C6 or C6/C7 spinal cord subhemisection. After severe initial paralysis of the ipsilesional hand, the average of success rate of grasping movements gradually recovered until 16 weeks after lesion except for one monkey with an extensive lesion and poor recovery. Under anesthetization with 0.8−1.0% isoflurane, fMRI data were acquired every week on a 3T MRI scanner with a custom-built monkey head coil. Responses of sensorimotor hand areas (SMHAs) to brushing of each hand were examined. In four monkeys with fair recovery, increased responses of the ipsilesional SMHA to contralesional hand stimulation were observed in 2-5 weeks after lesion, and the responses decreased over time. To examine network information in motor areas, eigenvector centrality that can represent hubness on recursively connected networks was computed. Changes in the responses of the ipsilesional SMHA were correlated with those in centrality of the bilateral ventral premotor cortex (PMv), supplementary motor area (SMA) but not the primary motor cortex. Responses of the ipsilesional sensorimotor cortex (SMC) and cerebellum to contralesional hand stimulation were correlated inversely with the success rate in the four monkeys with fair recovery, whereas those were extensively distributed across the temporal cortex, parietal cortex, and occipital cortex in a monkey with poor recovery. These findings suggested that the enhanced plasticity or disinhibition of the ipsilesional SMC might be associated with dynamics of centrality of PMv and SMA, which was prominent while ipsilesional hand movements were severely impaired but was declined as recovery progressed. The harmonized dynamics of the sensory responses with the centrality of higher-order motor networks might represent reorganization of sensory-motor circuits for promoting recovery after injury. The monkeys we used may be clinically classified as Brown-Séquard syndrome, classically characterized by ipsilesional hemiplegia and contralesional hemianalgesia but there are few reports on the “pure” syndrome. A dynamic reorganization process can mask the typical symptoms.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

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Program #/Poster #: PSTR337.13/X15

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NS110605

Title: Immune Response as a Potential Predictor of Locomotor Recovery: The Role of Dural Repair in SCI

Authors: *J. PAZ AMAYA[1], E. ABBOTT[3], M. ZABACK[1], J. RAJAVONG[2], T. J. CAMPION, III[2], M. A. LEMAY[1];
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Abstract: The reliability of the feline model has recently been questioned given the evidence of spontaneous locomotor recovery after a complete SCI. Interestingly, the study by Harnie et al. that presented this evidence reported transecting the dura along with the cord. Cutting the dura leaves the cord exposed to exogenous factors which likely increase the levels of BDNF-secreting activated microglia. BDNF being a known promoter of locomotor recovery in spinalized animals. We hypothesized that spontaneous recovery is correlated with the immune response associated with a transected dura. A group of 8 cats received a complete spinal transection at T11-T12 spinal segment. During the transection surgery, our control group (n=4) had their dura spared while the experimental group (n=4) had a transected dura. At 3 and 5 weeks post-transection, locomotor recovery was evaluated using high-speed motion capture. The experimental group was able to walk at higher speeds and showed better overall recovery in terms of plantar weight bearing and range of motion. After 6 weeks, spinal cord tissue was retrieved for histological analysis. Cross sections of the low thoracic cord were stained for activated microglia using calcium-binding protein (Iba-1). The best walking cats showed significantly more activated microglia near the transection site when compared to the worst walkers from the control group. The current evidence suggests a correlation between immune response at the injury site and spontaneous locomotor recovery. This study argues that a standard methodology for spinal transection is needed to compare treatment efficacy between studies.

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Poster

PSTR337. Spinal Cord Injury: Mechanisms

Location: WCC Halls A-C

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Program #/Poster #: PSTR337.14/X16

Topic: C.11. Spinal Cord Injury and Plasticity

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Rebecca F. Hammond Chair
KSCHIRT Funds

Title: Ventral and dorsal quadrant thoracic transections disrupt hindlimb gait features and inhibitory feedback

Authors: *L. M. KONAN[1], A. N. TRELL[2], A. DE BOEF[6], S. MCMURTRY[6], H. L. TRAN[3], W. A. O'STEEN[3], R. F. SHANTI[3], L. R. MONTGOMERY[3], R. L. VAN SANDT[3], G. E. BERTOCCI[4], T. R. NICHOLS[6], D. R. HOWLAND[5];
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Abstract: Gait relies on a complex interplay of pathways, including descending systems conveying supraspinal input and spinal sensory feedback circuitry, which governs mechanical limb properties. Post-thoracic hemisection (Hx), locomotion recovers, albeit with persisting deficits**.** Our work also shows an immediate and chronic reorganization of inhibitory force-related feedback (FFB), including 1b and possibly group II afferents, resulting in a biased inhibition of ankle extensors. Even so, our understanding of the contributions and interactions between descending pathways and spinal sensory FFB circuitry post-spinal cord injury (SCI) remains limited. This study aims to employ restricted lesions to determine if dorsal or ventral descending pathway(s) differentially influence gait recovery and modulate FFB. We studied hindlimb (HL) gait and FFB distribution in 14 animals; each received dorsal (dQ, n=7) or ventral quadrant lesions (vQ; n=7) at T9/10 and were followed for 6 or 12 weeks. Gait analysis was conducted on level and declined walkways at U Louisville, while FFB was tested during a terminal procedure using a decerebrate prep at GA Tech. Declines were used to augment eccentric extensor activity, which is regulated by FFB circuits. Gait features, such as percent stance, cadence, and angular kinematics, were examined with a Vicon System, 10 infrared cameras and custom scripts. FFB distribution was tested by recording individual muscle forces in response to varying stretch combinations delivered to muscle pairs, using a custom Matlab routine. Gait changes occurred after both lesions; however, the dQ group exhibited mainly bilateral changes, in contrast to predominantly ipsilateral effects after vQ. FFB flexibility was lost after both lesions; further, the single pattern of amplified, convergent inhibition of ankle extensors, was primarily observed ipsilaterally after dQ, while bilateral effects were evident after vQ. These results must be interpreted within the context of the condition under which they were obtained. Gait was in the presence of supraspinal systems that may not influence spinal circuitry after decerebration or may only be active during voluntary behavior. In contrast to FFB, gait is a symmetrical activity. Bilateral gait changes seen after dQ may reflect indirect cortical input via bilateral spared ventral tracts aimed at promoting symmetry. Exaggerated ankle flexion in early

stance aligns with the convergent FFB onto ankle extensors post-injury. The FFB changes after dQ and vQ suggest FFB circuitry is modulated by tracts in both quadrants. Abstract does not represent NIH views.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.01/X17

Topic: D.02. Somatosensation – Pain

Support: KLI 924

Title: Human heat sensation

Authors: F. RESCH[1], C. I. CIOTU[2], S. HEBER[3], ***M. J. M. FISCHER**[1];
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Abstract: Avoidance of potentially damaging heat is important, but not well understood in humans. There are several heat sensitive ion channels, whose manipulation led to more or less pronounced phenotypes in animals. Heat sensation was largely absent in triple knockout mice lacking TRPA1, TRPV1 and TRPM3. Additionally, Anoctamin 1 has emerged as a potential heat sensor that exhibits activation above 44°C in cellular models. We developed a human heat pain model with continuous intradermal injection of a solution with linearly increasing temperature in the range 44-52°C over a period of 150 s. The intradermal injection enables the delivery of substances to the same site as the thermal stimulus. Using a numerical rating scale, volunteers provided periodic ratings of the perceived pain experienced during the injection. Blinded injections showed substantial pain in heated injections in contrast to marginal pain induced by room temperature injections. The addition of lidocaine to the injected solution served as a positive control for pharmacological intervention in this heat pain model, effectively inhibiting the pain induced by heated injections. Through addition of an antagonist of TRPV1 we could demonstrate that it contributes to the detection of noxious heat over a broad temperature range. The TRPV1 antagonist effectively increased the temperature required to elicit a similar pain level and it reduced the maximal pain induced by the heat stimulus. However, substantial heat-induced pain remained, and further targets need to be investigated.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.02/X18

Topic: D.02. Somatosensation – Pain

Support: R01 NS115209
R01 NS086082
Arnold and Mabel Beckman Foundation
GSU Brains and Behavior

Title: Determination of Behavioral and Morphological Critical Periods in the Cold Nociceptive System

Authors: *K. J. DONALDSON[1], S. M. LATAILLADE[2], C. WILLIAMS[2], C. CUNNINGHAM[2], A. A. PATEL[2], D. N. COX[2];
[1]Neurosci., [2]Neurosci. Inst., Georgia State Univ., Atlanta, GA

Abstract: To ensure proper growth, development, and survival, organisms possess the ability to adapt to constantly changing sensory environments based on prior experience. Critical periods represent developmental stages when the nervous system is especially sensitive to specific stimuli and where abnormal input can cause dysfunction later in life. While experience-dependent plasticity has been previously studied in a variety of modalities and sensory systems, little is known about thermal sensation, specifically cold. *Drosophila* larvae predominantly respond to acute noxious cold (≤10°C) with a full body contraction (CT) behavior requiring activation of Class III multidendritic neurons (CIIIs) by cold stimuli. Previously, we've shown that prior cold experience (CE) in late development causes activity-dependent and neuronally driven cold-evoked behavioral plasticity, exhibited by increased magnitude of CT behavior. CE also induced an increase in C-bending behavior, a precursor to the rolling behavior typically associated with noxious heat stimulation and mediated by Class IV nociceptors (CIVs). Here, we sought to determine if critical periods exist in the cold nociceptive system by measuring behavioral responses to cold stimuli in late L3 larvae after undergoing 24 hours of CE at various developmental stages. Earlier CE caused desensitization of CT response, whereas later CE caused sensitization. To quantify additional, non-CT, behavioral changes and remove potential observer inconsistencies, it was necessary to improve upon existing analysis protocols by developing a novel pose-estimation model for *Drosophila* larvae using DeepLabCut. With this model, we are now able to greatly improve rigor and reproducibility of thermally-evoked larval behavior analysis. To investigate possible cellular mechanisms contributing to these behavioral effects, we quantified structural morphology of CIII dendritic arbors by imaging cell-type specific, genetic reporter lines which underwent the same CE paradigm as behavior experiments. CE led to activity-dependent changes in CIII dendritic arborization relative to non-CE controls. Together, this work demonstrates the existence of critical periods in the *Drosophila* cold nociceptive system while introducing a novel behavioral analysis method for larval nociceptive behavior.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.03/X19

Topic: D.02. Somatosensation – Pain

Support: 2022R1C1C1008226

Title: Amyloid Beta 1-42 Modulates Heat Pain Sensitivity in Aged Mice Through TRPV1 Inhibition via the LRP1-SHP2 Pathway

Authors: J. ROH[1], S.-M. HWANG[1], J. PAN[2], J. PARK[1], C.-K. PARK[1], *Y. KIM[1];
[1]Col. of Medicine, Gachon Univ., Incheon, Korea, Republic of; [2]Med. Sch. of Nantong Univ., Nantong, China

Abstract: With aging, there is an observed impairment in the ability to perceive heat, leading to risks such as an increased propensity for burns, compromised thermoregulatory function, and reduced ability to recognize signs of physical disorders, including infections and inflammation. This study aims to elucidate the mechanisms underpinning these alterations in heat sensitivity, providing a basis for strategies to manage associated risks. We investigated the role of amyloid beta (Aβ), a substance that accumulates with aging in peripheral tissues, in modulating heat sensitivity. Our findings indicate that aged mice show attenuated heat pain responses compared to younger ones at temperatures (45℃ and 50℃) known to activate transient receptor potential vanilloid 1 (TRPV1). Subsequent in vivo and in vitro experiments demonstrated that Aβ1-42 inhibits TRPV1 activation. Interestingly, we identified the role of the Low-density lipoprotein receptor-related protein-1 (LRP1) and Src homology region 2-containing protein tyrosine phosphatase 2 (SHP2) pathway in mediating this inhibitory effect of Aβ1-42 on TRPV1. Notably, Aβ1-42 administration attenuated heat hyperalgesia in the spared nerve injury (SNI) model, via the LRP1-SHP2-TRPV1 pathway, implying therapeutic potential. Collectively, our findings underscore the complex interplay between Aβ1-42, TRPV1, LRP1, SHP2, and thermal pain modulation, which may have significant implications for managing neurogenic pain in chronic pain patients with related conditions.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.04/X20

Topic: D.02. Somatosensation – Pain

Support: NS115209
NS086082
NS130970

Title: Non-canonical roles of odorant receptors in thermosensory nociception

Authors: *D. E. A. MOON, A. SAKURAI, A. A. PATEL, E. N. LOTTES, C. WILLIAMS, J. M. LETCHER, D. N. COX;
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Abstract: All organisms require sensory systems to transduce external stimuli into internal information. These sensory systems vary in complexity and mechanistic arrangement across species, however, organismal survival relies upon the ability to detect potentially damaging environments and respond appropriately. Nociception plays a critical role in adaptive behavioral responses to noxious stimuli, however the underlying mechanisms required to sense noxious cues remain incompletely understood, particularly in the case of thermosensory nociception to noxious cold stimuli. In a previously defined aversive cold behavioral assay, we implicated *Drosophila* class III multidendritic (CIII md) neurons in sensing cold nociceptive stimuli and demonstrated these neurons are both necessary and sufficient to elicit a stereotypical cold-evoked contraction (CT) behavior. Our previous studies have implicated TRP channels, metabotropic $GABA_B$ signaling coupled to calcium induced calcium release, and calcium activated chloride channel activity in mediating cold-evoked CT behavior, although mutating these pathways does not entirely abolish noxious cold behavior suggesting additional receptors may be involved in this process. To this end, we have used CIII md neuron-specific transcriptomics to identify potential non-canonical thermosensory nociceptive receptors. We performed a genetic screen of promising candidates among traditional chemoreceptor families for olfaction, odorant receptors (ORs). Intriguingly, selectively mutating CIII-enriched ORs revealed a significant role of Or65a and the obligatory co-receptor Orco in cold nociceptive behavioral response. We confirmed the impairment by testing multiple RNAi and mutants for our target genes. For further insight into the mechanistic roles of Or65a and Orco, we conducted functional assays to explore the requirements for these genes in cold-evoked electrical activity and calcium dynamics of CIII md neurons. Both *Or65a* and *Orco* knockdowns reduced cold-evoked calcium levels and decreased the firing rate with the *Orco* knockdown also shifting the firing pattern away from a bursting pattern and toward tonic spiking. This work is the first to exhibit a non-canonical role of ORs in thermal nociception. Further, this research situates ORs with the closely related multimodal gustatory receptors and ionotropic receptors, which have likewise been implicated in thermosensation, revealing functional roles that extend beyond labeled-line chemosensation.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

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Program #/Poster #: PSTR338.05/X21

Topic: D.02. Somatosensation – Pain

Support: Spanish AEI/10.13039/501100011033/PID2019-108194RB-100
Spanish AEI CEX2021-001165-S
Generalitat Valenciana GRISOLIA/2019/089
Generalitat Valenciana PROMETEO/2021/031

Title: The immunosuppressant Rapamycin is an agonist of the cold-activated TRPM8 ion channel

Authors: J. ARCAS[1], K. OUDAHA[1], A. GONZÁLEZ[1], J. CASTRO[1], J. FERNÁNDEZ-TRILLO[1], F. PERALTA[1], F. TABERNER[1], S. SALA[1], E. DE LA PEÑA[1], A. GOMIS[1], ***F. VIANA**[2];
[1]Inst. de Neurociencias, [2]Cell. and Systems Neurosci., Univ. Miguel Hernandez-CSIC, San Juan de Alicante, Spain

Abstract: Rapamycin (RAP) is a natural macrolide widely used in the clinic as an immunosuppressant. Topical application of RAP is also beneficial in ocular inflammatory disorders, including dry eye disease. Transient receptor potential melastatin 8 (TRPM8) is a non-selective cation channel activated by mild cold temperatures and cooling agents (e.g. menthol). TRPM8 is expressed in small-diameter DRG and trigeminal ganglion neurons, where it plays a critical role in the detection of environmental cold temperatures. Previously, we found that Tacrolimus, another macrolide immunosuppressant, activates TRPM8 channels (PMID: 30545944). Here we report that RAP, structurally similar to Tacrolimus, is also a TRPM8 agonist. In patch-clamp and calcium imaging experiments, RAP activated a typical TRPM8 current (i.e. non-selective, outwardly rectifying) and evoked calcium transients in HEK293 cells overexpressing mouse TRPM8. RAP also potentiated TRPM8-mediated cold responses in a dose-dependent manner. RAP induced a leftward shift in the voltage-dependent activation curve of TRPM8, bringing channel activation within physiological membrane potential values. These effects were also observed in different orthologs, including chicken and human TRPM8. In cell-attach recordings, RAP activated a non-selective cation channel of ≈100 pS. The menthol-insensitive mutant TRPM8-Y745H was also activated by RAP. Moreover, in the presence of RAP, the mutant channel showed the typical potentiation of cold-evoked responses. In cultured mouse DRG neurons, RAP (30 μM) increased intracellular calcium levels almost exclusively in cold-sensitive neurons. Lower concentrations (1 μM) potentiate cold responses. The effect of RAP on cold-sensitive neurons was fully prevented by the TRPM8 blocker AMTB. Consistent with these pharmacological results, in TRPM8 KO mice, responses to RAP were drastically blunted, while responses to other TRP agonist remained unaltered. In current-clamp recordings, RAP induced the firing of action potentials in TRPM8+ neurons. During whole-cell recordings at -60 mV, RAP activated an inward current and potentiated the current evoked by a cold ramp, causing a shift in the activation threshold. In anesthetized mice, application of RAP solutions (1

%) to the eye surface evoked tearing in wildtype animals while the effect was not observed in TRPM8 KO animals. Together, our results identify TRPM8 channels in sensory neurons as molecular targets of RAP, generalizing the agonist effect of macrolide immunosuppressants on this channel. These findings could help explain the anti-inflammatory effects of RAP after topical application to the skin and the eye surface.

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.06/X22

Topic: D.01. Somatosensation

Title: The paraventricular nucleus is required for histamine-induced itch

Authors: *J. KIM, S. HAN, H. KO;
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Abstract: Itch sensation induces scratching behavior to alleviate unpleasantness. Until now, many studies have revealed the neuronal circuit conveying pruriceptive information from the periphery to spinal cord and trigeminal nucleus, However, central mechanism of supraspinal level is largely unknown. The hypothalamic subregion, paraventricular nucleus (PVN) receives sensory inputs directly or indirectly from the periphery. The fMRI studies revealed that the PVN is activated by several pruritogen in human. Moreover, intrathecal oxytocin injection induces scratching responses in rodents. In this study, we tested whether chemogenetic inhibition of the PVN reduce histamine-induced scratching response. In addition, we examined which type of neuron in the PVN is associated with histamine induced itch sensation.

Disclosures: **J. Kim:** None. **S. Han:** None. **H. Ko:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

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Program #/Poster #: PSTR338.07/X23

Topic: D.01. Somatosensation

Support: the National Natural Science Foundation of China (31925017 and 31871087)

Title: Unraveling the pathogenesis of pruritus in intrahepatic cholestasis of pregnancy

Authors: *G. LAN, H. WANG, T. ZHAO, B. WU, Y. LI;
Peking Univ., Beijing, China

Abstract: Intrahepatic cholestasis of pregnancy (ICP) is a pregnancy-specific liver disorder that affects 0.3%-5.6% of pregnant women worldwide. The primary symptom of ICP is severe and gradually worsening pruritus. This and other complications can pose risks to the health and safety of both the mother and fetus. Unfortunately, there are currently no effective drugs for treating ICP, especially pruritus, as the molecular mechanism behind it remains unclear. This study reveals that specific sulfated progesterone molecules abnormally elevated in ICP patients can activate a human-specific itch receptor known as human MAS-related G protein-coupled receptor X4 (hX4). These molecules activate the hX4 receptor with an EC_{50} of about 0.7-5.3 μM in hX4-expressing HEK293T cells. Behaviorally, specific itch responses can be induced through these compounds in humanized hX4-expressing rats, but not in WT rats. More importantly, subcutaneous injection of sulfated progesterone can evoke itching in healthy individuals. Clinically, abnormally highly elevated levels of sulfated progesterone molecules are observed in the blood of ICP patients, whose levels could potentially serve as valuable biomarkers for early detection and prediction of ICP pruritus onset. In conclusion, sulfated progesterone molecules can activate hX4 to mediate ICP-related pruritus. This finding provides new effective targets for clinical drugs and could significantly help predict, diagnose, and treat ICP pruritus.

Disclosures: G. Lan: None. **H. Wang:** None. **T. Zhao:** None. **B. Wu:** None. **Y. Li:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.08/X24

Topic: D.01. Somatosensation

Support: NIH/NINDS R01:NS125413

Title: Compartmentalized signaling of MRGPRX1 and Transient Receptor Potential Vanilloid 4 (TRPV4) are both involved in the downstream signaling of itch.

Authors: J. S. RETAMAL[1], ***D. D. JENSEN**[2];
[1]Dept. of Biol., Univ. of Santiago De Chile, Santiago, Chile; [2]Translational Res. Ctr., NYU Col. of Dent., New York City, NY

Abstract: Chronic pruritus is a complex sensory modality that greatly impacts and diminishes quality of life. Chronic pruritus is associated with increased levels of anxiety, stress, depression,

and suicide. Unfortunately, the signaling mechanisms that initiate and maintain chronic itch are poorly understood. GPCRs are dynamic signaling receptors that are involved at all levels of itch sensation and regulation. The ability of GPCRs to signal from intracellular compartments is a new and exciting discovery that adds another level of control and new targets for the development of therapeutics to treat chronic disease states like pruritus. Here we investigated the role of endosomal trafficking and signaling of the Mas-related G-protein coupled receptor X1 (MRGPRX1) in itch and how endosomal signaling of MRGPRX1 alters TRPV4 sensitization. Methods: Fluorescence confocal microscopy, BRET, nbBRET, and FRET were used to investigate the internalization and signaling of MRGPRX1 from endosomes. Calcium imaging was used to characterize the sensitization of TRPV4, and itch behavioral assays were utilized to confirm the MRGPRX1 mediated TRPV4 sensitization in pruritus. Results: BRET, nbBRET and confocal microscopy showed MrGPRX1-activated by BAM8-22 induced a fast Gαq and βArr1 recruitment to the plasma membrane that is followed by internalization into RAB5-FYVE positive endosomes. nbBRET showed the recruitment of Gαq to MRGPRX1 in early endosomes and nuclear ERK FRET biosensors showed that MRGPRX1 signaling from the endosomal network induces a sustained increase of nuclear pERK, both of which are decreased by the endocytic inhibitor Dyngo4a. In addition, calcium influx mediated by MRGPRX1 activation is enhanced when TRPV4 is co-expressed in HEK293. The pre-stimulation of MRGPRX1 exacerbated the calcium response of TRPV4, which is switched to normal response in the presence of a TRPV4 antagonist (HC067) or a PKC inhibitor (GF109). Itch-induced by BAM8-22 *in vivo* was decreased by Dyngo4a and HC067 administration. Additionally, the coadministration of HC067 and Dyngo4a showed a potentiation, decreasing itch behavior induced by BAM8-22. Discussion: Activation of GPCRs like MRGPRX1 are directly linked to itch sensation. Here we show that MRGPRX1 is readily trafficked to and can signal from the endosomal compartment. Endosomal signaling of MRGPRX1 results in compartmentalized signaling in model cells and can lead to the sensitization of the TRPV4 ion channel. These results provide new insights into the mechanisms regulating GPCR signaling in pruritus and help identify new targets for therapeutic treatment.

Disclosures: **J.S. Retamal:** None. **D.D. Jensen:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.09/X25

Topic: D.02. Somatosensation – Pain

Support: JSPS Grant 22K06001

Title: Identification of TRPA1 channel activating substances from the mycelia of mycelium of the edible mushroom, Phlebia tremellosa.

Authors: *T. OHTA[1,2], K. TAKAHASHI[1,2], A. ISHIHARA[3,2];
[1]Dept. of Vet. Pharmacology, Fac. of Agr., [2]Fungus/Mushroom Resource and Res. Center, Fac. of Agr., [3]Dept. of Agricultural, Life and Envrn. Sciences, Fac. of Agr., Tottori Univ., Tottori, Japan

Abstract: Fungal mushrooms are a rich biological resource with millions of species estimated to exist. Many mushrooms are widely used not only for food but also for medicinal purposes. However, there remain many unidentified mushrooms and new active ingredients. The Fungus/Mushroom Resource and Research Center (FMRC), Tottori University, has been collecting and storing substances extracted from fungus/mushrooms and promoting their application and utilization in medicine. Nociceptive TRP channels, which are mainly expressed in peripheral sensory nerves, have important functions as pain signals. Among them, TRPA1, known as wasabi receptor, function as a responsible molecule for various pathophysiological pain. Therefore, identification of biological active substances acting TRPA1 has been forcefully conducted so far. In this study, 50 conserved strain extracts stored at FMRC were screened for TRPA1 channel activity. Because of the high Ca^{2+} permeability of this channel, we mainly used the Ca^{2+}-imaging for analyzing channel activity. In some experiments, patch-clamp analysis was also performed. In cells heterologous expressing TRPA1, the extract from mycelial culture medium of the *Phlebia tremellosus* increased cytosolic Ca^{2+} concentration. Using silica gel column chromatography and preparative HPLC, several compounds were isolated. Among them, we identified tremetriol, merulidial, and merulactone as active substances for TRPA1. Of these, merulidial showed the strongest activity. The responsiveness to merulidial was unchanged in the cysteine mutated TRPA1 channel, which abolishes the responsiveness to electrophiles, such as AITC. Furthermore, merulidial caused a TRPA1-dependent Ca^{2+}-increasing response in mouse sensory neurons. In contrast, these substances did not affect TRPV1, another nociceptive channel. These results suggest the presence of TRPA1-stimulating substances in the *Phlebia tremellosus* without modification of cysteine residues of TRPA1. These chemicals are expected to provide a tool for TRP channel research and fundamental insights into the development of new compounds targeting TRP channels.

Disclosures: T. Ohta: None. **K. Takahashi:** None. **A. Ishihara:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.10/Y1

Topic: D.01. Somatosensation

Support: NIH T34 GM131947
NIH R01 AR079713

Title: G-protein coupled receptor 35 (gpr35) expression and functional characterization using histological and cell-line based approach

Authors: *J. YU[1], A. LYFENKO[1], J. WHEELER[2], S. K. MISHRA[3];
[2]Mol. Biomed. Sci., [1]North Carolina State Univ., Raleigh, NC; [3]Mol. Biomed. Sci., The Col. of Vet. Medicine, NC State Unive, Raleigh, NC

Abstract: Itch is a major symptom of cutaneous diseases such as psoriasis and atopic dermatitis. Still, the mechanisms behind these debilitating conditions remain unknown, especially receptors that transduce itch signaling via these peripheral afferents innervating skin and send signals to the central nervous system. Earlier, we and others have reported an expression of G-protein Coupled Receptor 35 (GPR35) in the Dorsal Root Ganglia (DRG) sensory neurons, but their expression profile and function were less known. We used a transgenic line, immunological and cell-based assays to examine GPR35 expression and its function. DRGs from C57BL/6J and a transgenic line that expresses the fluorescent red protein (tdTomato) in the GPR35-promoter were collected under various fixation conditions and sectioned onto glass slides for immunohistological analysis in combination with different pruriceptive markers. Further, we examined the functional role of GPR35 using a heterologous expression system. We utilized Calcium Imaging to visualize the changes in the calcium concentration of HEK293 cells transfected with GPR35, TRPA1, and GPR35+TRPA1 and control (empty vectors) using agonists specific for receptor activation. In summary, we showed that GPR35 colocalized with TRPA1 and TRPV1-expressing neurons. We found increased calcium transients in cells co-transfected with GPR35 and TRPA1 compared to control in response to specific agonists. Our findings suggest a possible involvement of GPR35 in sensory transduction and may act as a potential therapeutic target.

Disclosures: J. Yu: None. **A. Lyfenko:** None. **J. Wheeler:** None. **S.K. Mishra:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.11/Web Only

Topic: D.01. Somatosensation

Support: National Natural Science Foundation of China 32030048
National Natural Science Foundation of China 31970938
Natural Science Foundation of Jiangsu Province BK20191448

Title: Descending dopaminergic pathway facilitates itch signal processing via activating spinal $GRPR^+$ neurons

Authors: Z.-J. ZHANG, H.-Y. SHAO, C. LIU, H.-L. SONG, X.-B. WU, D.-L. CAO, ***Y.-J. GAO**;
Nantong Univ., Nantong, China

Abstract: The A11 dopaminergic neurons regulate somatosensory transduction by projecting to the spinal cord, but the function of this descending projection in itch remains elusive. Here we

report that A11 dopaminergic$^{A11\text{-}SDH}$ neurons are activated by pruritogens. Inhibition of these neurons alleviates itch-induced scratching behaviors. Furthermore, chemogenetic manipulation of spinal dopamine receptor D1-expressing ($DRD1^+$) neurons changes acute or chronic itch-induced scratches. Mechanistically, spinal $DRD1^+$ neurons are excitatory and mostly co-localized with gastrin-releasing peptide (GRP), an endogenous neuropeptide for itch. In addition, $DRD1^+$ neurons form a synapse with GRP receptor-expressing ($GRPR^+$) neurons and activate these neurons via AMPA receptor (AMPAR). Finally, the spontaneous itch and the enhanced acute itch induced by activating spinal $DRD1^+$ neurons are blocked by antagonists for AMPAR and GRPR. Thus, the descending dopaminergic pathway facilitates spinal itch transmission via activating $DRD1^+$ neurons and releasing glutamate and GRP, which directly augments GRPR signaling. Interruption of this descending pathway may be used to treat chronic itch.

Disclosures: Z. Zhang: None. **H. Shao:** None. **C. Liu:** None. **H. Song:** None. **X. Wu:** None. **D. Cao:** None. **Y. Gao:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.12/Y2

Topic: D.01. Somatosensation

Support: SRNSF #217076
SRNSF #FR-21/2322

Title: Thermal hyperalgesia and mechanical allodynia in itch sensation: involvement of TRPV1 and TRPA1 channels

Authors: *M. G. TSAGARELI[1], I. NOZADZE[1], M. IODI CARSTENS[2], G. GURTSKAIA[1], E. CARSTENS[2];
[1]Pain and Analgesia, Ivane Beritashvili Ctr. of Exptl. Biomedicine Ctr., Tbilisi, Georgia;
[2]Neurobiology, Physiol. and Behavior, Univ. of California at Davis, Davis, CA

Abstract: Itch is defined as an unpleasant skin sensation associated with the desire to scratch, thereby removing exogenous stimuli such as parasites and plant particles. Previous studies have shown that intraplantar injection of pruritogens in mice elicited thermal hyperalgesia and mechanical allodynia suggesting that histamine and non-histaminergic pruritogens elicit painful as well as itchy dysesthesias. In this study, we tested if histamine, chloroquine, the bovine adrenal medulla peptide (BAM8-22), and septa-peptide Ser-Leu-Ile-Gly-Arg-Leu (SLIGRL) resulted in thermal hyperalgesia and mechanical allodynia in adult male mice. Methods and Results. The latency of hindpaw withdrawal from a noxious heat stimulus (Hargreaves test) and the threshold for hindpaw withdrawal from a mechanical stimulus were measured (von Frey test). In the first set of experiments, each group of mice received two intraplantar injections either saline or one of the three concentrations of a given pruritogens. Following the injection,

animals were tested in either Hargreaves or the von Frey tests. In the second set of experiments, the effect of intraplantar pretreatment with two different doses of the TRPV1 antagonist AMG-517 or two doses of the TRPA1 antagonist HC-030031 on thermal or mechanical withdrawals elicited by intraplantar injection of two doses of each pruritogen was tested. Intraplantar injection of histamine resulted in significant thermal hyperalgesia ($p < 0.001$) and mechanical allodynia ($p < 0.001$) ipsilaterally that persisted for 1 h. Pretreatment with two doses of the TRPV1 antagonist AMG-517, but not the TRPA1 antagonist HC-030031, significantly attenuated the magnitude and time course of thermal hyperalgesia and mechanical allodynia elicited by histamine ($p < 0.001$ for both), indicating that these effects are mediated by TRPV1. In contrast, pretreatment with the TRPA1 antagonist significantly reduced thermal hyperalgesia and mechanical allodynia elicited by chloroquine ($p < 0.001$ for both), BAM-822 ($p < 0.01$, $p < 0.001$, respectively) and SLGRL ($p < 0.05$, $p < 0.001$, respectively), indicating that effects elicited by these non-histaminergic itch mediators require TRPA1. Conclusions. These findings indicate that itch and pain coexist simultaneously and that histamine, BAM8-22, and SLIGRL can acutely elicit hyperalgesia and allodynia. TRPV1 and TRPA1 channel inhibitors might prove to be useful in the clinical treatment of increased pain and allodynia which may be symptoms in patients suffering from chronic itch. Acknowledgments. This work was supported partially by grants from the Rustaveli National Science Foundation of Georgia (#217076, FR21/2322).

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Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.13/Y3

Topic: D.01. Somatosensation

Support: NSFC 61890952
NSFC 31825013
National Science and Technology Innovation 2030 Major Program (2021ZD0204404)

Title: An atlas of itch-related neural dynamics in the mouse brain

Authors: W. CHEN[1], ***Y. SUN**[2];
[1]Inst. of Neuroscience, State Key Lab. of Neuroscience, Ctr. for Excellence in Brain Sci. & Intelligence Technology, Chinese Acad. of Sci., Shanghai, China; [2]Inst. of Neuroscience, CAS, Shanghai, China

Abstract: Neural dynamics in the brain play a crucial role in generating the itch-scratching cycle. However, our understanding of the neural dynamics during this cycle remains limited. In this study, we examined the neural dynamics of 126 mouse brain regions using fiber photometry.

Our investigation uncovered numerous novel response patterns in the mouse brain during the itch-scratching cycle, and we discovered that the neural dynamics in response to histamine and histamine-independent pruritogens show significant differences. Interestingly, we found that a group of brain regions displayed activation at the end of histamine-induced scratching behavior. Additionally, several brain regions demonstrated transient activation only at the onset of scratching induced by chloroquine. Furthermore, the functional neural network in the cortex differs between the two itch models, suggesting distinct neural mechanisms underpinning histamine- and histamine-independent itch. In summary, our study provides a valuable dataset that paves the way for further exploration into the neural mechanisms underlying the itch-scratching cycle.

Disclosures: W. Chen: None. **Y. Sun:** None.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.14/Y4

Topic: D.01. Somatosensation

Title: Assay Development and Profiling of Glial Cell Derived Neurotrophic Factor Receptor Alpha (GFRα) 2/3 Inhibitors for Pain and Itch

Authors: *K. GUPTA, K. OUK, M. TOH, G. MISSIG, S. JOHNSON, M. WANG, J. LIM, H. NGUYEN, D. STONE, S. PIN;
Cerevel Therapeut., Cambridge, MA

Abstract: The glial derived neurotropic factor (GDNF) family of endogenous ligands, including GDNF, neurturin (NRTN), artemin (ARTN), and persephin (PSPN), are proteins that play a diverse role in development and maintenance of peripheral and central neurons. Receptor signaling occurs through ligand binding to a complex consisting of rearranged during transfection (RET) receptor tyrosine kinase and one of four glycosyl-phosphatidylinositol-anchored co-receptors, namely GDNF receptor-α (GFRα) 1-4. GFRα2 and GFRα3 are highly expressed in primary sensory neurons and a convergence of genetic, preclinical, and clinical evidence implicates activation of these receptors in inflammatory and neuropathic nociception. These data suggests that inhibition of GFRα2/GFRα3 protein interactions may serve as a therapeutic avenue for pain and itch treatment. The aim of this study was to develop a high fidelity GFRα2/GFRα3 RET in-vitro functional assay to understand GFRα-mediated RET antagonism as well as profile binding of potential small molecule inhibitors of GFRα2 and GFRα3. Multiple high-throughput screens were conducted to search for novel chemical matter. This included a binding-based DNA-encoded library (DEL) screen of 70 billion compounds, a functional screen of a 60k chemical library, AI-based screen of 13 billion compounds, and a fragment library of 3.3k compounds. Confirmed hits were further triaged and analyzed in-depth at both the binding and functional levels to determine active compounds that selectively inhibit

GFRα2/GFRα3 mediated RET phosphorylation. Chemiluminescence signal from the induced receptor dimerization of activated RTK-PK causing complementation of two beta galactosidase enzyme fragments was used as a measure of GFRα inhibition. U2OS cells that expressed either GFRα1, GFRα2, GFRα3, or a constitutively active form of RET, enabled determination of receptor selectivity. Biophysical-based Surface Plasmon Resonance (SPR) assay was used to evaluate binding and revealed that the DEL hits indicated the best inhibitors out of all the compound screens based on profile. As a result, the first selective (GFRα3>2>1), sub-micromolar (~500nM) inhibitors with no direct RET inhibition were identified. Together, these results demonstrate the utility of the assay for identification of small molecule inhibitors of GFRα2/GFRα3 and identify compounds for potential further development as therapeutics in pain and itch.

Disclosures: **K. Gupta:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **K. Ouk:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **M. Toh:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **G. Missig:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Johnson:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **M. Wang:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **J. Lim:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **H. Nguyen:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **D. Stone:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics. **S. Pin:** A. Employment/Salary (full or part-time):; Cerevel Therapeutics.

Poster

PSTR338. Itch, Thermosensation, and ThermoTRP Channels

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR338.15/Y5

Topic: D.01. Somatosensation

Title: The localization and function of Cbln1 protein in dorsal root ganglion neurons

Authors: *S. TAKASUGI[1], M. YUZAKI[2];
[2]Physiol., [1]Keio Univ., Tokyo, Japan

Abstract: Dorsal root ganglion neurons are a population of neurons that receive inputs not only from external stimuli but also from visceral organs such as intestines and exchange substances with the bloodstream, thereby establishing a network with non-neural organs. MrgprA3-expressing neurons in the dorsal root ganglia have been well studied as a specific population that conveys the histamine-independent itch sensation elicited by the agonist of MrgprA3, chloroquine. Cbln1 is one of the well-known synaptic organizers in the central nervous system. Cbln1 is released into the synaptic cleft at the granule cell-Purkinje cell synapse in the cerebellum to induce synapse formation when neuronal activity of granule cells is acutely increased, while chronic hyperactivity results in gene transcription arrest and reduces the number of synapses.

Interestingly, Cbln1 is known to be highly expressed in dorsal root ganglia, specifically in the MrgprA3-expressing neurons, but little is known about its detailed localization and function. To investigate the role of Cbln1 in the transduction of chloroquine-evoked pruritus sensation, we analyzed the localization of the Cbln1 protein in dorsal root ganglion neurons and its function using the mice in which the Cbln1 gene was knocked out.

Disclosures: S. Takasugi: None. **M. Yuzaki:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.01/Y6

Topic: D.02. Somatosensation – Pain

Support: TNFR2 sex differences and EAE: R01NS124123 (NINDS)

Title: Role of male sex hormone and sex-chromosome complement on the therapeutic efficacy of a TNFR2 agonist in EAE

Authors: *S. GUPTA[1], K. A. SWANSON[1], R. FISCHER[2], J. R. BETHEA[1];
[1]Drexel Univ., Philadelphia, PA; [2]resano GmbH, BioNTech SE, Mainz, Germany

Abstract: Approximately 80% of multiple sclerosis (MS) patients experience chronic neuropathic pain (CNP) while 50% suffer from paralysis, with no effective therapies available. Neuroinflammation and neuropathology are major pathophysiological hallmarks of MS. Recognizing that, we tested whether TNFR2 activation (an anti-inflammatory and neuroprotective immune response) could be a potential therapeutic for this devastating disease. Pharmacological and genetic studies determined that TNFR2 signaling is therapeutic for CNP in both male and female mice with experimental autoimmune encephalomyelitis (EAE). However, TNFR2 signaling was only therapeutic for paralysis in female mice. To interrogate these sex-dependent mechanisms resulting in the limited therapeutic efficacy of TNFR2 signaling in EAE males, we performed experiments with wild-type and four core genotype (FCG) mice with and without surgical and pharmacological manipulation of testosterone. We utilized 10 weeks old wild type males and females along with gonadectomized males with and without testosterone replacement. In addition, age-matched gonadally intact males were either administered with flutamide (anti-androgen) or a placebo. XX^{sry+} (testis bearing) FCG males were used to study if the male sex-chromosome complement (XY) limited the TNFR2 signaling efficacy in mitigating paralysis. All animals were immunized using the nonPTX-EAE method. A novel TNFR2 agonist (10mg/kg) was administered on days 10, 13, and 16 after immunization. Mechanical allodynia (Von Frey test) was evaluated weekly to assess CNP and paralysis was scored daily based on open field testing until 30 DPI. Surprisingly, the TNFR2 agonist did not alleviate CNP in gonadectomized EAE males, with or without testosterone replacement. However, the TNFR2 agonist was able to alleviate CNP in gonadally intact males administered with flutamide.

Additionally, we found that there was a significant delay in paralysis onset and its severity in EAE XX^{sry+} males treated with the TNFR2 agonist as compared to EAE XY males. Our data indicates that alleviation of CNP by TNFR2 agonist in EAE males is dependent on male gonads and is independent of the presence of testosterone. Moreover, we found that the sex-dependent characteristic, which limited the efficacy of TNFR2 agonist in mitigating paralysis in EAE males is the presence of the XY sex-chromosome complement. Understanding the sex-mediated mechanisms which limit the efficacy of TNFR2 agonist in males is important because, unlike other therapies which have high side effects, TNFR2 agonist could promote endogenous anti-inflammatory and repair pathways, making it a feasible therapy for MS.

Disclosures: S. Gupta: None. **K.A. Swanson:** None. **R. Fischer:** A. Employment/Salary (full or part-time):; Employment/Salary: full-time, resano GmbH. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents regarding TNFR2 agonist technology. **J.R. Bethea:** 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.; TNFR2 agonist from resano GmbH.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.02/Y7

Topic: D.02. Somatosensation – Pain

Support: DOD CP200074 to JRB

Title: Tissue specific tumor necrosis factor receptor 2 (TNFR2) signaling is crucial for resolution of chronic pain

Authors: *S. ARNAB[1], V. BRACCHI-RICARD[1], R. FISCHER[2], J. R. BETHEA[1];
[1]Biol., Drexel Univ., Philadelphia, PA; [2]Inflammation & Tissue Damage, resanoGmbH, Mainz, Germany

Abstract: Injury to the somatosensory nervous system can lead to chronic neuropathic pain (CNP) often resulting from persistent neuroinflammation and maladaptive plasticity. Patients with CNP oftentimes suffer from comorbidities such as depression, anxiety, and neurocognitive alterations. Despite affecting a substantial number of populations treating neuropathic pain has proven challenging, because of its complexity and multifaceted nature. CNP is also refractory to many analgesic treatments, therefore no effective therapies to treat or cure CNP are available on the market . Our lab has shown an increase in tumor necrosis factor (TNF) at the site of injury in animal models of peripheral nerve injury and in CNP. This suggests that TNF signaling plays a crucial role in CNP. *In vitro* studies from our lab have also shown that tumor necrosis factor receptor 2 (TNFR2) signaling on cortical neurons promotes neurite outgrowth and endogenous

repair programs. To further study the role of TNFR2 in modulating chronic neuropathic pain, we performed a localized knock out of TNFR2 using a tamoxifen inducible tissue specific line. We then performed chronic constriction injury (CCI) after 3 weeks of tamoxifen induction. In addition, we used a specific TNFR2 agonist administered intraperitoneally at days 7, 10 and 13 post-CCI. Using two different pain tests (Von frey and conflict of avoidance) we show that (i) mice treated with TNFR2 agonist do not experience chronic neuropathic pain (ii) TNFR2 agonist is not therapeutic in conditional TNFR2 KO mice . To gain insight into the therapeutic effects of TNFR2 agonist, we performed an RNAseq analysis of the TNFR2 agonist-treated mice compared to vehicle-treated mice 6 weeks following CCI. We have identified a significant number of genes changed between groups in particular tissue regions from the RNAseq data analysis. Taken together our data suggests TNF signaling in specific localized regions is critical to the establishment of chronic neuropathic pain with TNFR2 playing a pivotal role.

Disclosures: S. Arnab: None. **V. Bracchi-Ricard:** None. **R. Fischer:** A. Employment/Salary (full or part-time):; full-time, resano GmbH. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on patents regarding TNFR2 agonist technology. **J.R. Bethea:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); receipt of drugs from resano GmbH.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.03/Y8

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS051709
DoD Grant W81XWH2110599

Title: Sex-specific regulation of chronic neuropathic pain via TNFR1/p38αMAPK signaling in Nex+ supraspinal neurons

Authors: *K. NGUYEN, K. A. SWANSON, S. GUPTA, J. RICARD, J. R. BETHEA; Drexel Univ., Philadelphia, PA

Abstract: Upregulation of soluble tumor necrosis factor (sTNF) cytokine signaling through TNF receptor 1 (TNFR1) and subsequent neuronal hyperexcitability are observed in both animal models and human chronic neuropathic pain (CNP) (Clark et al., 2013; Empl et al., 2001; Ji et al., 2018; Lindenlaub and Sommer, 2003) To test the hypothesis that supraspinal circuitry is critically linked to pain chronification, we studied the intersect between supraspinal TNFR1 mediated neuronal signaling and sex specificity by selectively removing TNFR1 in Nex+ neurons in adult mice ($NexCre^{ERT2}$::$TNFR1^{f/f}$). We determined that following chronic constriction injury (CCI), pain resolves in males; however, female acute pain transitions to

chronic. Subsequently, we investigated two downstream pathways important in TNFR1 signaling and injury response, p38MAPK and NF-κB. We detected p38αMAPK and NF-κB activation in male cortical tissue; however, p38αMAPK phosphorylation was reduced in NexCre^{ERT2}::TNFR1$^{f/f}$ males. Following CCI in NexCre^{ERT2}::p38αMAPK$^{f/f}$ mice we observed similar behavioral results. Previously, we established estrogen's ability to modulate sTNF/TNFR1 signaling in CNP, which may contribute to female CNP prevalence (Bouhassira et al., 2008; Claiborne et al., 2006; de Mos et al., 2007; Del Rivero et al., 2019; Li et al., 2009). To explore the potential relationship between estrogen and inflammation in CNP we used a combination therapy of an estrogen receptor β (ER β) inhibitor with a sTNF/TNFR1 or general p38MAPK inhibitor. We determined both combination therapies lend "male-like" therapeutic relief to females following CCI. These data suggest that TNFR1/p38αMAPK signaling in Nex+ neurons in CNP is male-specific and lack of therapeutic efficacy following sTNF inhibition in females is due to ER β interference. These studies highlight sex-specific differences in pathways important to pain chronification and elucidate potential therapeutic strategies that would be effective in both sexes.

Disclosures: K. Nguyen: None. **K.A. Swanson:** None. **S. Gupta:** None. **J. Ricard:** None. **J.R. Bethea:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.04/Y9

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01DA047157
NIH Grant R01DA047089
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NIH Grant R01DA34749

Title: Tlr4, lcn2, and cb1/2 are involved in HIV-related neuropathic pain model in mice

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Abstract: The opioid epidemic is a health and social issue in the U.S. Many of the HIV-infected population are intravenous drug users. Emerging clinical data suggests that repeated use of opioid pain medications can increase neuropathic pain in people living with HIV. Thus, it is significant to elucidate the exact mechanisms of the persistent and complexed chronic pain state. Evidence shows that proinflammatory factors (such as TLR4 and LCN2) are involved in the neuroinflammation in different diseases. Recent studies have indicated that cannabinoid receptor

type 1/2 (CB1/2R) agonists reduce neurodegeneration through anti-inflammatory activity, such as through inhibition of TLR4. In the present study, we investigated the role of TLR4 and LCN2 in HIV-related neuropathic pain model in mice. Neuropathic pain was induced by repeated intrathecal administration of recombinant HIV glycoprotein gp120 with morphine (gp120/M) in mice. Using Aldh1l1^{CreERT2}::**TLR4$^{fl/fl}$** mice, we selectively knocked out the expression of *tlr4* gene in the astrocytes under the induction of tamoxifen in gp120/M model. Conditional knockout of TLR4 reduced neuropathic pain in mice. Similarly, using Aldh1l1^{CreERT2}::**LCN2$^{fl/fl}$** mice, we found that conditional knockout of LCN2 reduced neuropathic pain in mice. LCN2 KO mice showed less pain responses in von Frey test and hot plate test. In the cultured astrocytes, TLR4 agonist treatment induced LCN2 expression. Either CB1 receptor agonist ACEA or CB2 receptor agonist LY2828360 suppressed neuropathic pain in mice. Current preliminary data suggest that TLR4, LCN2, and CB1/2 are involved in HIV-related neuropathic pain model in mice.

Disclosures: X. Zhu: None. **J. Gu:** None. **S. Liu:** None. **K. Hayashi:** None. **M. Pardo:** None. **H. Li:** None. **R.C. Levitt:** None. **S. Hao:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: D.02. Somatosensation – Pain

Support: DoD grants W81XWH2210266 to L.B.
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Title: A β-arrestin-biased positive allosteric modulator at NTSR1 abrogates physiological and pathological pain through central and peripheral mechanisms

Authors: *R. GUO[1,3], O. CHEN[3,2], Y. ZHOU[2], L. M. SLOSKY[5], W. WETSEL[2,4], L. S. BARAK[2], R.-R. JI[3,2,6];
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Abstract: Neurotensin (NT) is a tridecapeptide and it binds to the G protein-coupled receptors: NT receptor 1 (NTSR1) and NT receptor 2 (NTSR2). NTSR1 and NTSR2 are widely distributed in the brain and peripheral tissues. NT has been shown to produce antinociceptive effects in both acute and chronic pain models. However, poor oral bioavailability and CNS penetration, as well as severe side effects preclude the clinical application of NTSR ligands. Recent research has revealed that by biasing NTSR1 signaling towards the β-arrestin (βArr) pathway while simultaneously antagonizing NTSR1 Gq protein signaling, it effectively mitigates the side effects typically associated with balanced NTSR1 agonism. In this study, we demonstrate potent

antinociceptive and analgesic actions with the new NTSR1 βArr-biased positive allosteric modulator, SBI-810.Administration of SBI-810(i.p.) increased mechanical thresholds in naive mice for up to 3 hr. This effect was abolished in *Ntsr1*$^{-/-}$mice and *Arrb2*$^{-/-}$mice, indicating that both NTSR1 and βArr2 are required for the antinociceptive actions of SBI-810. Intrathecal injection of SBI-810 extended the antinociceptive effect to 5 hr. Furthermore, intrathecal or systemic delivery of SBI-810 reduced mechanical and cold allodynia in neuropathic pain models of spared nerve injury (SNI) and chemotherapy-induced peripheral neuropathy. Intriguingly, SBI-810 failed to induce conditioned place preference (CPP) in naive mice, suggesting that this compound may not activate reward circuitry in the brain. By contrast, SBI-810 induced significant CPP in mice with neuropathic pain, suggestive of the rewarding effects of inhibition of spontaneous pain after nerve injury. Patch-clamp recordings in spinal cord slices revealed that SBI-810 perfusion inhibited spontaneous excitatory postsynaptic currents in dorsal horn neurons. Calcium imaging of dissociated DRG demonstrated that SBI-810 inhibited TRPV1 but not TRPA1 signaling.
Therefore, SBI-810 serves as a biased NTSR1 agonist and selectively engages NTSR1/β-arrestin2 signaling, producing analgesic actions through both central and peripheral mechanisms.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.06/Y12

Topic: D.02. Somatosensation – Pain

Support: CIHR grant FDN-154336
CIHR Canada Graduate Scholarships Doctoral Award

Title: Pain hypersensitivity is dependent on autophagy protein Beclin 1 in males but not females

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Abstract: BACKGROUND: Pathological pain involves numerous cellular and molecular changes within the dorsal horn of the spinal cord. Autophagy is an intracellular degradation pathway and autophagy dysfunction is implicated in many neuropathologies. Yet, whether autophagy regulates pain is unknown. Here we investigated whether autophagy is involved in pain processing by targeting a protein critical in autophagy initiation, beclin 1 (Becn1). METHODS: Mice underwent intraplantar injection of complete Freund's adjuvant (CFA) to model inflammatory pain or spared nerve injury (SNI) surgery as a model of neuropathic pain. Both pain models induce hypersensitivity to mechanical stimuli as assessed by von Frey assay. RESULTS: Mice with reduced expression of beclin 1 due to monoallelic deletion of *Becn1*

(*Becn1*$^{+/-}$) have greater CFA-induced mechanical hypersensitivity compared to wild type. This was observed in males, but not in females. Moreover, intrathecal administration of a beclin 1-activating peptide (tat-beclin 1) reversed mechanical hypersensitivity induced by CFA in wild type male mice, but had a limited effect in females. Tat-beclin 1 also reversed SNI-induced mechanical hypersensitivity in males, but not females. We previously demonstrated that the male-specific pain pathway is dependent on brain-derived neurotrophic factor (BDNF)-induced upregulation of NMDA receptors in the spinal dorsal horn. Here, we found that tat-beclin 1 prevented mechanical hypersensitivity induced by exogenous BDNF. Furthermore, *Becn1*$^{+/-}$ mice expressed higher levels of the GluN2B subunit of the NMDA receptor in the dorsal horn compared to wild type, in males but not females. In line with GluN2B upregulation, dorsal horn neurons from male *Becn1*$^{+/-}$ mice have greater NMDA receptor-mediated miniature excitatory post-synaptic currents compared to neurons from wild type male mice. CONCLUSION: Taken together, we conclude that Beclin 1 regulates inflammatory and neuropathic pain signaling pathways in a sex-dependent manner. In males, downregulation of beclin 1 enhances NMDA receptor-mediated currents in dorsal horn neurons, which increases pain hypersensitivity.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.07/Y13

Topic: D.02. Somatosensation – Pain

Title: Selective manipulation of the claustrum modulates acute and chronic pain

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Abstract: The anterior cingulate cortex (ACC) is integral for the cognitive integration of nociception. Chronic pain is associated with ACC hyperactivity while inhibition alleviates allodynia, highlighting an importance in mediating pathological pain hypersensitivity. The causes underlying these changes are not understood. The claustrum (CLA) is a narrow subcortical structure that restricts ACC activity by activating inhibitory interneurons. Though its function is unknown, early studies point to regulation of cortical network activity in sensory processing. We hypothesize that acute nociceptive stimuli will activate CLA projection neurons leading to an attenuation of ACC activity and that loss of this function will lead to pain hypersensitivity. Chronic inflammatory pain was modeled by injecting 0.1ml complete Freund's adjuvant (CFA) or saline into the hind paw of adult male and female C57Bl6/J mice (n=8/group). Experimenters were blinded during analysis. Neuronal activity in ACC projecting CLA neurons (CLA_{ACC}) was assessed through retrograde tracing and cFOS immunohistochemistry. Acute nociception significantly activated the CLA, however chronic inflammatory pain reduced this

activation. *In-vivo* fiber photometry revealed peak CLA activation occurred after pain behaviors resolved. Using a viral vector strategy in male mice, complimentary gain- and loss- of function studies were performed by expressing HM3Dq or caspase-3 in CLA_{ACC} neurons respectively along-side viral controls. Lesioning this pathway significantly decreased mechanical pain thresholds and exacerbated the onset of chronic pain. However, chemogenic activation did not affect nociceptive thresholds but did rescue mechanical allodynia in the CFA model. This work has identified a new neural circuit that participates in pain processing, highlighting a potential dysfunction in chronic pain that could be manipulated to resolve pain hypersensitivity. Further characterizing this pathway will provide greater insight into how the CLA influences cortical function which will deepen our understanding of pain processing, the establishment of chronic pain, and sensory processing in general.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

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Program #/Poster #: PSTR339.08/Y14

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS057499
NIH Grant R21NS122347

Title: Regulation of neuropathic pain by microglial Orai1 channels

Authors: *K. DEMEULENAERE[1], S. TSUJIKAWA[1], M. YAMASHITA[1], K. STAUDERMAN[2], A. APKARIAN[3], M. SALTER[4], M. PRAKRIYA[1];
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Abstract: Neuropathic pain is a debilitating condition that can be brought on by disease or injury to the nervous system. A major step in this process is neuroinflammation mediated by microglia in the spinal dorsal horn, but the molecular checkpoints for microglial reactivity after nerve injury remain poorly understood. Furthermore, the contributions of microglia for driving neuropathic pain have been found to be sex-dependent (in males only), and it is unknown if this sexual dimorphism arises from differences in spinal cytokine levels, cell-autonomous differences in microglia, or different pro-inflammatory mediators from adaptive immune cells, such as T cells. Store operated calcium entry (SOCE) is a key mechanism for Ca^{2+} entry and production of proinflammatory mediators in immune cells. SOCE is mediated by the Orai family of ion channels via interactions with STIM1, the ER Ca^{2+} sensor. Here, we investigated the role of Orai1 in microglia and its contribution to the inflammatory landscape of the spinal cord after nerve injury. Ablation of Orai1 in microglia attenuated microglial Ca^{2+} signaling and reduced

pro-inflammatory cytokine production following LPS stimulation. Following nerve injury, conditional deletion of microglial Orai1 channels in mice attenuated microglial proliferation in the dorsal horn and cytokine levels in the lumbar spinal cord. Microglial Orai1 cKO mice showed partial mitigation of allodynia after nerve injury and this effect was also seen in WT mice administered with a small molecule inhibitor of Orai1, CM4620. However, mitigation of allodynia was only observed in male but not female mice. To investigate if this sexual dimorphism is due to differences in adaptive immune cell contributions to the inflammation, mice with deletion of Orai1 in $CD4^+$ T cells were tested for changes in allodynia following nerve injury, but no differences were detected in these T-cell KO mice. Together, these results indicate that Orai1 channels are critical regulators of the sex-specific microglia mediated neuroinflammation that underlies neuropathic pain.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Program #/Poster #: PSTR339.09/Y15

Topic: D.02. Somatosensation – Pain

Support: Seale Fund

Title: Low-intensity focused ultrasound to human insular and dorsal anterior cingulate cortex affects measures of central sensitization and autonomic processing

Authors: *A. IN[1], A. STROHMAN[2], W. LEGON[3];
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Abstract: Central sensitization is the abnormal amplified response to pain associated with many chronic pain conditions. Several lines of evidence demonstrate abnormally increased insula and dorsal anterior cingulate cortex (dACC) activity to noxious stimuli in chronic pain patients. Unfortunately, the insula and dACC lie deep to the cortex making it difficult to target non-invasively with spatial selectivity. Low-intensity focused ultrasound (LIFU) is a novel form of non-invasive neuromodulation with high spatial resolution combined with deep focal lengths that can precisely target the anterior insula (AI), posterior insula (PI) and dACC independently. We examined the effects of LIFU to the AI and PI as well as dACC on measures of pain and central sensitization including conditioned pain modulation (CPM) and temporal summation of pain (TSP). We enrolled 20 healthy participants (13F / 7M) and stereotaxically targeted LIFU to either the AI, PI or sham in a cross-over design. We also enrolled 18 healthy participants (12F /

6M) and targeted the dACC with 2 arms (dACC, sham). All participants (ages 20-45) underwent pain thresholding as well as CPM and TSP tasks. For CPM, the conditioning stimulus (CS) was a cold pressor test to the left hand and the test stimulus (TS) a brief heat stimulus to the right hand. Participants were required to rate the TS on a 0-9 pain scale after the CS. This procedure was performed three times. For TSP, 10 brief heat stimuli were administered to the right forearm separated by 2 seconds. Participants rated each of the 10 stimuli immediately after they occurred. Both CPM and TSP procedures were completed before and after LIFU or sham intervention to the AI, PI or dACC. Electrodermal response (EDR) and two lead electrocardiogram (ECG) was continuously collected to monitor autonomic nervous system changes assessing for effect from LIFU. We found a significant effect of LIFU to PI for CPM and TSP tests. LIFU to PI increased CPM 0.5±0.181 compared to AI and sham conditions ($p=0.024$). LIFU to PI also reduced perceived pain during the TSP task compared to sham and AI ($p=0.0004$). We also found a significant difference between dACC and sham conditions for the TSP test of -0.55±0.05 ($p=0.0009$). Preliminary analysis of the autonomic data suggests that LIFU to AI and dACC but not PI affects metrics of heart-rate variability including the standard deviation of the N-N interval (SDNN) and low-frequency power. LIFU is an effective non-invasive method to target deep brain structures like the insula and dACC to affect pain processing and measures of central sensitization. We demonstrate LIFU as a promising tool for pain amelioration that could be developed for clinical therapies.

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Poster

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS104964

Title: An unexpected player in the circuitry for mechanical allodynia revealed through studies of diabetic neuropathy

Authors: *M.-C. NOH[1,2], K. A. CORRIGAN[1,2], S. P. WILLIAMS[3], C. PEIRS[1,2,5], X. ZHAO[1,2], D. F. S. SANTOS[4,2], B. K. TAYLOR[4,2], R. P. SEAL[1,2];
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Abstract: Over 250 million individuals with diabetes suffer from chronic polyneuropathic pain. The persistent hyperglycemia in diabetes triggers peripheral sensitization, which in turn leads to central sensitization. We have previously identified unique dorsal horn circuits involved in mechanical allodynia as a function of injury type. We showed that calretinin neurons are involved in inflammatory injuries; by contrast, protein kinase C gamma neurons and the enzyme

itself are involved in neuropathic injuries. Finally, cholecystokinin neurons are engaged in both inflammatory and neuropathic injuries. To date, no spinal interneuron population important for mechanical allodynia in diabetic polyneuropathy has been identified. Herein, we aimed to elucidate the mechanical allodynia circuitry for diabetic neuropathy and compare it with the mechanical allodynia circuitry induced by the other two types of injuries. Streptozotocin (STZ) was injected to trigger persistent hyperglycemia in both male and female mice. Recombinant adeno associated virus vectors containing excitatory or inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) were intraspinally injected to selectively manipulate spinal interneurons. Von Frey and cotton swab tests were used to measure static and dynamic mechanical sensitivity. One week following the behavior tests, mice were walked on a treadmill to induce c*fos* expression. Interestingly, none of the previously studied excitatory populations were involved in STZ induced mechanical allodynia. Our *cfos* analysis suggests involvement of more superficial neurons such as vertical cells. Parvalbumin (PV) inhibitory interneurons were shown to gate vertical cells. Therefore, we tested whether activation of PV neurons would impact mechanical allodynia induced by STZ. Both punctate and dynamic allodynia were significantly reduced when PV neurons were activated. Furthermore, the activation also decreased *cfos* expression in the superficial dorsal horn. Gastrin releasing peptide receptor (GRPR) neurons have a vertical cell morphology and are implicated in itch and pain. *Grpr* neurons expressed *cfos* in the STZ model and had significantly diminished *cfos* with PV activation. To confirm *Grpr* neuron's involvement in mechanical allodynia, we silenced *Grpr* neurons in the STZ model, and in neuropathic and inflammatory pain models. Remarkably, inhibition of *Grpr* neurons significantly reduced both punctate and dynamic allodynia in all pain models. Given its critical role in the spinal itch circuit, *Grpr* neurons may serve as an attractive therapeutic target for both itch and chronic pain.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS091296, DE 02773, DE029946, DE029187

Title: Plastic changes of serotonergic system in DRN-ACC neural circuit and its loss of ascending inhibition after nerve injury contribute to the maintenance of neuropathic pain

Authors: *C. BIAN, T. LI, J. YANG, W. GUO, S. ZOU, M.-K. CHUNG, K. REN, F. WEI; Dept. of Neural and Pain Sciences; Ctr. to Advance Chronic Pain Res., Univ. Maryland, Baltimore, MD

Abstract: The 5-HT-containing neurons in the dorsal raphe nucleus (DRN), representing the main source of ascending serotonergic projecting to the forebrain including the anterior cingulate cortex (ACC), are involved in a broad physiological function. Dysfunction of the serotonergic system in the brain is associated with central mechanisms of chronic pain. Laboratory data has shown that DRN or its 5-HT neurons participate in endogenous pain inhibition, which is the main basis for the clinical trials of antidepressants such as SSRIs for pain management. Although these drugs may help relieve depression and certain types of pain, new clinical trials have explored a "shocking" lack of evidence for their efficiency in the alleviation of neuropathic pain. In fact, whether plasticity in ascending 5-HT fibers and the complex 5-HTR expressions present in the ACC after nerve injury remains still poorly unclear. In this study, we examined the distribution of ascending serotonergic fibers and the expression of some 5-HTR subtypes in the ACC in the mouse CCI-ION model. After microinjection of AAV9-Flex-tdTomato into the DRN of sert-cre mice, we observed a time course-dependent increase of the intensity of 5-HT fibers in the bilateral ACC at 2 and 6 w after the unilateral CCI. Brush stimulation in the ipsilateral V2 area of the face induced c-Fos expression in 5-HT DRN neurons in CCI animals but not the sham group. Meanwhile, we found that optogenetic activation of DRN-derived 5-HT terminals in the ACC induced analgesia in naïve mice, which remained at 1-2 w but was totally lost at 4-8 w after CCI. These findings suggest that ascending 5-HT system in the DRN-ACC circuit is inhibitory for pain in physical conditions. Although the progressive increase of 5-HT fibers in the ACC occurs after nerve injury, there is a loss of ascending pain inhibition in the maintenance but not induction of neuropathic pain. Using two-photon calcium imaging in ACC slices, we further demonstrated a temporal switch of 5-HT effects on ACC neuronal activity from the majority of inhibition to excitatory tone after CCI when compared to that in naïve animals. Finally, consistent with delayed downregulation of 5-HTR1b expression in the ACC, intra-ACC injection of its agonist CP-94,253 (0.1-1 μM) significantly attenuated CCI-induced mechanical hypersensitivity at 2 w but not 8 w after CCI, indicating that a loss of inhibitory tone from some anti-nociceptive 5-HTR subtypes in the ACC mediates the loss of 5-HT-dependant ascending pain inhibition. Combined with enhanced 5-HT-dependent descending facilitation after injury, our data may provide a new explanation for why most antidepressants to treat chronic pain are not helpful.

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Poster

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Topic: D.02. Somatosensation – Pain

Support: R01NS116759

Title: Disruption of mitochondrial pyruvate oxidation in dorsal root ganglia leads to long-lasting pain and profound transcriptome modifications

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Abstract: Disruption of mitochondrial pyruvate oxidation in dorsal root ganglia leads to long-lasting pain and profound transcriptome modifications
Md Mamunul Haque, Panjamurthy Kuppusamy, Ohannes K. Melemedjian
Department of Neural and Pain Sciences, School of Dentistry, University of Maryland, Baltimore, MD, USA
Disclosures: **Md Mamunul Haque**: None. **Panjamurthy Kuppusamy**: None. **Ohannes K. Melemedjian**: None.
Metabolism is inextricably linked to every aspect of cellular function and defects in metabolic pathways are associated with a variety of neoplastic, cardiovascular, and neurodegenerative diseases. Additionally, polymorphisms and inborn errors in metabolic genes are associated with a multitude of chronic pain conditions. Nerve growth factor (NGF) is another factor that is associated with a multitude of chronic pain conditions, but its potential role in regulating the metabolism of sensory neurons is largely unknown. Hence, we explored whether intraplantar NGF injection in mice alters the metabolism of sensory neurons. We determined that intraplantar NGF injection disrupts mitochondrial pyruvate oxidation leading to an increased extrusion of lactate and protons. These changes were driven by the enhanced expression of pyruvate dehydrogenase kinase 1 (PDHK1) and lactate dehydrogenase A (LDHA). Moreover, pharmacological, and genetic blockade of PDHK1 and LDHA attenuated NGF-induced tactile allodynia. Direct disruption of mitochondrial pyruvate oxidation in dorsal root ganglia was sufficient to cause tactile allodynia that lasted at least 3 months - unlike NGF-induced allodynia which resolves within 72 hours. Full-length transcript analysis revealed that the disruption of mitochondrial pyruvate oxidation significantly altered the transcription of a large number of genes, reduced the poly-A tail length of mRNAs, and modified the alternative splicing of hundreds of transcripts. These findings demonstrate that aberrant mitochondrial pyruvate oxidation is a crucial mechanism for the development and maintenance of chronic pain.
Keywords: chronic pain, NGF, metabolism, RNA-seq, DRG.
R01NS116759

Disclosures: M. Haque: None. **P. Kuppusamy:** None. **O.K. Melemedjian:** None.

Poster

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant P50 DA044121
NIH Grant R01AR074274-01A1

Title: Mechanical hyperalgesia and neuropathic pain qualities in osteoarthritis are associated with poorer outcomes after total knee replacement

Authors: *O. CONG[1], C. BONIN PINTO[3], J. BARROSO[1], A. VIGOTSKY[1], J. PEREZ[4], K. K. PETERSEN[5], L. ARENDT-NIELSEN[5], K. HARDT[6], D. MANNING[6], A. APKARIAN[7], P. BRANCO[2];
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Abstract: Knee osteoarthritis (KOA) is one of the most common persistent pain conditions worldwide. Total knee replacement (TKR) surgery is the only treatment for end-stage OA, yet a significant minority of patients develop chronic post-TKR pain. More recently, quantitative sensory testing (QST) has been used to assess pain sensitivity in KOA patients, indicating its potential to be utilized in identifying high-risk patients for development of chronic postoperative pain. We sought to explore whether QST parameters differed between KOA and HC subjects, to determine their ability to predict chronic postoperative pain at each timepoint, and to examine whether QST parameters would change over time.
QST was performed on 77 symptomatic KOA patients and 41 age- and sex-matched pain-free healthy controls (HC) at baseline. Postoperatively, QST measures were repeated at 3- and 6-months after surgery and pain-related questionnaires at 3, 6 and 12-months. Pressure pain detection threshold (PDT) and tolerance thresholds (PTT), conditioned pain modulation (CPM) and temporal summation (TS) were assessed at the lower leg using cuff algometry. Mechanical hyperalgesia, namely pain for a single prick (prick1) and 10 consecutive pricks (prick10), and TS were also assessed at the medial site of the patella using a von-Frey pinprick test.
In the cross-sectional analysis, KOA patients showed a lower cuff algometry PTT; however, all other measures did not differ between groups. For measures collected with the von-Frey pinprick stimulator, KOA patients reported significantly higher pain for prick1 and prick10. For the predictive analysis, prick1 predicted pain at 3- and 12-months and prick10 predicted pain at 12-months after surgery, with higher mechanical hyperalgesia at baseline leading to higher pain ratings both pre- and post-TKR. Moreover, we found that preoperative neuropathic pain scores, as measured by the PainDETECT questionnaire, were predictive of pain at 6- and 12-months. Lastly, for the longitudinal analysis, postoperatively, higher pinprick values were associated with higher pain intensity. Together, our findings show that KOA patients exhibit pronounced mechanical hyperalgesia and that this hyperalgesia is predictive of chronic postoperative pain. These results have important mechanistic and clinical insights regarding pain persistence after knee replacement that can be readily implemented.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.14/Y20

Topic: D.02. Somatosensation – Pain

Support: GACR 21-17085S
GAUK 198923

Title: The role of auxiliary subunit KCTD16 of GABAB receptor in nociception

Authors: D. VASCONCELOS[1], M. HELES[1], P. ADAMEK[1], ***J. PALECEK**[2];
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Abstract: Gamma aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system acting through the ionotropic (GABAAR) and metabotropic (GABABR) receptors. The metabotropic receptors are heterodimers composed of GABAB1 and GABAB2 which function is thought to be modulated by the potassium channel tetramerization domain proteins (KCTD) from clade F (KCTD16; KCTD12 and KCTD8). In our experiments we explored the role of the KCTD16 in modulation of nociceptive synaptic transmission in different pain models using KCTD16 KO and WT mice (obtained from B.Bettler, Uni. Basel) and transgenic mutant mice (VGAT-ChR2-EYFP crossed with KCTD16 KO) enabling optical stimulation of inhibitory neurons. Behavioral testing of mechanical and thermal sensitivity, whole-cell patch clamp recordings from spinal cord dorsal horn neurons (miniature postsynaptic excitatory currents mEPSC, light evoked inhibitory currents leIPSC) and calcium imaging from dorsal root ganglion (DRG) neuronal cultures were performed. Our data suggest that under control conditions the thermal and mechanical thresholds do not differ between the WT and KCTD16 KO mice, while the analgesic effect of the GABABR agonist (Baclofen) was significantly more pronounced in the WT animals. The excitatory and inhibitory currents recorded from the dorsal horn neurons did not differ significantly between the WT and the KO animals. In animals after carrageenan induced peripheral inflammation the behavioral testing did not show any significant differences (WT vs KO) although the inhibitory effect of Baclofen on mEPSC and leIPSC was reduced in neurons from the KCTD16 KO mice. Chronic constriction injury model (CCI) of neuropathic pain induced significant changes in thermal and mechanical thresholds in both WT and KO animals. However, the KCTD16 KO animals showed reduced thermal hyperalgesia during the later stages 21, 28 and 35 days after the CCI induction, while in the WT animals the hyperalgesia was still pronounced. Altogether our data suggest the importance of the KCTD16 in modulation of GABABR function in the pain pathway, especially under pathological conditions. Further experiments are needed to elucidate the downstream cascade events involving interaction with GIRK, VGCC and potentially TRPV1 and to understand the role of the KCTD16 in modulation of nociception and pain. This work was supported by Grant Agency of the Czech Republic GACR 21-17085S and GAUK 198923.

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Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.15/Z1

Topic: D.02. Somatosensation – Pain

Support: Rita Allen Scholar Award in Pain
Hetzler Foundation Pilot Grant
T32 GR0025759
R01DA049924

Title: Hypoexcitability of accumbal-projecting dopamine neurons is associated with decreased reward sensitivity in a model of chronic pain.

Authors: *Y.-H. CHANG[1], J. M. THOMPSON[2], M. CREED[3];
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Abstract: Chronic pain is a common and costly healthcare burden that is significantly associated with development of negative affective states, complicating pain management. Decreased activity of ventral tegmental area (VTA) dopamine neurons has been directly suggested to underlie development of pain-related anhedonia and amotivation. However, direct evidence for this hypothesis in models of chronic pain is lacking, and its underlying mechanisms are not well understood. The VTA is a heterogeneous structure with discrete dopaminergic projections that have distinct roles in reward learning. The dopaminergic projection to the nucleus accumbens (VTA->NAc) is critical for computing reward prediction errors and updating relative reward value. Thus, we hypothesized that hypoactivity of the VTA->NAc circuit contributes to the chronic pain-related hypodopaminergic state and correlates to impairment of dopamine-dependent effort and reward-based behaviors. We performed patch-clamp recordings from identified VTA->NAc neurons in mice that had undergone spared nerve injury (SNI)- or sham surgery. Intrinsic excitability of VTA->NAc neurons was decreased in SNI mice compared to sham controls, manifested as a significant reduction in the ability to burst fire. Based on these findings, we hypothesized that performance on tasks requiring sustained effort in pursuit of rewards or updating behavior under conditions of reward uncertainty would be impaired following SNI, which we corroborated using homecage-based, closed economy behavioral assays. These results suggest that pharmacological or neuromodulation strategies that could rescue these cellular adaptations in VTA->NAc neurons would be important for treating affective symptoms of chronic pain.

Disclosures: Y. Chang: None. **J.M. Thompson:** None. **M. Creed:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.16

Topic: D.02. Somatosensation – Pain

Support: MOST-109-2320-B-182-008-MY3
CMRPD1K0081
BMRPB96

Title: Human brain neural oscillations in respiratory sensory gating

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Abstract: Background: Human respiratory sensory gating is a neural process associated with inhibiting repetitive respiratory mechanical stimuli in the central nervous system. With a paired transient inspiratory occlusion paradigm, respiratory sensory gating is demonstrated by a diminished N1 peak amplitude for the second stimulus (S2) compared to that for the first stimulus (S1) in scalp EEG recorded respiratory-related evoked potential (RREP). However, brain neural oscillations mediating respiratory sensory gating still remain unclear. The purpose of the present study was to examine the frequency-specific oscillatory components contributing to respiratory sensory gating. **Methods:** A group of healthy adults participated in the present study. Two transient 150-ms inspiratory occlusions with a 500-ms inter-stimulus-interval were provided every 2 to 4 breaths randomly. The participants were instructed to count the number of respirations occluded during the experiment. At least 100 paired inspiratory occlusions were collected for data analysis. The perceived level of breathlessness and level of unpleasantness of the occlusions were measured after the experiment. **Results:** The S1 amplitude for the N1 peak was significantly larger than the S2 RREP N1 peak, as expected. For both the evoked- and induced-oscillations, time-frequency analysis showed higher theta and alpha activations in response to S1 compared to S2. The averaged respiratory sensory gating S2/S1 ratio for the N1 peak amplitude was approximately 0.66. The induced theta in response to S1 and S2, together with perceived dyspnea unpleasantness accounted for approximately 43% of variance in the linear regression model for predicting respiratory sensory gating ratio. **Conclusions:** Our results suggested that theta and alpha oscillations confirmed the “gating” phenomena in respiratory sensation regardless of evoked or induced powers. The induced theta oscillations together with the rated level of dyspnea unpleasantness are critical in predicting respiratory sensory gating performance. Our findings serve as a foundation for future investigations in the underlying mechanisms of respiratory sensory gating, particularly in diseased populations.

Disclosures: P. Chan: None. **K. Liang:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.17/Z2

Topic: D.02. Somatosensation – Pain

Support: DoD grant W81XWH2110885

Title: Gpr37l1 protects neuropathic pain by regulating astrocyte gliosis and synaptic transmission in the spinal cord

Authors: *J. XU[1,2], Z. YAN[3], S. BANG[2], D. VELMESHEV[3], R.-R. JI[2,3];
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Abstract: Astrocytes play important role in regulating synaptic formation and synaptic functions. Astrocyte activation and astrogliosis in the spinal cord has been implicated in the pathogenesis of chronic pain, including neuropathic pain after nerve injury and chemotherapy. Thus, neuropathic pain can result from both neuropathy and gliopathy. However, the diversity of spinal cord astrocytes in neuropathic pain development has not been characterized. Identification of the specific markers in astrocytes will provide new insight into the diversity and function of astrocytes and may also lead to novel therapeutic targets for chronic pain management. G protein-coupled receptors (GPCRs) are the largest family of transmembrane proteins in eukaryotes and represent nearly half of the therapeutic drug targets. GPR37L1 is an orphan GPCR and its function in pain regulation remains unknown. Here we show that *Gpr37l1* is highly expressed in spinal cord astrocytes and plays an essential role in protecting against the development and maintenance of neuropathic pain in mice. Using single-nucleus RNA sequencing (snRNA-seq) and in situ hybridization, we revealed that *Gpr37l1* is specifically expressed in astrocytes of the spinal dorsal horn (SDH) of mice, and *Gpr37l1* represents one of the highest expressed GPCR transcripts in SDH. Importantly, our snRNA-seq analysis identified a unique astrocyte subcluster marked by *Gpr37l1*. In *Gpr37l1* knockout mice or after siRNA treatment in SDH, we find that loss of *Gpr37l1* resulted in pain hypersensitivity and astrogliosis, and snRNA-seq confirms an enhanced astrogliosis in *Gpr37l1* knockout mice after SNI compared to SNI alone. Furthermore, we found a possible interaction of GPR37L1 with glutamate transporter 1 (GLT-1) in astrocytes, and partial loss of GPR37L1 resulted in enhanced excitatory synaptic transmission. Finally, selective overexpression of GPR37L1 in SDH astrocytes with the AAV vector in mice with nerve injury was able to reverse neuropathic pain and astrogliosis. Taken together, our findings suggest that targeting GPR37L1 in astrocytes may provide a new therapeutic option for treating neuropathic pain.

Disclosures: J. Xu: None. **Z. Yan:** None. **S. Bang:** None. **D. Velmeshev:** None. **R. Ji:** None.

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.18/Z3

Topic: D.02. Somatosensation – Pain

Support: JSPS KAKENHI Grant JP22K15206 (K.Koga)
JSPS KAKENHI Grant JP20H04043 (H.F.)
The collaborative study Grant with Daiichi Sankyo Co., Ltd. (H.F.)

Title: Involvement of the voltage-gated calcium channel subunit, $\alpha_2\delta$-1, expressed in spinal dorsal horn excitatory neurons for facilitation of excitatory synaptic transmission and mechanical hypersensitivity after peripheral nerve injury.

Authors: *K. KOGA[1], K. KOBAYASHI[2], M. TSUDA[3], K. KUBOTA[4], Y. KITANO[5], H. FURUE[1];
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Abstract: Neuropathic pain is an intractable pain condition that commonly occurs after nerve damage, and caused by aberrant excitability of spinal dorsal horn (SDH) neurons. It is known that the current therapeutic drugs, gabapentinoids, reduce spinal neurotransmitter releases, and alleviate neuropathic pain by binding to $\alpha_2\delta$-1 subunits. Although $\alpha_2\delta$-1 is expressed both in the primary afferent and SDH neurons, the contribution of $\alpha_2\delta$-1 in SDH neurons to neuropathic pain conditions after nerve injury is not fully understood. In this study, we investigated whether $\alpha_2\delta$-1 in SDH neurons is involved in mechanical hypersensitivity and aberrant synaptic transmission after peripheral nerve injury. Using in situ hybridization technique, we found that *Cacna2d1*, mRNA coding $\alpha_2\delta$-1, was mainly colocalized with *Slc17a6*, an excitatory neuronal marker, but not with *Slc32a1*, an inhibitory neuronal marker in the SDH. Using clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9 system, we showed that SDH neuron-specific ablation of *Cacna2d1* alleviated mechanical hypersensitivity following nerve injury. We further found that excitatory post-synaptic responses evoked by electrical stimulation applied to SDH were significantly enhanced both in the nerve injured mice and in the presence of inhibitory neurotransmitter antagonists, and these enhanced responses were significantly suppressed by the SDH neuron-specific ablation of *Cacna2d1*. These results suggest that $\alpha_2\delta$-1 expressed in SDH excitatory neurons enhances spinal nociceptive synaptic transmission and contributes to the development of peripheral nerve injury-induced mechanical hypersensitivity.

Disclosures: K. Koga: None. **K. Kobayashi:** None. **M. Tsuda:** None. **K. Kubota:** A. Employment/Salary (full or part-time):; Daiichi Sankyo Co., Ltd. **Y. Kitano:** A. Employment/Salary (full or part-time):; Daiichi Sankyo Co., Ltd. **H. Furue:** 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.; Daiichi Sankyo Co., Ltd..

Poster

PSTR339. Central Mechanisms of Neuropathic Pain I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR339.19/Z4

Topic: B.09. Glial Mechanisms

Support: NIAAA grants- R01 AA029694
NIAAA grants- R01 AA025967
NIAAA grants- P50 AA022534.

Title: Morphine treatment in prenatal alcohol-exposed mice prolongs NLRP3 inflammasome-mediated proinflammatory immune actions increasing the chronic allodynia from peripheral nerve injury.

Authors: *A. A. PASMAY[1], A. PRITHA[2], J. CARTER[3], L. FAUZI[3], M. S. SUN[5], A. K. FERNANDEZ-OROPEZA[3], D. C. JIMENEZ[3], M. MURPHY[3], D. D. SAVAGE II[6], C. F. VALENZUELA[8], N. MELLIOS[7], E. D. MILLIGAN[4], S. NOOR[3];
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Abstract: *In utero* alcohol exposure can lead to Fetal Alcohol Spectrum Disorder (FASD) resulting in a range of neurobehavioral deficits. Prenatal Alcohol exposure (PAE) can result in chronic central nervous system (CNS) immune dysfunction during adulthood. Prior work demonstrated that adult PAE rat offspring with minimal nerve injury develop pathological light touch sensitivity or allodynia due to aberrant peripheral immune and spinal glial actions through the release of pain-inducing proinflammatory cytokines such as interleukin (IL)-1β. Our data suggests that PAE-related adaptations "prime" CNS glial-immune cells to over-respond to subtle challenges from endogenous cell-stress signals through the innate immune receptors, Toll-like receptor 4 (TLR4) and Nod-like receptor family pyrin domain-containing 3 (NLRP3). Interestingly, morphine known to be the standard opioid used as a pain therapeutic; in addition to signaling via μ-opioid receptors, can activate the glial TLR4-NLRP3 pathway resulting in increased production of mature IL-1β protein. These overlapping immune interactions between PAE and morphine led us to hypothesize that morphine treatment following adult-onset peripheral nerve injury may paradoxically prolong allodynia in PAE offspring mediated by aberrant NLRP3 activation. Our data suggest that morphine treatment (10 mg/kg, 5 sequential days) in nerve-injured PAE mice with an established allodynia *prolongs* the duration of set allodynia for another 20-28 days. Morphine-induced prolongation of allodynia is evident in both sexes; however, it is more pronounced in male PAE mice. Moreover, treatment with a small-molecule inhibitor of NLRP3, MCC950 (i.p. 10 mg/kg) induces full reversal of allodynia, regardless of sex. MCC950-mediated reversal of allodynia is observed as soon as 90 minutes post-injection and persisted to 24 hours post- injection. Ongoing work is focusing on evaluating the differential expression of immune molecules related to the TLR4-NLRP3 pathway in the presence of morphine, with or without MCC950 treatment. Together, these data provide

evidence that PAE and morphine interactions involve aberrant NLRP3 activation, which may be predictive of adverse responses to opioids intended to treat pain in individuals with FASD.

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Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.01/Z5

Topic: D.03. Somatosensation – Touch

Support: NSF Grant 1818140
Phi Sigma RD Weigel grant

Title: The AMsh glia modulate touch-induced escape response in C. elegans

Authors: *T. AWE, J. ADAMS, L. KELLY, S. NIHA, W. STEIN, A. VIDAL-GADEA; Sch. of Biol. sciences, Illinois State Univ., Normal, IL

Abstract: Glia are non-neuronal cells in the nervous system that play a vital role in supporting the function and structure of neurons and in modulating neuronal activity. The amphid sheath glia of *C. elegans* (AMsh) surrounds and supports a dozen sensory neurons in the head of the worm. The AMsh were previously shown to modulate neuronal activity and behavior. For example, AMsh glia respond to noxious volatiles, and use GABA to modulate aversive odorant avoidance. In addition, AMsh glia respond to mechanosensory stimulation of the animal's head (nose touch). Nose touch results in a rapid aversive behavioral response in *C. elegans*. AMsh, however, respond to nose touch in a time scale longer than a minute, which is far longer than expected to mediate a meaningful aversive response. The molecular mechanism and behavioral significance of this glial touch response remains unresolved. We used mutant analysis, RNA interference, behavioral and calcium ratiometry to investigate the molecular machinery responsible for AMsh's nose touch response, and its behavioral relevance. Our results indicate that AMsh responds to touch using a mechanism partially conserved with odor detection, which includes OSM-9, ITR-1, and MEC-12. Interestingly, we found that AMsh touch-induced activation peaks coincide with the end of escape reversals induced by nose touch and by gentle anterior touch. AMsh was also found to peak at the end of spontaneous (unprompted) reversals. Additionally, AMsh modulation of touch-induced reversal duration is mediated by GABA signaling. Our results indicate that AMsh glia acts on multiple neuronal circuits associated aversive stimuli, suggesting a role in arousal-mediated behavioral modulation.

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Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.02/Z6

Topic: D.03. Somatosensation – Touch

Support: JSPS KAKENHI 20H05960

Title: Auxetic mechanism hypothesis of mechanoreceptor Ruffini endings in touch reception

Authors: *M. NAKATANI;
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Abstract: In this study, we addressed our investigation of the mechanisms of transduction in Ruffini endings, with a focus on their morphology and mechanical mechanism. Ruffini endings are the preferential responders to skin stretching, among other external cutaneous stimuli. However, it is not straightforward to detect the knobby nature of a flexible body in an anisotropic and heterogeneous elastic body, such as cutaneous skin. This observation may suggest not only the ability of Ruffini endings to detect physical stimuli on the skin as mechanoreceptors, but also that there is a mechanical mechanism by which Ruffini ending morphology can respond sensitively to skin stretch.

Although there are only limited studies on the morphology of Ruffini endings, super-resolution morphological analysis using electron microscopy and investigations combining immunohistochemical staining and confocal microscopy observations to analyze the structure of multi-cellular tissues remain to be explored. There remains plenty of room for further study regarding large scale morphological observations in Ruffini endings.

The ability of Ruffini endings to respond more dominantly to horizontal skin stretch rather than to vertical displacement given against the skin suggests that this mechanoreceptive structure may have mechanical properties in common with auxetic metamaterials. Auxetic metamaterials exhibit mechanical properties that are different from those of common linear elastic materials. For example, the common property of linear elastic materials is that they expand laterally when compressed vertically, but auxetic metamaterials can have the property of shrinking laterally in response to mechanical compression in the vertical direction.

Therefore, we conceived several basic structures of auxetic metamaterials using a 3D printer, and produced the real-world model of basic structures and the Rufini endingl-like shape with those basic inner structures. We then studied the deformation of these 3D printed shapes when we compressed them vertically. The results showed that the helical structure among the developed auxetic metamaterials could transform the vertical deformation of the skin into a lateral deformation.

There is a need to examine from an anatomical perspective whether such a mechanistic mechanism of Ruffini endings actually exists and function in the skin tissues. Further research will then be tied to the mechanistic mechanism to analyze the biophysics of how the mechanoreceptor channels are arranged in the sensory nerves that populate the Ruffini endings.

Disclosures: M. Nakatani: None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.03/Z7

Topic: D.03. Somatosensation – Touch

Title: Piezo2 channels contribute to the detection of hair movement and eliciting dynamic responses

Authors: *Y. BABA, K. NGUYEN, E. A. LUMPKIN;
Univ. of California Berkeley, Berkeley, CA

Abstract: PIEZO2 channels have been identified as critical mediators of mechanosensation and proprioception. Mouse studies have demonstrated that PIEZO2 is responsible for detecting light touch and pressure in the skin and play a crucial role in proprioception. Similarly, human mutations in *PIEZO2* have been linked to loss of touch discrimination and proprioception. Although the importance of PIEZO2 is well recognized, little is known about the contribution of PIEZO2 in various physiological response profiles observed in sensory neurons. In this study, we utilized a $CDX2^{cre}$ driver to selectively knock out *Piezo2* in mice to carefully examine the impact of *Piezo2* deletion on the response characteristics of sensory neurons. The $CDX2^{Cre+}$;$Piezo2^{fl/fl}$ conditional knockout mice (CKO) are viable but lack PIEZO2 in all caudal tissues, including peripheral sensory neurons. To assess the influence of PIEZO2 channels on the physiological properties of A-fibers, such as rapid and slow adaptation, we used an ex vivo skin-nerve preparation. First, we observed a complete absence of responses to brush stimulation on the skin in CKO mice compared to littermate controls (CONT). Second, sensory neurons of CKO mice displayed minimal to no response to low force indentation (<1 mN), compared with $CDX2^{Cre-}$, consistent with previous studies (median von Frey threshold, CONT: 0.69 mN, n=106; CKO: 5.88 mN, n=87). Third, CKO mice showed no responses to hair-follicle deflection, specifically the absence of responses from guard hair afferents, D-hair afferents, and low threshold C-fibers. The lack of responsiveness in hair follicle-innervating sensory neurons in CKO mice indicates that PIEZO2 serves as the primary mechanosensor for detecting hair movements. By contrast, A mechanonociceptors (AM) (von Frey threshold ≥1 mN) of CKO mice demonstrated responses to skin indentation similar to those observed in CONT. Notably, the key differences in the response profiles between CONT and CKO A-fibers were primarily observed in the dynamic phase of the response. CKO mice exhibited minimal responses to dynamic stimuli, resulting in the absence of RA responses. Conversely, no notable differences were observed in the characteristics of the static phase of AM afferents as the average instantaneous firing frequency were similar between CKO and CONT (mean±SD, CONT: 17.1±11.4 Hz; CKO: 18.4±10.8 Hz; P=0.54; Student's unpaired t-test, two-tailed). Together, these findings indicate that PIEZO2 primarily contributes to the dynamic phase of mechanosensation and not to the sustained phase of firing. Moreover,

these results suggest the presence of a high-threshold mechanosensitive ion channel within hairy skin.

Disclosures: Y. Baba: None. **K. Nguyen:** None. **E.A. Lumpkin:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.04/Z8

Topic: D.03. Somatosensation – Touch

Support: ETRI Grant 23ZB1100

Title: Evaluation on the Encoding and Decoding model of Slow Adapting Mechanoreceptors to Pressure Stimuli

Authors: Y. KANG[1], Y. CHOI[2], H. CHO[3], K.-H. PARK[1], S. JUNG[4], *S. LEE[1];
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Abstract: There is a growing interest in implementing natural touch sensations in virtual reality applications, but achieving this goal remains a challenging problem. Some studies are exploring the encoding of virtual touch sensations using mechanically driven stimuli. However, physical encoding methods have limitations, such as device weight. In contrast, electrical stimulation encoding offers an advantage in terms of device weight as it eliminates the need for separate mechanical actuators. This study presents foundational research on implementing virtual touch sensations through electrical stimulation and proposes a electrophysiological decoding/encoding model to achieve realistic touch sensations in virtual spaces. To decode the response of mechanoreceptors to stimuli, electrophysiological experiments were conducted on the saphenous nerves of mice under physical pressure conditions. Spike signals from the saphenous nerves were recorded using Au electrodes in an environment where they were electrochemically separated from paraffin oil and synthetic interstitial fluid (SIF) solution. C57bl/6 mice, aged 8-10 weeks, were used in the experiments. The stimulus intensity ranged from 10 mN to 100 mN with 10 mN increments, and each stimulation period lasted 6 seconds, followed by a 40-second rest period. Regression analysis was performed on the recorded electrical signals from the saphenous nerve. The decoding regression model exhibited a high level of similarity with the electrical signal patterns (R-squared value = 0.98). The decoding regression model effectively represented the spike firing patterns of SA receptors, which showed concentrated spike firing at the beginning of the stimulus. Furthermore, statistical analysis of the inter-spike interval (ISI) values of the recorded electrical signals from the saphenous nerves was conducted to develop an encoding electrical stimulation model based on the pressure stimulus intensity. Based on the statistical

analysis results and the decoding regression model, we developed an encoding electrical stimulation model that corresponds to the intensity of pressure stimuli. Using this encoding model, we applied electrical spike trains of approximately 400uA to the skin and confirmed a precise one to one matching response in saphenous nerve. Furthermore, measuring local field potentials in mice's somatosensory cortex validates the effectiveness of the developed decoding/encoding model by showing discernible discrimination in changes in beta wave activity with varying pressure intensity.

Disclosures: **Y. Kang:** None. **Y. Choi:** None. **H. Cho:** None. **K. Park:** None. **S. Jung:** None. **S. Lee:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.05/Z9

Topic: D.03. Somatosensation – Touch

Title: Vagal sensory nerves require PIEZO2 for sensing mechanical distention in the esophagus.

Authors: *S. S. NAIR, S. HADLEY, M. PATIL, T. TAYLOR-CLARK;
Mol. Pharmacol. and Physiol., Univ. of South Florida, Tampa, FL

Abstract: The esophagus is densely innervated by sensory afferent nerves projected from both the vagal and dorsal root ganglia, which are capable of sensing mechanical and chemical stimuli. Previous research has demonstrated that certain vagal afferents innervating the esophagus act as low-threshold mechanoreceptors, regulating homeostatic peristalsis and reflexes such as swallowing and vomiting. In mammals, PIEZO2 is the bona fide mechanosensitive ion channel essential for sensing lung and bladder inflation. Although PIEZO2 expression has been detected in a subset of vagal sensory neurons, its role in esophageal mechanosensation remains unclear. Therefore, we hypothesize that PIEZO2 is necessary for vagal esophageal afferent fibers to perceive esophageal distention. To test our hypothesis, we employed two-photon imaging of neuronal GCaMP6s fluorescence in an *ex-vivo* vagal-esophageal preparation. Using a gravity-driven perfusion system to distend the esophagus, we measured the activation of vagal esophageal afferents (increase in cytosolic Ca^{2+}). Our proof-of-concept studies were conducted on the transgenic Pirt-GCaMP6s, obtained through the crossbreeding of Pirt-Cre mice with the Cre-dependent, ROSA26-based, GCaMP6s reporter *B6.129S6-Gt(ROSA)26Sor-tm96(CAG-CGaMP6s)Hze*. Our preliminary data from Pirt-GCaMP6s mice showed a profound increase in Ca^{2+} influx in a subset of vagal neurons upon esophageal distention at pressures of 5, 10, and 30 mmHg. To evaluate the specific role of Piezo2, we conducted a dual injection protocol; we administered a retrograde AAV (rgAAV2) vector encoding Cre recombinase with a tdTomato reporter into the esophagi of $Piezo2^{fl/fl}$mice, along with an intravagal injection of an AAV9 vector encoding GCaMP6s calcium indicator. Following cre-mediated knockout of Piezo2, the tdTomato+ neurons exhibited a reduction of >85% in Ca^{2+} influx in response to esophageal

distention at all three intraesophageal pressure conditions. Our preliminary findings support the conclusion that Piezo2 plays a crucial role in esophageal mechanosensation mediated by vagal esophageal neurons.

Disclosures: S.S. Nair: None. **S. Hadley:** None. **M. Patil:** None. **T. Taylor-Clark:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.06/Z10

Topic: D.03. Somatosensation – Touch

Support: NIH intramural funding

Title: Impaired mechanosensation caused by PIEZO2 hypomorphism

Authors: *A. NICKOLLS, G. O'BRIEN, S. SHNAYDER, S. DONKERVOORT, D. SAADE, D. BHARUCHA, X. HU, C. BONNEMANN, A. CHESLER;
NIH, Bethesda, MD

Abstract: Our senses of touch and proprioception (limb position) are mediated by PIEZO2, a mechanosensitive ion channel. PIEZO2 converts mechanical forces on the skin, muscle, and tendons into ionic current by sensing plasma membrane stretch. Here, we report a case study on an apparently hypomorphic allele of PIEZO2 caused by an asparagine-to-lysine (N◊K) missense variant. This variant was identified in two sibling patients and inherited from their parents in compound heterozygosity with a loss-of-function variant. These patients presented with mild body coordination deficits and missed motor milestones throughout childhood. Based on *in vitro* heterologous expression assays, the PIEZO2$^{N◊K}$ variant is trafficked properly to the plasma membrane but has a 5-fold diminished response to membrane stretch. Amino acid substitution experiments and *in silico* structural analyses suggest that the wildtype asparagine residue makes a critical hydrogen bond to support mechanotransduction domains within PIEZO2. Finally, knock-in mice and rats homozygous for PIEZO2$^{N◊K}$ phenotypically match the human patients, exhibiting strikingly uncoordinated motor control and touch sensation alongside greatly diminished mechanically evoked currents in their sensory neurons. Altogether, this study identifies a new class of genetic variant in PIEZO2 that renders a hypomorphic ion channel and impairs the transduction of touch and proprioceptive stimuli.

Disclosures: A. Nickolls: None. **G. O'Brien:** None. **S. Shnayder:** None. **S. Donkervoort:** None. **D. Saade:** None. **D. Bharucha:** None. **X. Hu:** None. **C. Bonnemann:** None. **A. Chesler:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.07/Z11

Topic: D.03. Somatosensation – Touch

Support: DFG - EXC-2049 – 390688087
Humboldt Universität zu Berlin

Title: 3D architecture of the rat vibrissa innervation revealed by DiI-CT and synchrotron X-ray radiation

Authors: *B. GERHARDT[1], J. REICHMANN[2], L. EIGEN[1], J. ALFKEN[2], M. MUGNAINI[1], S. HECHT[3], T. SALDITT[2], M. BRECHT[1];
[1]Bernstein Ctr. For Computat. Neuroscinece Berlin, Berlin, Germany; [2]Inst. für Röntgenphysik, Georg-August-Universität Göttingen, Göttingen, Germany; [3]Dept. of Chem. & IRIS/CSMB Adlershof, Humboldt Univ. Berlin, Berlin, Germany

Abstract: Rodent vibrissae are essential for tactile sampling of the environment and enable elaborate behaviors, ranging from texture discrimination to navigation and social interactions. Studying sensory transduction in the vibrissa follicle is difficult, however, due to the inaccessibility of vibrissa tissue to fluorescence imaging and histology techniques. In our study, we address this issue and analyze the three-dimensional neuroarchitecture of functionally different rat vibrissa follicles stained with DiI-CT microinjections - a novel neural tracer for X-ray microscopy - from phase contrast X-ray nano computer tomography (nanoCT) volume images obtained at the DESY synchrotron source. Our volume imaging approach allowed us to resolve the vibrissa innervation at the axonal level, revealing the afferent system in unprecedented detail. We find that the deep vibrissal nerve enters the follicle at fixed angles after which it engulfs the vibrissa shaft radially asymmetric, mirror symmetrically matching the C-shape of the ring wulst as it branches into ~30 arms before terminating in mechanosensitive nerve endings. Further, comparison of functionally different rat vibrissae shows a patterning of ring wulst geometry, an anatomical structure to which the most sensitive mechanoreceptors - the club-like endings - are associated, which are arranged in semi-circles across the vibrissa pad with the wind sensitive supra-orbital having the closest and E-row/ arch-4 vibrissae the most open ring wulst. We conclude that functional specialization begins at the sensory periphery, as functionally different vibrissae display distinct follicle anatomy, likely reflecting their differential engagement during behavior.

Disclosures: B. Gerhardt: None. **J. Reichmann:** None. **L. Eigen:** None. **J. Alfken:** None. **M. Mugnaini:** None. **S. Hecht:** None. **T. Salditt:** None. **M. Brecht:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.08/Web Only

Topic: D.03. Somatosensation – Touch

Title: Evaluating gene expression in skin tissue and plasma proteins related to the morphology of peripheral nerve fibers

Authors: *F. O. UCHIMURA, T. TOHGASAKI, X. YU, S. KONDO, T. SAKURAI;
FANCL Res. Inst., Yokohama, Japan

Abstract: Human cutaneous peripheral nerves elongate and branch along scaffolding proteins of the extracellular matrix (ECM), and their terminals are distributed from the dermis to the epidermis. Cutaneous sensation is attenuated with a decrease in peripheral nerve fiber density with age. Recently, a three-dimensional analysis using immunofluorescence and decolorization technology reported that the morphology of intraepidermal nerve fibers changes with age; shortening of fiber length, reduction in the number of branching fibers, and complexity. However, the cause of age-related peripheral nerve fiber loss in the skin around the peripheral nerve fibers, is unclear.
Therefore, this study aimed to investigate the relationship among the morphology of peripheral nerve fibers, genes from dermal tissues, and plasma proteins. Abdominal skin tissue and plasma samples from Caucasian women were used. Peripheral nerve fibers in the skin were observed under a confocal microscope using fluorescence immunostaining and decolorization technology. 3D structural analysis of peripheral nerve fiber morphology was performed to quantify the length, volume, and number of fibers as structural parameters. Gene expression in dermal tissues was quantified using microarray analysis. The expression levels of 3092 plasma proteins were determined using the Olink Proximity Extension Assay. Using these datasets, transcriptome and proteomic analyses, GO enrichment analysis and Kyoto Encyclopedia of Genes and Genomes KEGG pathway analysis, were performed to identify the genes and proteins associated with each structural parameter of the peripheral nerve fibers in the skin.
Intergroup analysis identified genes commonly involved in inflammatory responses in dermal tissue. From plasma, a proteome analysis identified some proteins associated with skin inflammation and immunity, including cytokine signaling and extracellular secretion regulation. In addition, protein-protein interaction enrichment analysis of the plasma detected Interleukin-1alpha and 8, which are proteins related to inflammation.
These results suggest that morphological changes in peripheral nerve fibers are associated with the inflammatory response of the dermis and systemic chronic inflammation. Inflammation causes ECM degeneration, altering its physical properties and quantities. Additionally, plasma inflammatory factors would associate with ECM degeneration, which may affect peripheral nerve elongation and branching in the skin. Further study of the relationship between nerve structure, ECM and inflammation is expected to clarify the mechanism of the peripheral nerve fiber loss in the skin.

Disclosures: F.O. Uchimura: None. **T. Tohgasaki:** None. **X. Yu:** None. **S. Kondo:** None. **T. Sakurai:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.09/Z12

Topic: D.02. Somatosensation – Pain

Support: VR Start to MS
MS is a fellow of Wallenberg Academy

Title: Piezo2-dependent rapid pain system in humans.

Authors: G. CARBALLO[1], O. BOUCHATTA[1], M. BRODZKI[1], A. MARSHALL[2], H. MANOUZE[1], J. LILJENCRANTZ[3], F. M. DE-FARIA[1], E. FRANGOS[4], D. SADE[6], C. BONNEMANN[5], A. T. CHESLER[7], H. OLAUSSON[1], S. S. NAGI[1], *M. SZCZOT[8]; [1]BKV/CSAN, Linkopings Universitetet, Linkoping, Sweden; [2]Univ. of Liverpool, Liverpool, United Kingdom; [3]Univ. of Goteborg, Göteborg, Sweden; [4]NIH, NIH, Rockville, MD; [5]NIH, Bethesda, MD; [6]Univ. of Iowa, Iowa City, IA; [7]NCCIH/NINDS, NIH/NCCIH, Bethesda, MD; [8]BKV/CSAN, Linkoping Univ., Linköping, Sweden

Abstract: The ability to perceive and respond to our environment is essential for survival. While progress has been made in understanding nonpainful mechanical sensations, the neural mechanisms behind painful mechanical sensations remain less explored. Using human psychophysics techniques, we investigated a distinct submodality of mechanical pain evoked by hair pulling. First, we found that patients with PIEZO2 deficiency syndrome do not perceive hair pulling as painful. Moreover, we observed that preferential blockade of Aβ fibers in healthy participants eliminated hair-pulling pain, suggesting that Aβ fibers are essential for pain sensation induced by hair pulling. Using *in vivo* calcium imaging, we established that even though pulling of the single hair recruits multiple classes of sensory neurons, only a subset shows selective tuning to hair pulling and a lack of sensitivity to other physiological stimuli, including skin stretch. We show that in genetically defined murine hair-pull nociceptors, Piezo2 sets the threshold for mechanical sensitivity, and we uncover a shared transcriptomic identity between murine hair-pull nociceptors and a previously uncharacterized class of human neurons which we found to be associated with hair follicles. Using microneurography and targeted pharmacology, we elucidate that these human neurons represent a new class of rapidly conducting nociceptors that show selective tuning to painful hair-pull stimuli. This system enables precise signaling of mechanical pain evoked by hair pulling and exhibits molecular, cellular, and functional specialization.

Disclosures: G. Carballo: None. **O. Bouchatta:** None. **M. Brodzki:** None. **A. Marshall:** None. **H. Manouze:** None. **J. Liljencrantz:** None. **F.M. de-Faria:** None. **E. Frangos:** None. **D. Sade:** None. **C. Bonnemann:** None. **A.T. Chesler:** None. **H. Olausson:** None. **S.S. Nagi:** None. **M. Szczot:** None.

Poster

PSTR340. Touch: Mechanosensory Neurons and Piezo2

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR340.10/Z13

Topic: D.02. Somatosensation – Pain

Support: NIH grant NS097344
NIH grant AT011447
HHMI
Harvard Medical School Shapiro predoctoral fellowship

Title: Characterizing genetically defined fast-conducting high threshold mechanoreceptors that innervate mouse glabrous skin

Authors: *K. LEZGIYEVA[1], A. EMANUEL[1], L. QI[1], K. NGUYEN[1], N. SHARMA[2], D. D. GINTY[1];
[1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Pharmacol. and Systems Biol., Columbia Univ., New York, NY

Abstract: A large diversity of sensory neurons enables the sense of touch by encoding different features of stimuli acting on the skin. Much is known about low threshold mechanoreceptors (LTMRs) of fast and slow conduction velocities, and most of our understanding of intense force sensation comes from the study of slow C-fiber high threshold mechanoreceptors (HTMRs). Yet, there also exists a less-well understood group of neurons that have high mechanical thresholds and fast conduction velocities, termed Aδ high-threshold mechanoreceptors (Aδ-HTMRs). Our lab has recently generated *Smr2*^{Cre} and *Bmpr1b*^{Cre} mouse lines, and we hypothesized that they label distinct classes of Aδ-HTMRs that innervate non-hairy, or glabrous, skin. To assess their physiological properties, we performed *in vivo* targeted loose-patch electrophysiological recordings and calcium imaging of dorsal root ganglia (DRG) of genetically labeled mice and found that *Smr2*^{Cre}-labeled neurons indeed have high mechanical thresholds (>40 mN) and fast conduction velocities (~8 m/s). We've also shown that these neurons respond repetitively to continued stimulation, that they have large, overlapping receptive fields, and that they appear to be temperature-insensitive in glabrous skin. Recordings from *Bmpr1b*^{Cre}-labeled mice are yet to be completed but previous studies suggest that *Bmpr1b*^{Cre}-labeled neurons that innervate hairy skin have lower force thresholds compared to *Smr2*^{Cre}-labeled neurons. Anatomical analyses showed that neurons labeled by both genetic lines densely and uniformly innervate glabrous skin. In contrast to their distinct endings within hairy skin, in glabrous skin, both neuron types are broadly similar: they have large anatomical receptive fields, they penetrate the epidermis and form free nerve endings. Central branches of *Smr2*^{Cre} and *Bmpr1b*^{Cre}-labeled neurons terminate in both superficial and deep laminae of the spinal cord dorsal horn. Strikingly, deep terminals of *Smr2*^{Cre}-labeled neurons often appear to form dense cage-like structures around one or several cell bodies of dorsal horn neurons. Optical activation of both HTMR subtypes in hindpaw glabrous skin leads to a fast and robust nocifensive response. Around 25 ms after stimulus onset, single action potentials in paw-innervating neurons initiate a behavioral cascade that includes rapid paw withdrawal, kicking, and jumping. These Aδ-HTMR genetic tools thus provide an

opportunity to characterize these neurons at multiple levels, from primary sensory neuron properties to central connectivity to roles in behavior.

Disclosures: K. Lezgiyeva: None. **A. Emanuel:** None. **L. Qi:** None. **K. Nguyen:** None. **N. Sharma:** None. **D.D. Ginty:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.01/Z14

Topic: D.03. Somatosensation – Touch

Support: NIH Grant AG065290
Neurodegeneration Consortium

Title: Intrinsic and synaptic mechanisms mediate reduced thalamic reticular nucleus activity in a mouse model of Alzheimer's disease

Authors: *N. RIVERA-RAMIREZ[1], R. JAGIRDAR[2], J. CAMPBELL[2], M. SILVA-PÉREZ[2], J. CHIN[2], M. BEIERLEIN[1];
[1]McGovern Med. Sch. at UTHealth, Houston, TX; [2]Baylor Col. of Med., Houston, TX

Abstract: The thalamic reticular nucleus (TRN) provides inhibition to other thalamic nuclei and regulates sleep, attention, sensory processing and memory consolidation. Thalamic and cortical glutamatergic afferents are the main sources of excitatory inputs to TRN. In addition, neuromodulators such as Acetylcholine (ACh) are thought to play important roles in the regulation of TRN activity. Previously, we found reduced TRN activity and sleep deficits in 3-4 months old amyloid precursor protein transgenic mice (APP mice), a model commonly used to study Alzheimer's disease (AD), suggesting a key role of TRN dysfunction in AD progression. At this age there is no neuronal loss or plaque pathology in the TRN, indicating that reduced activity results from altered intrinsic or synaptic properties. To identify potential mechanisms of TRN hypoactivity we used electrophysiology in thalamic slices of wild-type and APP mice. Confirming previous findings, TRN neurons displayed dramatically different firing patterns based on their location within TRN, with core neurons showing T-type Ca-channel dependent persistent firing and robust rebound bursting, while shell neurons never displayed persistent firing and showed weaker rebound activity. Furthermore, APP mice displayed reduced peak firing frequencies specifically in core neurons. To determine cell-type specificity and functional roles of cholinergic synaptic inputs we activated cholinergic afferents in the presence of antagonists for fast glutamatergic and GABAergic synaptic transmission. We found that stimulus-evoked ACh release led to biphasic excitatory-inhibitory postsynaptic responses in both TRN core and shell neurons, with an initial short-latency α4β2 nicotinic receptor (nAChR) EPSC followed by a long-lasting IPSC mediated by M2 muscarinic ACh receptors (mAChRs). Previous studies have indicated that cholinergic afferent activity increases excitability in TRN

neurons. By contrast, we found that ACh release only led to a brief increase but a prolonged pause in TRN firing, indicating that cholinergic inputs to TRN neurons act primarily inhibitory. In APP mice, mRNA and protein levels for mAChRs were increased, while mRNA and protein levels for α4 nAChRs remained unchanged. In agreement, we found that biphasic cholinergic signaling in TRN core neurons from APP mice was shifted towards stronger mAChR-mediated inhibition. Our results suggest that during early stages of AD changes in both intrinsic and synaptic properties can mediate reductions in TRN neuronal firing, thereby contributing to sleep disruptions.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.02/Z15

Topic: D.03. Somatosensation – Touch

Support: NIH R01-NS100016

Title: Distinct corticothalamic integration by primary and higher-order inhibitory cells of the thalamus

Authors: C. PUZZO[1], R. MARTINEZ-GARCIA[3], H. LIU[1], W. GILBERT[1], ***S. J. CRUIKSHANK**[2];
[1]Neurobio., [2]Univ. of Alabama at Birmingham, Birmingham, AL; [3]Neurobio., Howard Hughes Med. Institute, Harvard Med. Sch., Boston, MA

Abstract: * Most sensory information undergoes processing and modulation in the thalamus before being transmitted to the neocortex. Interestingly, the neocortex itself exerts a top-down influence on this modulation via extensive descending projections to the thalamus. Central to these bidirectional interactions is the GABAergic thalamic reticular nucleus (TRN). The corticothalamic (CT) projections that descend from the neocortex excite TRN, leading to disynaptic inhibition of thalamic relay cells, gating the flow of thalamocortical signals.
* Recent evidence has shown that cells in sensory sectors of TRN are organized into primary and higher-order (HO) subpopulations, named according to their reciprocal connections with either primary or HO thalamic relay neurons. In addition to having distinct connections, these TRN cell subtypes have distinct physiological properties, resulting in pathway-specific variation in the way they process their thalamic inputs. Nevertheless, our understanding of functional roles of primary and HO TRN cells, and even basic knowledge about the organization and impact of CT systems on these distinct subpopulations, remains limited.
* Here, we investigate the integration of CT inputs in primary and HO cells of somatosensory TRN. Our findings indicate that layer 6 of somatosensory cortex (S1) drives distinct spiking

patterns in the two classes of TRN cells, due to variations in both synaptic and intrinsic properties. Primary TRN cell spiking is strong and depressing; this results from robust synaptic input summating with powerful but transient T-type calcium currents that undergo inactivation during repetitive stimulation. On the other hand, HO TRN spiking is modest and facilitating, owing to weak but facilitating synaptic input and minimal T-type current. We also find that HO cells, but not primary cells, integrate convergent novel inputs from S1 layer 5 and motor cortex (M1). The synaptic currents evoked by layer 5 inputs are strong and depressing - hallmarks of layer 5 CT inputs to other nuclei. In contrast synaptic currents evoked by M1 inputs are initially weak but facilitate with repetitive activation, consistent with layer 6 CT pathways.
* Thus, in contrast to primary cells, which predominantly receive input from the canonical layer 6 sensory CT pathway, HO TRN cells integrate a mix of inputs from multiple CT pathways, mirroring the input patterns of HO thalamic relay neurons. The stark differences in organizational and integrative properties between TRN cell subtypes are likely to have important implications for CT disynaptic inhibition and top-down regulation of primary and HO thalamic subnetworks.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.03/Z16

Topic: D.03. Somatosensation – Touch

Title: Fine changes in cortical global activity distributions induced by weak tactile inputs measured as covariance patterns across multi-channel ECoG

Authors: *A. MELLBIN, U. RONGALA, H. JORNTELL, F. L. BENGTSSON;
Dept. of experimental medical science, Lund Univ., Lund, Sweden

Abstract: Despite decades of research on the brain circuits, our understanding of the neural network architectures underlying brain function is still evolving and changing. For a long time, the brain has been considered an organ with discrete functional localizations. However, recent studies indicate that the neocortex operates as a global network of functionally interconnected neurons. Studies of the functional interconnectivity of the cerebral cortex at the single neuron level indicate that sensory input indeed can cause input pattern-dependent changes widely across the neocortex. The wide spread of information is also potentially highly useful for clinical investigations, as underlying pathology in the function of the circuit would lead to abnormal activity distributions globally, which could therefore be more easily picked up with less precise tools, but with multiple recording locations, such as EEG. Here we used an eight-channel electrocorticogram (ECoG) to record global activity in anaesthetized rats (n=27) during spontaneous activity and activity during single pulse electrical tactile stimulations. We then

applied principal component analysis (PCA) to study signal covariance patterns across the ECoG channels, rather than the more traditional analysis of frequency or information content of individual channels. We used k-nearest neighbour (kNN) to quantify the difference between spontaneous and stimulated activity clusters extracted by PCA. We found that the tactile stimulation, of either the forepaw or hind paw skin, could cause substantial and significant changes in the covariance patterns across the ECoG channels globally, also in cases where the S1 electrodes displaying an evoked field potential to the tactile stimulation was excluded (random permutation test p<0,01). These results suggest that even weak sensory input can have global effects on the activity distribution in the cortical network. Importantly, it appears that this method has a high sensitivity for the detection of such changes, making the EEG covariance patterns a potential method to detect changes in brain activity relating to neurological disorders.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Program #/Poster #: PSTR341.04/Z17

Topic: D.03. Somatosensation – Touch

Support: NIH BRAIN Grant RF1NS128896
NIH BRAIN Grant R01NS104928
Howard Hughes Medical Institute Gilliam Fellowship for Advanced Study
NSF Graduate Research Fellowship Program

Title: The recruitment of layer six corticothalamic neurons in sensory behavior

Authors: E. DIMWAMWA, ***C. WAIBLINGER**, N. CHANG, G. B. STANLEY;
Georgia Inst. of Technol., Atlanta, GA

Abstract: Amid the traditionally studied feedforward neuronal pathways that enable perception through our senses are numerous feedback processes. Corticothalamic feedback from layer 6 of cortex (L6CT) is one such process that provides extensive input to the thalamus, in addition to direct intracortical inputs. L6CT neurons are well-positioned to play a key role in thalamocortical sensory signaling for perception. Indeed, with the expansion of tools for the selective and causal manipulation of L6CT neurons, increasing evidence highlights L6CT neurons as context-dependent, gain modulators of thalamocortical sensory responses, with implications for the perception of sensory inputs. However, there are also numerous reports that L6CT neurons are sparsely active, so it remains unclear when and how L6CT neurons would conduct such modulatory functions. To investigate how exactly L6CT neurons are recruited during sensory perception, we conducted extracellular recordings of L6CT neurons in NTSR1-cre mice selectively expressing channelrhodopsin-2 in L6CT neurons. These mice were engaged in a simple tactile detection task where they lick a spout for a water reward upon perception of a

whisker deflection. As a first step, we are measuring the spiking properties of opto-tagged identified L6CT neurons. Initial observations highlight non-sparse spontaneous and sensory stimulus-evoked activity. Further, we observe behavioral state-dependent modulation, such that the sensory response of L6CT neurons is suppressed while the animal is actively whisking compared to quiescence. Next, we are measuring the spiking response of L6CT neurons in conditions when the animal is engaged in the behavioral task compared to when the animal is not engaged (no reward, lick spout removed). Preliminary results show higher spontaneous firing rates of L6CT neurons in the disengaged compared to the engaged condition. Similarly, we observe higher spontaneous firing rates of L6CT neurons on error trials (misses) compared to successfully detected trials (hits), suggesting that L6CT spontaneous activity is predictive of the animal's choice. These data suggest that L6CT neurons are indeed recruited in sensory behavior. Their responses are modulated by the behavioral state of the animal, thus enabling L6CT neurons to then modulate ongoing thalamocortical activity in accordance with behavioral needs.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Program #/Poster #: PSTR341.05/Z18

Topic: D.03. Somatosensation – Touch

Support: PHS NIH R01AA027754 and R01AG072900

Title: Prenatal alcohol exposure alters form and functionof the mouse somatosensory cortex.

Authors: *Z. SIDDIQUI, P. YEH, H. YEH;
Dartmouth Col., Hanover, NH

Abstract: Atypical tactile responses are implicated in Fetal Alcohol Spectrum Disorders (FASD). Our lab reported disrupted form and function of the somatosensory cortex (SSC), associated with diminished tactile sensitivity in young adolescent mice with history of prenatal alcohol exposure (PAE). Here we asked whether specific layers of the SSC and/or subclasses of its resident pyramidal neurons (PNs) were affected. In rodents, tactile information is relayed somatotopically to SSC via thalamocortical (TC) afferents that express vesicular glutamate transporter 2(vGlut2) and aggregate in the form of ‘barrels’ in L4, in addition to innervating layer (L)5/6, L3, and L1. In our PAE paradigm, 5% (w/w) alcohol (ethanol) is administered in a liquid diet to pregnant mice on gestational days (E)13.5-16.5, at the height of PN genesis and migration in embryonic SSC development. We investigated immunohistochemically the changes in the pre- (TC) and postsynaptic (PN) partners involved in processing tactile information in the SSC. With PAE, there was a significant main effect with two-way ANOVA of vGlut2 immunofluorescence intensity across the SSC in postnatal day (P)7 mice. Bonferroni-corrected

post hoc analysis of the two-way ANOVA revealed a significant decrease in L1. In L4, we found the average barrel width to be significantly larger (unpaired t-test) with PAE, resulting in fewer barrels within an operationally-defined region of interest. Thus, PAE disrupts barrel development, consistent with previous studies that albeit employed different PAE paradigms. In addition, PAE affected the disposition of Special AT-rich sequence-binding protein 2(Satb2)-expressing callosal and COUP TF1-interacting protein 2(Ctip2)-expressing subcortical projection PNs in the SSC. There is a significant main effect with two-way ANOVA on Satb2 and Ctip2 immunofluorescence intensity across the SSC. Bonferroni-corrected post hoc analysis of the two-way ANOVA revealed a significant increase in Satb2 immunofluorescence in layers 2/3 to 6 of the SSC and Ctip2 immunofluorescence in layers 4 to 6, indicating upregulated expression with PAE. Building on these immunohistochemistry-based findings, ongoing studies are employing electrophysiology to determine whether the neuroanatomical changes seen in the cortical layers are commensurately accompanied by altered synaptic activities in the SSC. Preliminary data indicate that, with PAE, there is a trend toward lowered glutamatergic amplitude and frequency as well as GABAergic frequency in L4 excitatory cells. Behavioral studies assessing sensorimotor function are also planned to complement the neuroanatomical and functional studies.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Topic: D.03. Somatosensation – Touch

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Title: Processing of tactile inputs in the mouse perirhinal cortex

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Abstract: The perirhinal cortex (PER) is a region in the parahippocampal formation that interfaces sensory cortical systems with the entorhinal-hippocampal memory system in both rodents and primates. Because of its involvement in object recognition tasks and its connectivity to the lateral entorhinal cortex, PER has been placed in the “what” pathway along the cortico-hippocampal network. However, the role of PER in integrating sensory stimuli is not well understood. Previous work in mice and rats has demonstrated that the PER receives inputs from the somatosensory areas; however, the topographical organization of these inputs to PER, the

circuits processing tactile inputs in PER and how tactile stimuli modulate the activity of neurons in PER remain unclear. In this work we investigate the origin of somatosensory inputs to PER and their topographical organization along its rostro-caudal axis using neuroanatomical tracing. Our retrograde tracing experiments revealed that somatosensory areas of the cortex mainly target the rostral portion of PER. These projections arise from layers 2 and 5A of the barrel cortex and from the secondary somatosensory cortex, in a typical feedforward architecture. We are currently characterizing the circuits integrating inputs from barrel cortex in PER with *in vitro* electrophysiology. Moreover, to understand how perirhinal neurons respond to tactile stimuli we performed *in vivo* electrophysiological recordings with Neuropixel probes in head-fixed animals targeting both PER and barrel cortex to characterize population activity using a passive whisker deflection paradigm. Whisker stimulation was provided randomly either ipsi-, contra-, or bilaterally. Passive tactile stimulation of the whiskers positively modulated a higher percentage of neurons in barrel cortex as compared to PER. In PER about 50% of single units showed a decrease in firing rate upon whisker stimulation, a higher proportion than in barrel cortex. Furthermore, modulated PER units displayed different patterns of activity based on the direction of tactile stimulation (i.e., ipsi, contra or bilateral stimulation); finally, we have observed differences in activity in response to tactile stimuli across layers in PER. We are currently exploring how tactile information is processed in the PER at the population level. Our results show a topographical organization of somatosensory inputs in the PER cortex along its rostro-caudal axis. Furthermore, we show *in vivo* how PER neuron's activity is modulated in response to tactile stimuli, contributing to sensory information processing in the parahippocampal region.

Disclosures: L.F. Cobar: None. **R.F. Sali:** None. **A. Foroughi:** None. **E.J. Courcelles:** None. **K. Kjelsberg:** None. **M.J. Nigro:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.07/Z20

Topic: D.03. Somatosensation – Touch

Support: NIH R00NS119739
HHMI
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Title: Tactile feature tuning during locomotion in mouse primary somatosensory cortex

Authors: *A. J. EMANUEL[1,2], D. APONTE[2], N. L. PETTIT[2], J. HUA[2], M. M. DELISLE[2,3], I. D'ALESSANDRO[2], D. D. GINTY[2,3], C. D. HARVEY[2];
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Abstract: Diverse arrays of mechanoreceptors have been ascribed distinct functions based on their characteristic responses to force stimuli. Two mechanoreceptors essential for light, discriminative touch in glabrous skin are the Aβ rapidly adapting type 1 low threshold mechanoreceptor (RA1-LTMR) and the Aβ slowly adapting (SA-) LTMR. The Aβ RA1-LTMR responds when the force on the skin is changing, and greater forces result in larger receptive fields. In contrast, the Aβ SA-LTMR responds during static indentation and its receptive field changes minimally with variations in stimulus intensity. It has therefore been postulated that Aβ RA1-LTMRs are motion sensors and Aβ SA-LTMRs detect tactile shapes. However, we have only recently been able to selectively disrupt signals from these mechanoreceptor subtypes to investigate their contributions to tactile processing. Here, we investigated the contributions of Aβ LTMR subtypes to cortical processing of texture information during locomotion. We developed a treadmill that is fabricated by 3D printing and is composed of exchangeable sections, which allows us to parametrically determine the surface texture. Head-fixed mice ran on this treadmill while we used multi-channel electrodes to record spiking in the forepaw region of primary somatosensory cortex (S1). We found that S1 firing rate changes were highly heterogeneous in their magnitudes, durations, and signs when aligned to the timepoints when the forepaw contacts the treadmill. This heterogeneity is strikingly unlike the mostly homogeneous S1 responses to passive force stimuli that we reported previously. The treadmill consisted of four segments each with a different orientation of raised sinusoidal gratings. By relating the firing rate at the time of the footfall to the orientation, we found that ~25% of S1 neurons in control mice exhibited tuning to the grating orientation. Mice lacking Meissner corpuscles (*Avil*^{Cre}*;TrkB*$^{flox/flox}$), and therefore Aβ RA1-LTMR responses from glabrous skin, had similar fractions of cells with tuning to the grating orientation as in control mice, suggesting that Aβ RA1-LTMRs are dispensable for detecting the orientation of tactile stimuli. In contrast, in mice lacking Merkel cells (*K5-Cre;Atoh1*$^{flox/flox}$), which are critical for normal Aβ SA-LTMR signals, only ~5% of S1 units had distinct firing rates for forepaw contacts with gratings of different orientations. These results reveal a requirement for Merkel cells for orientation selectivity in S1, consistent with a role for Aβ SA-LTMRs in delineating object shapes. Overall, we have established a paradigm for dissecting tactile feature tuning during locomotion.

Disclosures: **A.J. Emanuel:** None. **D. Aponte:** None. **N.L. Pettit:** None. **J. Hua:** None. **M.M. DeLisle:** None. **I. D'Alessandro:** None. **D.D. Ginty:** None. **C.D. Harvey:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.08/Z21

Topic: D.03. Somatosensation – Touch

Title: Functional modules in rat S1 forelimb cortex

Authors: *O. FAVOROV[1], T. CHALLENER[2], R. W. MURROW[3];
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Abstract: Just as in cats and primates (Favorov and Diamond, J. Comp. Neurol. 298:97-112, 1990), the rat granular S1 forelimb cortex is partitioned into a mosaic of discrete place-defined macrocolumns, with each macrocolumn processing input from one of the forelimb subdivisions, such as individual fingers or interdigital, thenar or hypothenar pads. Each macrocolumn also receives marginal input from neighboring forelimb subdivisions, To study functional relations among neighboring macrocolumns, optical intrinsic signal (OIS) imaging and extracellular recordings of spiking activity were collected in response to 25Hz sinusoidal skin indent (flutter) stimulation of the tips of digits 2 and 3 (D2 and D3). Neural recordings were obtained under 0.5% isoflurane in nitrous oxide/ oxygen anesthesia using 4-shank linear NeuroNexus arrays (0.2mm shank spacing, 8 recording sites per shank, 0.1mm apart). The probe was inserted into the D2 and D3 macrocolumns, with most of recording sites in either D2 or D3 macrocolumn. The OIS and single-unit recordings revealed that while in addition to stimulation of its own digit, each macrocolumn responded to flutter stimulation of the other digit, this marginal response was small and was still further reduced during 0.5s of continuous stimulation, and spike timing was significantly delayed in the stimulus cycle, resulting in a prominent funneling of the S1 response to the more directly stimulated macrocolumn. When D2 and D3 were both synchronously but unevenly stimulated, the size of the phase delay of spike firings in the more weakly stimulated column depended on the relative amplitudes of the two stimuli. However, even at matching flutter amplitudes, spike timings were more tightly synchronized among neurons in the same macrocolumn than across macrocolumns, and were partially shifted in the stimulus cycle. These findings are consistent with a view that discrete place-defined macrocolumns are competitive functional modules aiming to represent sensory events best fitted in their periphery-viewing window. Such event representations in a macrocolumn take a form of a spatial pattern of spikes emitted synchronously in the population of feature-tuned neurons composing the macrocolumn; i.e., a spike vector in the macrocolumn's feature space.

Disclosures: O. Favorov: None. **T. Challener:** None. **R.W. Murrow:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Program #/Poster #: PSTR341.09/Z22

Topic: D.07. Visual Sensory-Motor Processing

Support: NIH R01NS129794
Imagine More

Title: Sensory cortical control of locomotion after spinal cord injury

Authors: *H. SWEETMAN[1], D. GRAHAM[1], A. MILSCH-KRONER[1], S. GOSGNACH[3], C. M. OLSEN[2], K. SATKUNENDRARAJAH[1];
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Abstract: The primary somatosensory cortex (SI) has direct neural control on the locomotor central pattern generator relayed through cervical excitatory interneurons (SI locomotor pathway) independent of the motor cortex. Here, we use in vivo calcium imaging to decode how SI-pyramidal neurons of the locomotor pathway participate in the onset of movement and play a role in the speed programming of movement. Detailed mapping of SI-pyramidal neurons projecting to the cervical region demonstrates collateral projections to subthalamic and brainstem regions involved in locomotor control. Notably, circuit-specific monosynaptic tracing reveals direct inputs to cervical projecting-layer 5 pyramidal neurons from the auditory cortex, visual cortex, basal ganglia, and thalamus indicating a greater impact of external sensory stimuli in initiating movement. In fact, activating the SI-pyramidal neurons with optogenetic stimulation is sufficient in initiating and enhancing movement. To date, stimulation of the motor cortex has only resulted in modest improvements in locomotor function after spinal cord injury (SCI). In this study we demonstrate that part of the SI-locomotor circuitry is preserved after thoracic SCI and selective optogenetic and chemogenetic stimulation of the SI-cervical pyramidal neurons after SCI promotes locomotor recovery. Long-term chemogenetic activation of the SI-cervical pyramidal neurons after spinal cord injury restores partial locomotor recovery. Importantly, the recovered locomotor function after SCI is attributed to the neuromodulation of the sensory cortical-locomotor pathway. Thus, modulating the SI locomotor pathway enhances movement in health and represents a promising therapeutic approach for promoting functional locomotor recovery after SCI.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.10/Z23

Topic: D.03. Somatosensation – Touch

Title: Somatosensory feedback guides motor correction of tongue movements.

Authors: J. KIM, D. O'CONNOR;
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Abstract: Accurate motor actions require timely corrections based on sensory feedback. Licking behavior in mice involves cortical control of flexible and complex orofacial movements. Here I present a behavioral task that examines how somatotopic sensory feedback at the tongue guides

licks to unseen targets. Inspired from corrective saccade tasks in non-human primates, the somatosensory error task directs head-fixed mice to lick a motorized port undergoing a random walk (right or left) between licks. With each random walk, licks touch the target on the lateral sides of the tongue, creating a somatotopic error signal. In this task, mice adjust lick angle based on somatosensory error in one or two lick cycles. The sign of the somatosensory error (right or left shift) directs corrective licks to the ipsilateral side of the error touch. Optogenetic inhibition of the tongue and jaw regions of the primary somatosensory cortex (S1TJ) bilaterally disrupts lick angles and accuracy while optogenetic inhibition of unilateral S1TJ cortex biases lick angles ipsilaterally. Notably, single-lick cycle optogenetic inhibition of S1TJ leads to slightly hypometric misses depending on the lick cycle phase, hinting at the role of sensory input to central pattern generators. Initial Neuropixel recordings in S1TJ show strong lick-modulated activity. A working model of neural dynamics in this task is a set of point-attractors corresponding to discrete motor command states, between which transitions are governed by sensory inputs.

Disclosures: **J. Kim:** None. **D. O'Connor:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR341.11/Z24

Topic: D.03. Somatosensation – Touch

Support: NIGMS T32 GM007347

Title: Does sensorimotor integration across the upper-lower and right-left face halves occur at the level of the cortex?

Authors: *K. S. BRESS[1], C. J. CASCIO[2];
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Abstract: Facial expression is a complex sensorimotor behavior which requires coordinated action across both the upper-lower and right-left face halves. Facial expressions which accurately represent one's internal sensations and emotions are critical for social communication. Facial expression differences are commonly observed in autism and contribute significantly to social communication challenges. These differences may be related to sensorimotor deficits in autism; however, the exact neural mechanisms remain unknown. While integration of facial sensory input with corticobulbar facial motor control pathways is necessary for control of facial expression, it is unclear at what level(s) of the nervous system this integration occurs. In this study, resting state functional magnetic resonance images (fMRI) were acquired from n=59 typically-developing and n=71 autistic adults. Seed-based resting state connectivity analysis was performed to interrogate pairwise connections between the upper and lower somatotopic face areas of the primary sensory (S1) and primary motor (M1) cortices. Results demonstrate that in

both groups, the upper face areas of S1 have equivalent functional connectivity (FC) with the upper and lower face areas of M1. In addition, the right and left face areas of S1 have equivalent FC with the ipsilateral and contralateral face areas of M1. In the typically-developing sample, lower face areas of S1 demonstrate greater FC with the lower face areas of M1, compared to the upper face areas of M1 ($p=0.009$). These data suggest that while sensory information from the upper face is integrated with motor control of the upper and lower face at the level of the cortex, sensory information from the lower face may not be integrated with motor control of the upper face at the level of the cortex. Preserved somatotopic specificity in lower face S1-to-M1 projections may reflect the involvement of the lower face in more complex actions such as vocalization and mastication, necessitating fine sensorimotor control. Repetition of this analysis with a larger autistic sample is necessary to confirm findings. Future directions will also address integration between sensory thalamic nuclei and cortex.

Disclosures: K.S. Bress: None. **C.J. Cascio:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

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Topic: D.03. Somatosensation – Touch

Support: European Research Council (ERC) (FeelAgain grant agreement No. 759998)
Swiss National Science Foundation (SNSF) (MOVEIT No. 197271)

Title: Brain correlates of electrically restored sensations in diabetic peripheral neuropathy

Authors: *L. CHEE[1], N. GOZZI[1], I. ODERMATT[1], S. KIKKERT[1], C. ZIPSER[2], N. PFENDER[2], N. WENDEROTH[1], S. RASPOPOVIC[1];
[1]Swiss Federal Inst. of Technol. Zurich, Zürich, Switzerland; [2]Balgrist Univ. Hosp., Zürich, Switzerland

Abstract: Diabetic Peripheral Neuropathy (DPN) is a slowly progressing disease characterized by peripheral nerve damage starting distally and moving proximally. Sensory loss is one of the main symptoms of DPN and has a variety of consequences including reduced balance and mobility as well as an increased risk of ulcer formation. Transcutaneous electrical nerve stimulation (TENS) can proximally target the nerves that innervate the areas of sensory loss, suggesting the ability to restore this lost sensation non-invasively. We developed a fully portable neuroprothesis employing non-invasive smart-stimulation to proximally stimulate the distally damaged nerves of DPN patients. The sensations restored by the neuroprothesis were characterized both subjectively and objectively with fMRI in healthy and diabetic subjects to understand how the artificially induced electrical sensations are perceived in the brain. To do this, three distinct target regions (peroneal, medial tibial, and sural nerves) in both healthy and

diabetic subjects were targeted with electrodes placed to elicit a somatotopic sensation. Once these sensations were subjectively characterized, a local foot sensation (overlapping somatotopic) and ankle-only sensation (not overlapping somatotopic) were characterized for each of the three target regions resulting in 3 conditions in each of the 3 locations. The three conditions were compared to understand how the somatotopic sensations provided by our neuroprosthesis compare to sensations in the foot. Somatotopic sensations provided by our neuroprosthesis were found to be similar to in foot sensations and different from ankle-only sensations in healthy participants. Foot sensations in diabetic patients were found to elicit significantly less brain activity than in healthy controls, confirming reported sensation loss. These results suggest that neural stimulating prostheses are able to elicit distal sensations with proximal stimulation that is perceived the same way as distal stimulation in the cortex. This highlights the importance of intuitive sensations and the difference between remapped sensations and restorative sensations. These findings take steps towards the development of an intuitive at home usable system for treating long term DPN symptoms.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

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Program #/Poster #: PSTR341.13/Z26

Topic: D.03. Somatosensation – Touch

Support: JSPS KAKENHI Grant number JP20K23298

Title: Short latency afferent inhibition effects on the motor representation area of the hand muscles in healthy adults

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Abstract: Delivering the electrical stimulation to the peripheral nerve prior to transcranial magnetic stimulation (TMS) over the primary motor cortex induces inhibition of the motor evoked potential (MEP), known as short latency afferent inhibition (SAI), which reflects sensorimotor interaction in the brain. Previous work has assessed SAI by stimulating a single site of the motor cortex (i.e., hot spot) of a target muscle. However, it is possible that electrical afferent inputs from peripheral nerves alter cortical excitability in the motor representation area of the target muscle, not just in the hot spot. Therefore, evaluating the size of the representation area when electrical stimulation is applied to peripheral nerves would clarify the effects of afferent inputs on the cortical excitability that cannot be captured by stimulating hot spot. In

addition, it is controversial between studies that the type of peripheral nerves affects the magnitude of SAI. Here we aimed to investigate the SAI effects on the motor representation using TMS mapping method and the effects of the type of nerve electrically stimulated.Sixteen healthy young adults participated in this study. We stimulated i) the hot spot of the first dorsal interosseous (FDI) (SAI_{single}) and ii) random locations of the left motor cortex ($SAI_{mapping}$) using TMS to elicit MEPs from the right FDI muscle and the abductor pollicis brevis (APB) muscle. We evaluated SAI by delivering a single-pulse TMS alone (unconditioned TMS) or TMS preceded by electrical stimulation of either the median nerve (innervating the APB muscle) at just lateral to the tendons of the wrist or the ulnar nerve (innervating the FDI muscle) at the ulnar side of the wrist, approximately 19-23 ms before each TMS pulse (conditioned TMS). We compared the amplitude of MEPs elicited by conditioned and unconditioned TMS for SAI_{single}, and the area size calculated from the motor map produced by conditioned and unconditioned TMS for $SAI_{mapping}$ in the median or the ulnar nerve stimulation condition, separately. SAI_{single} was observed only in the FDI muscle with the median nerve stimulation. Importantly, $SAI_{mapping}$ (i.e., a decrease in the area size produced by conditioned TMS) was observed not only in the FDI muscle but also in the APB muscle with the median nerve stimulation, but not with the ulnar nerve stimulation. Our results suggest that evaluating the area of motor representation may reveal effects of SAI that cannot be captured by stimulating a single site. Furthermore, the spread of inhibition might depend on the cutaneous innervation or the proximity of the stimulated location to the target muscle rather than the nerve innervation to the muscle.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Topic: D.03. Somatosensation – Touch

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Title: Effects of prior stimulation on tactile evoked epidural field potentials in rat S1 cortex

Authors: A. AKDENIZ-KARATAY[1,2], ***B. GUCLU**[1];
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Abstract: Tactile exploration and mammalian navigation involve sequential limb movements which are accompanied by a continuous stream of somatosensory inputs. In the tactile modality, temporal relationship between those inputs may produce psychophysical masking or summation depending on various stimulus parameters. This work investigates the neural effects of a

vibrotactile stimulus (duration: 1 s) presented prior to the vibrotactile test stimulus (duration: 0.5 s) with a temporal gap (0.1 or 0.3 s). The stimuli were applied on the hind paw glabrous skin (plastic probe diameter: 1.9 mm) of anesthetized rats at various combinations of vibrotactile frequencies (5, 40, 250 Hz) and amplitudes (zero-to-peak 210-480 μm of sinusoidal displacements). Contralateral epidural field potentials (EFPs) were recorded from the rat S1 cortex by using platinum 16-channel μECoG electrodes (each active site diameter: 200 μm) and ×1000 amplification (bandwidth: 0.7-300 Hz). The EFPs were digitized, further band-pass filtered (2nd order, zero-phase Butterworth, 4-150 Hz), and time-averaged across 10 trials to study evoked activity of the test stimulus for each experimental condition. The vibrotactile stimuli produced robust EFPs at stimulus onset and ended within 0.15 s. The presentation of the prior stimulus suppressed the EFPs for the test stimulus, such that their amplitudes decreased and latencies increased compared to those in trials with only the test stimulus. This suppression decreased as the temporal gap increased, and there were also significant main and interaction effects of prior/test stimulus amplitudes and frequencies. For example, higher prior stimulus levels produced higher suppression; and the greatest suppression occurred when the prior and test vibrotactile frequencies were 250 and 5 Hz, respectively. These results may be considered as the neural correlate of vibrotactile forward masking (albeit not at threshold level) and highlight the interaction between the effects of sequential vibrotactile inputs. This work can help improve the design and implementation of somatosensory neuroprostheses and brain-machine interfaces.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: Wellcome Trust
Academy of Medical Sciences
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Title: Learning-induced plasticity of secondary somatosensory and visual thalamus

Authors: G. H. PETTY[1], ***R. M. BRUNO**[2];
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Abstract: Each sensory modality has its own primary and secondary thalamic nuclei. While primary thalamic nuclei are well understood to relay sensory information from the periphery to the cortex, the function of secondary sensory nuclei is elusive. One hypothesis has been that

secondary nuclei may support feature-based attention. If this is true, one would expect the activity in different nuclei to reflect the degree to which modalities are or are not behaviorally relevant to a learned task.
We trained head-fixed mice to attend to one sensory modality while ignoring a second modality, namely attend to touch and ignore vision (or vice versa). Arrays were used to record simultaneously from secondary somatosensory thalamus (POm) and secondary visual thalamus (LP, the mouse homolog of primate visual pulvinar). In mice trained to respond to tactile stimuli and ignore visual stimuli, POm was robustly activated by whisker touches and largely unresponsive to visual stimuli. The reverse pattern was observed when mice were trained to respond to visual stimuli and ignore touch, with POm now more robustly activated during visual trials. This POm plasticity was not explained by differences in movements (i.e., whisking, licking) resulting from the two tasks (respond to vision vs respond to touch). Post hoc histological reconstruction of array tracks through POm revealed that subregions varied in their degree of plasticity. LP exhibited similar phenomena. We conclude that behavioral training reshapes activity in secondary thalamic nuclei. Secondary nuclei may then serve as "control knobs" on sensory processing and plasticity in their corresponding sensory cortical areas, such as primary somatosensory and primary visual cortex.

Disclosures: G.H. Petty: None. **R.M. Bruno:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Topic: D.02. Somatosensation – Pain

Support: IBS-R015-D1

Title: Investigating Modulation of Laminar Activity in Attended and Unattended Regions of the Human Somatosensory Cortex with 7T BOLD fMRI

Authors: *D. KIM[1], S. HAN[2], S. EUN[1], C. WOO[1], M.-S. KANG[3], S. KIM[1];
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Abstract: The human brain's capacity for information processing is finite, limiting its ability to handle vast amounts of data concurrently. Attention assists in filtering relevant information and disregarding unnecessary stimuli. Key to this is lateral inhibition, a neurobiological mechanism that attenuates activity in surrounding neurons, thus heightening sensory perception by prioritizing more significant sensory input over weaker or irrelevant signals. However, the specifics of how this mechanism operates within the diverse cortical layers of the primary sensory cortex (S1) remain obscure. We aim to demystify the role of lateral inhibition in

downplaying irrelevant stimuli, thereby enhancing our understanding of sensory information processing. In our research, we deployed Spin echo-planar imaging (EPI) to assess the effects of attention on irrelevant stimuli within S1. Participants experienced tactile stimulation on their fingers and wrists within an fMRI scanner, and we concurrently presented either tactile or thermal stimuli on their wrists as unattended stimuli. It's important to note that the somatosensory system employs unique receptors and pathways to process various types of sensory information. To establish a universal mechanism, we conducted two separate experiments. The first involved eight participants and used a tactile stimulus as the unattended stimulus. The second included the same eight participants plus one more, and used a thermal stimulus at 44 degrees Celsius, known to activate nociceptors. Our aim was to differentiate between neural responses during active task engagement and passive states. Our results reveal that attention significantly modifies activity in the superficial layer of the sensory cortex, suggesting a layer-specific impact. Additionally, we noticed a complete suppression of S1 activity in response to unattended stimuli across all layers, potentially indicating a role for thalamo-cortical feedback in diminishing the processing of unattended stimuli in S1. This research highlights the intricate interaction between cortical and subcortical mechanisms in sensory information processing. Our discovery of layer-specific effects and the potential involvement of thalamo-cortical feedback underscore the complexity of sensory processing under the influence of attention.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: R01MH126676
R01NS112266
R35NS122181

Title: An Amygdalar Local Oscillator Coordinates Cellular and Behavioral Rhythms

Authors: *Q. LIU[1], T. KIM[2], J. XIONG[2], S. LEE[2], B. BELL[2], C. ALEXANDRE[2], S. BLACKSHAW[2], A. LATREMOLIERE[2], M. WU[2];
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Abstract: In mammals, daily biological rhythms are organized by a circadian timing system, consisting of a master pacemaker in the suprachiasmatic nucleus (SCN) that coordinates local clocks throughout the body. Work over the past several decades has led to a deep understanding of the molecular and cellular mechanisms underlying SCN function and revealed the importance

of peripheral oscillators in metabolism. However, the function and cellular identity of local oscillators in the brain remain poorly understood. In particular, while cycling of core clock genes is observed across many brain regions, it is unclear whether specific neurons within a given brain region play a special role in generating cyclical behaviors and organizing cellular rhythms. We previously identified the clock output molecule WIDE AWAKE (WAKE) in *Drosophila* and characterized its role in clock neurons, where it upregulates ion channels/pumps at night to promote rhythmic excitability. A single homolog of WAKE is present in mice (mWAKE/ANKFN1), which is expressed in the SCN and a distributed set of brain regions, many of which are thought to house local brain oscillators. Because extra-SCN oscillators are defined by cycling electrical activity, in addition to clock gene expression, we hypothesized that mWAKE defines neural circuits that function as local circadian oscillators in the mouse brain. Here, we find that mWAKE defines a subpopulation of lateral amygdala (LA) neurons that exhibits rhythmic excitability and coordinates sensory perception and emotional state in a time-dependent manner. Surprisingly, clock function within LA^{mWAKE} neurons is required for cycling of clock gene expression throughout the LA. Taken together, these data suggest a hierarchical system within extra-SCN brain regions, where local clocks function in specific neural networks to control behavioral and cellular rhythms.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.18/Web Only

Topic: D.03. Somatosensation – Touch

Support: JSPS KAKENHI 21H03788
JSPS KAKENHI 19K22585
JSPS KAKENHI 21H03532
JSPS KAKENHI 19K22990

Title: Effects of local cortical temperature on the P1 and N1 components of somatosensory evoked potentials

Authors: M. GOTOH[1,2,3], S. DEZAWA[1,2,4], I. TAKASHIMA[1,2,5], *S. YAMAMOTO[1,2];
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Abstract: Local brain temperature is one of the key factors in neural information processing. However, whether and how cortical temperature affects sensory processing is yet underexplored.

To address this issue, we examined the effect of cortical temperature on somatosensory evoked potentials (SEPs) and how GABAergic inhibitory components contribute to the temperature dependency of SEPs in anesthetized rats. We controlled the local cortical temperature using a custom-made thermal control chamber containing either 100 µL saline or an antagonist solution placed above the somatosensory cortex. Electrical stimulation was delivered to the contralateral forepaw, and the SEPs were recorded using a silver ball electrode on the dura surface above the somatosensory cortex. We determined the effects of local cortical temperature on the SEPs by evaluating the amplitudes of the first positive (P1) and the subsequent negative deflections (N1) of the SEPs at each temperature. The amplitudes were defined as the absolute values of the peaks from the baseline (average for 50 ms before the onset of electrical stimulation). When the chamber only contained saline with no antagonist (N = 24), the N1 amplitudes and the cortical temperature showed a significantly negative correlation at temperatures ≥ 27.5 °C ($R = -0.67$, $p < 0.05$) and a significantly positive correlation at temperatures ≤ 27.5 °C ($R = 0.64$, $p < 0.05$). Thus, the N1 amplitude plotted against the cortical temperature showed an inverted-U relationship. The P1 amplitude, which was smaller than N1 amplitude, also showed a similar relationship with its maximum at 27.5 °C. The P1 amplitudes and the cortical temperature showed a significantly negative correlation at temperatures ≥ 27.5 °C ($R = -0.36$, $p < 0.05$) and a slight but insignificant positive correlation at temperatures ≤ 27.5 °C ($R = 0.15$, $p > 0.05$). However, both N1 and P1 amplitudes did not show significant negative correlations at temperature ≥ 27.5 °C, as observed in the saline condition ($R = 0.0013$, $p > 0.05$, and $R = -0.21$, $p > 0.05$), when the chamber contained 10 µM gabazine, a $GABA_A$ receptor antagonist (N = 8). This clearly shows that somatosensory processing is affected by the local cortical temperature. Our data suggest that the GABAergic inhibitory inputs are more susceptible to cortical temperature than the excitatory inputs, and differences in the susceptibility to temperature causes the negative correlation observed between P1/N1 amplitudes and the cortical temperature.

Disclosures: **M. Gotoh:** None. **S. Dezawa:** None. **I. Takashima:** None. **S. Yamamoto:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.19/AA3

Topic: D.03. Somatosensation – Touch

Support: P01/AG078116

Title: In vigilo age- and sex-dependent alterations in S1 neuronal network dynamics may contribute to gait dysregulation

Authors: *R.-L. LIN, S. CASE, O. THIBAULT;
Dept. of Pharmacol. and Nutritional Sci., Univ. of Kentucky Col. of Med., Lexington, KY

Abstract: Over the past 30 years, the calcium (Ca^{2+}) hypothesis of brain aging has provided clear evidence that hippocampal neuronal Ca^{2+} dysregulation is a key biomarker of aging. Indeed, age-dependent Ca^{2+}-mediated changes in intrinsic excitability, synaptic plasticity, and activity have helped identify some of the mechanisms engaged in memory and cognitive decline. However, much of this work has been done at the single-cell level, mostly in slice preparations, and in restricted structures of the brain. Recently, our lab identified age- and Ca^{2+}-related neuronal network dysregulation in the cortex of the anesthetized animal. Still, investigations in the awake animal are needed to test the generalizability of the Ca^{2+} hypothesis of brain aging. Here, we used two-photon imaging of awake, ambulating mice, to acquire GCaMP8f signal from the hindlimb somatosensory cortex (S1HL) region, during ambulation and while stationary. In order to investigate age- and sex-related changes in the neuronal network, a continuous wavelet transform-based binarization and pair-wise correlation coefficient analysis was introduced in MATLAB to extract measures of network communication at single-cell across hundreds of neurons. Following imaging, gait behavior was characterized to test for changes in locomotor stability. During ambulation, in both young (~4 months) and aged mice (~22 months), an increase in network connectivity and synchronicity was noted. An age-dependent increase in network synchronicity was observed in ambulating males only. Additionally, females displayed a greater number of active neurons, area-under-curve, and neuronal activity compared to males, particularly during ambulation. These results suggest S1HL Ca^{2+} dynamics and network synchronicity are potential contributors of locomotor stability. We believe this work raises awareness of central elements at play in S1, where neuronal network dysregulation is seen with aging, perhaps highlighting potential therapeutic targets that may help offset age-dependent increases in falls.

Disclosures: R. Lin: None. **S. Case:** None. **O. Thibault:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.20/AA4

Topic: D.03. Somatosensation – Touch

Support: P01 AG078116

Title: Central processes contributing to age- and sex-dependent alterations in ambulatory stability

Authors: *O. THIBAULT, J. E. RHINEHART, S. L. CASE, N. A. WRIGHT, R.-L. LIN; Pharmacol. and Nutritional Sci., Univ. of Kentucky, Lexington, KY

Abstract: Recently published work from our lab has identified primary somatosensory cortex (S1) network dysregulation in aged mice, particularly during ambulation, and mostly in males. This analysis was conducted on calcium transients (gCamp8f) obtained in awake ambulating and

non-ambulating animals (Neurotar) undergoing two-photon imaging. While network alterations based on pairwise correlation coefficients were noted using a continuous wavelet transform, analysis of individual neuronal calcium transients also showed sex-dependent increases in activity and area-under-the-curve in aged ambulating females. Here therefore, we tested the hypothesis that several calcium-fluxing proteins might be able to reflect on these alterations in individual transients. We used S1 samples in combination with Western blot analyses to quantify L-VGCC (CaV1.2), NMDAR (GluN1) and ryanodine receptors (RyR2) across 43 samples from young-adult (4 months) and aged (22 months) C57BL/6J males and females. Using standard SDS-PAGE protocols in combination with primary and secondary antibodies we report here on age-dependent elevations in GluN1 proteins in both sexes, along with a trend in CaV1.2 proteins increasing in aged females only. RyR2 also showed a strong trend for an age-dependent increase in both sexes. Along with these results, it is interesting to note that females displayed better performance on the walking task (stride length, stride time deviance index) compared to males. The results appear to be clinically relevant based on evidence for a decreased risk of falls, along with lower stride time deviance and greater stride length, in older females, compared to older males. We hope our work also raises awareness about the central components of gait dysregulation with age, focusing on network alteration in S1 and the influence of neuronal calcium-centric processes.

Disclosures: O. Thibault: None. **J.E. Rhinehart:** None. **S.L. Case:** None. **N.A. Wright:** None. **R. Lin:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Program #/Poster #: PSTR341.21/AA5

Topic: D.03. Somatosensation – Touch

Support: NRF Grant 2022-11-0700

Title: Vpm thalamic responses to sensory stimuli during motivated state

Authors: *H. JEONG, H. KWAK, C. PARK, E. CHEONG;
yonsei university, Seoul, Korea, Republic of

Abstract: Animals can receive various stimuli from the environment through sensory organs. Among various stimuli, they have to accept the stimuli needed in the current situation more accurately and quickly to survive. Motivation and attention reinforce information about stimuli necessary for the situation and suppress information about stimuli that are not needed. This increases the sensitivity to the necessary stimuli, enabling a more detailed recognition of the corresponding sensation. Mice use active whisking in order to perceive surrounding objects. The whisker plays the same role as a fingertip to humans and enables them to obtain delicate touch information. Touch information can be controlled by motivation in the barrel cortex, an area that

accepts and processes sensory information from the whisker. Also, stimulus information is controlled by learning.It is known that this is controlled through interaction between layers in the barrel cortex and through interaction with S2. However, the mechanisms of regulation in the subcortical area is not well known. VPm thalamus neurons, which lie in the Whisker-related sensory pathway, can be regulated by NE, and this neuromodulator system exhibits internal state-dependent activity.This suggests that the VPm thalamus can modulate sensory information according to its internal state. To find out whether thalamic responses can be modulated by state change, we developed a closed loop texture touch task for head fixed mice to analyze perceptual performances and neuronal activity only in motivated trials — meaning the subjects are involved in trials only when they intently move forward (i.e. motivated to move). Using textures with various roughness levels, we found that the closed loop task can show various perceptual performance levels. In this task, thalamus neurons mainly showed 4 subtypes - Activated/inhibited touch units, Activated/inhibited touch units pressure units.

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Poster

PSTR341. Touch: Thalamic and Cortical Processing

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Topic: D.03. Somatosensation – Touch

Support: NIH Grant 1R01NS125435-01A1
NIH Grant 1R01_NS124222-01

Title: Controllability of S1 cortical activity using upstream intrathalamic microstimulation with different electrode site areas

Authors: *B. A. SEE, L. DICKEY, W. A. WALKER, J. T. FRANCIS;
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Abstract: Somatosensory neuroprosthetic devices could one day restore lost sensations due to nerve damage or sensory feedback to a robotic limb by electrically stimulating the representation of the corresponding area in the sensory regions of the brain. One issue faced by this approach is the generation of paresthesias, abnormal sensations that do not replicate the feel of natural touch percepts. This can be attributed, at least in part, to the elicited activity being dissimilar to naturally evoked activity. Previously, we have demonstrated a model based control methodology for stimulation in the ventral posterolateral (VPL) thalamus based on natural activity in the primary somatosensory cortex (S1) in response to localized touch sensations in the rat forepaw. Optimized intrathalamic microstimulation (ITMS) based on this model generated activity in the S1 cortex substantially similar to natural tactile stimulus evoked activity. Another cause of paresthesias from electrical stimulation is the lack of specificity for the neurons with activity that correlates with a specific sensory percept, which may be caused to some extent by the size of the

area stimulated by electrodes. In this study, we aim to test the effect of implanted electrode site area in the forelimb representation in the VPL thalamus on the downstream activated region in S1 and its controllability. Male 9-11 week old Lewis rats have been chronically implanted with electrode arrays in the VPL thalamus and the S1 cortex. The cortical electrodes are 16 channel microwire arrays with 5mm length and 33µm wire diameter, while the 16 channel thalamic arrays have a single 10mm shank, and either 177µm2 or 703µm2 electrode site areas. Utilizing otherwise identical electrode designs in the VPL will enable us to determine the specific effect of electrode site area. Natural tactile stimulus will be provided to different touch sites using a motorized precision tactor synchronized with a Tucker-Davis Technologies BioAmp Processor. Randomized single pulse, biphasic, bipolar stimulation trains will be applied to the thalamus while recording from the cortex. Once recorded, a model will be trained relating cortical output to the stimulation input in the VPL thalamus, then used to deliver optimized stimulation to match activity from the natural touch experiments. Percent Variance Explained, Mahalanobis distances, and the accuracy of different classifiers will be used to assess the controllability of S1 cortical activity in response to ITMS. These data will be used to demonstrate the benefits of smaller electrode sites in conjunction with optimized ITMS for generating naturalistic sensory percepts.

Disclosures: **B.A. See:** None. **L. Dickey:** None. **W.A. Walker:** None. **J.T. Francis:** None.

Poster

PSTR341. Touch: Thalamic and Cortical Processing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR341.23/AA7

Topic: D.03. Somatosensation – Touch

Title: Short-latency somatosensory evoked potentials following vibrotactile stimulation

Authors: *E. FUEHRER[1,2], L. K. MAURER[3,2], K. FIEHLER[1,2];
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Abstract: Somatosensory evoked potentials (SSEPs) are typically elicited through electrical nerve stimulation yielding short- (P9, N9, P11, N11, P13/P14, N18, P20, N20, N30, P40) and long-latency (P100, N150, P250, P350) components. However, for certain experimental paradigms, researchers may prefer using more naturalistic vibrotactile stimuli. While long-latency SSEPs can be reliably evoked using vibrotactile stimulations, there is limited information available on short-latency SSEPs due to their smaller amplitudes. To address this gap and facilitate future SSEP research, we aimed to characterize the short-latency potentials produced by brief vibrotactile stimulations at different amplitudes. Twenty participants received 200 repetitions each of sinusoidal vibrotactile stimulations (20 ms, 280 Hz) at four supra-threshold amplitudes (42, 97, 134, and 190 µm) in a randomized order. The inter-stimulus interval was set to 2200 ms. Our findings reveal a centro-parietal short-latency (44 - 48 ms) positive peak

corresponding to a P45 at the CP3 electrode, located near the somatosensory cortex contralateral to the stimulation. The latency of the P45 component did not differ significantly among the four stimulation amplitudes. However, a preliminary analysis of the averaged signal during the P45 peak (36-56 ms) demonstrated a significant difference in SSEP amplitude at CP3 based on the stimulation amplitudes. To better contrast short-latency SSEPs elicited by electrical and vibrotactile stimulations, we plan to retest the participants using electrical stimulations. Additionally, we intend to conduct further detailed analyses of all components and their topographies, aiming to present a comprehensive overview of SSEPs following electrical versus vibrotactile stimulations.

Disclosures: E. Fuehrer: None. **L.K. Maurer:** None. **K. Fiehler:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.01/AA8

Topic: D.05. Auditory & Vestibular Systems

Support: German Center for Neurodegenerative Diseases (DZNE)
German Research Foundation (DFG)
SFB 1089
The Forschungsfonds Nachwuchsforschende of the University of Basel
Swiss National Science Foundation

Title: Ensemble state changes in sensory thalamus represent learned outcomes

Authors: M. HASEGAWA[1], ***Z. HUANG**[2], J. GRÜNDEMANN[2];
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Abstract: Subcortical thalamic brain areas play an important role in adaptive behaviors. Nevertheless, the neural population dynamics and plasticity of thalamic sensory relays during behavioral learning across sensory modalities remains unknown. Using a cross-modal sensory reversal learning paradigm in combination with longitudinal deep brain two-photon calcium imaging of large populations of auditory thalamus neurons (medial geniculate body, MGB), we identified functional classes of MGB neurons that align with distinct task periods and behavioral outcomes both, dependent and independent of sensory modality. In addition, during non-sensory delay periods, MGB ensembles developed coherent neuronal representation as well as distinct co-activity network states reflecting task outcome. Our results demonstrate the flexible cross-modal ensemble coding capacity of auditory thalamus during adaptive learning and highlight its importance in brain-wide computations for complex behavior.

Disclosures: M. Hasegawa: None. **Z. huang:** None. **J. Gründemann:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

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Program #/Poster #: PSTR342.02/AA9

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD (NIH) R01-DC-018561

Title: A candidate mechanism for benefits in signal-in-noise detection in auditory cortex revealed using auditory associative learning in rats

Authors: *N. ATESYAKAR[1,2], A. SHANG[1,2], K. BIESZCZAD[1,2,3,4];
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Abstract: Comprehension of sound in noise is a remarkable feat of auditory systems. Learning experiences with salient sounds are known to enhance signal-in-noise detection performance. For example, music training was found to improve speech-in-noise performance. Learning experiences, moreover, involve physiological changes to receptive fields in the auditory cortex (ACx) (Bieszczad & Weinberger, 2010). As such, cortical neurophysiological plasticity induced by learning about sound cues may facilitate hearing those salient signals in noisy backgrounds, i.e., relative to novel or insignificant sounds. Given the well-established association between experience-dependent changes in receptive fields in ACx and memory formation, the role of experience-dependent physiological changes in ACx to signal processing merits research to understand successful signal-in-noise detection. Several ACx mechanisms have emerged as key candidates of modulation, including changes to receptive field properties like sound-evoked threshold and bandwidth. In this regard, learning-induced changes to receptive fields that mimic ACx function in noisy backgrounds may effectively promote sound signal detection from noise and facilitate adaptive behavior. Recent investigations have also shown that learning-induced ACx plasticity can be facilitated by treating subjects with an HDAC-inhibitor (histone deacetylase 3, HDAC3i) while they learn an association between a signal acoustic frequency and reward (Bieszczad et al., 2015; Shang & Bieszczad, 2022). However, the extent to which the effect of HDAC3i persists in novel backgrounds of noise remains unknown. Thus, the current study utilizes a rodent (rat) model of sound-reward learning for a 5.0 kHz (60 dB) pure tone frequency cue with *in vivo* auditory cortical (A1) multiunit electrophysiological recordings (as in Rotondo & Bieszczad, 2020). We assessed behavioral and A1 responses to signal and non-signal frequency cues presented under different signal-to-noise ratios (SNR) (+0, +20 and +40dB SNR). The results revealed that both high and low noise have a suppressive effect on selectivity of frequency tuning in trained animals. Yet, HDAC3i protects suppressive effect of low but not high noise on frequency tuning bandwidth. Comparing A1 responses of naïve animals to trained animals will reveal the potential role of learning-induced cortical plasticity in signal-in-noise detection. The findings will enable us to build a comprehensive model of the representational

plasticity underlying long-term memory for sounds, which will improve the ability to achieve successful hearing-related therapeutics in real world environments.

Disclosures: **N. Atesyakar:** None. **A. Shang:** None. **K. Bieszczad:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

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Program #/Poster #: PSTR342.03/AA10

Topic: D.05. Auditory & Vestibular Systems

Support: DFG, Germany`s Excellence Strategy EXC 2177/1

Title: Hearing loss in adult rats leads to less ultrasound vocalization during social interaction and cognitive disturbances in visuospatial attention

Authors: *M. STENZEL, M. ALAM, J. JELINEK, J. KRAUSS, K. SCHWABE, M. JOHNE; Neurosurg., Hannover Med. Sch., Hannover, Germany

Abstract: Background: Hearing loss in the elderly has been associated with difficulties in speech comprehension and cognitive decline. Not least, it is a possible risk factor for dementia. In adult rats we already showed that hearing loss leads to reduced neuronal activity in the medial prefrontal cortex (mPFC). To investigate the impact of hearing loss on cognitive function and communication, we here tested adult rats in behavioral paradigms for motor activity, attention, and impulse control, as well as social interaction, including ultrasound vocalization (USV). **Methods:** In a cohort of adult male Sprague Dawley rats, hearing loss was induced under general anaesthesia with intracochlear injection of neomycin (n=10). Naive (n=10) and sham-operated rats (n=7) served as control. Hearing loss was verified after surgery with auditory brainstem response (ABR) measurement. Furthermore, the rats were tested for motor activity (Open Field), motor coordination (Rotarod), and social interaction before surgery and at week 1, 2, 4, 8, 16, and 24 after surgery. From week 8 onwards, the rats were tested in the Five Choices Serial Reaction Time Task (5CSRTT) for visuospatial attention, impulse control, learning, and memory. In this paradigm, rats have to react to a light stimulus in one of five holes of the aperture, which is shortened from session to session. **Results:** In the Open Field, deaf rats moved significantly faster and a longer distance in total than the naive and sham-operated controls (both $p<0.05$). Moreover, the motor coordination tested on Rotarod was disturbed in deaf rats ($p<0.05$). Although social interaction was not altered, the frequency of ultrasound vocalization was significantly less in deaf rats compared to the control group ($p<0.05$). Learning the paradigm of the 5CSRTT was significantly impeded in the deaf group for the first training session ($p<0.05$). Although shortening the light stimulus in the subsequent sessions had no effect, the accuracy, which is associated with attention, was reduced in deaf rats ($p<0.05$). Retesting in week 20 and 24 did not indicate a long-term memory deficit in the deaf group**. Conclusion:** Hearing loss in adult rats leads to hyperlocomotion, less USV while social interaction, and

deficits in initial visuospatial attention, and learning, which may be related to compromised neuronal activity in the mPFC. Therefore, this model may be used to test the effect of neuromodulatory stimulation on cognitive decline attributed to hearing impairment.

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Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.04/AA11

Topic: D.05. Auditory & Vestibular Systems

Support: ERC

Title: Signaling of sequence violations in mouse auditory cortex in the absence of adaptation

Authors: ***S. JAMALI**[1], S. BAGUR[1], S. DEHAENE[2], T. VAN KERKOERLE[2], B. BATHELLIER[1];
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Abstract: The brain can detect violations of temporal regularities in incoming stimuli, an ability that may reflect the predictions it generates. This ability is often studied based on single-tone violations and with short intervals between stimuli, which may be explained, at least in part, by stimulus-specific adaptation mechanisms. Here, we show that mouse auditory cortex represents surprise responses to local regularity violations of short sound sequences with a very long inter-sequence interval of 30s. Although, at the level of a single trial, we find no effect of adaptation to commonly played sequences, responses to rare stimuli are still greater than responses to common stimuli. This effect is suppressed by eliminating sequence structure. By contrast, we were unable to observe any violation response to pure global sequence regularities using short or long inter-sequence intervals. Thus the global prediction effects observed so far only in awake monkeys and humans do not seem to exist in mouse auditory cortex. VIP neurons were found to mainly implement a sequence termination code independent of sequence identity. These results provide new insights into an underlying circuit logic of stimulus prediction that does not only rely on adaptation.

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Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

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Topic: D.05. Auditory & Vestibular Systems

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NIH Training Fellowship 5TL1TR001447
NIH Diversity Supplement 5R01DC003937-21

Title: Neuroplasticity and early cochlear implant use in adult deafened humans and rats.

Authors: *A. E. HIGHT[1], J. NEUKAM[2], N. H. CAPACH[1], E. G. GLENNON[1], J. K. SCARPA[3], Y.-S. CHENG[4], M. INSANALLY[5], S. VALTCHEVA[6], M. A. SVIRSKY[7], R. C. FROEMKE[1];
[1]NYU Grossman Sch. of Med., New York, NY; [2]Vanderbilt Univ., Nashville, TN; [3]Weill Cornell Med., New York, NY; [4]Massachusetts Eye and Ear Infirmary - Harvard Med. Sch., Boston, MA; [5]Otolaryngology, Univ. of Pittsburgh, Pittsburgh, PA; [6]Univ. of Cologne, Cologne, Germany; [7]New York Univ. Sch. of Med., New York, NY

Abstract: Cochlear implants are neuroprostheses that restore hearing and speech perception in humans with severe to profound hearing loss. Attaining adequate speech perception with CIs, however, does not happen instantaneously and can take weeks, months, and in some cases years. We hypothesize that the 'bottom-up' encoding of CI stimuli improves during early CI use resulting in improved spectral acuity and speech perception. Here we track spectral acuity and relate these measures to speech perception (humans) and sensory encoding in the auditory cortex (A1, rats) during early CI use.
Methods ***(Human)*** Following initial activation of their CI, we sent subjects (N=3) home with a tablet loaded with validated tests of spectral acuity (quick-spectral modulation detection, QSMD). Subjects completed these tasks every other day for the first 30 days following activation. Speech perception was tested periodically in-lab using standard tests of word and sentence recognition (CNC 30). ***(Rodent)*** Acute: we performed intercranial Electroencephalography (iEEG) recordings of the auditory cortex (A1) of normal hearing (tone-evoked, N=7) and deafened rats (CI-evoked, N=7). Chronic: we trained rats on a 2-alternative forced choice (2AFC) task for auditory-stimulus identification, first using tones (N=18) and then, after deafening, using stimuli via CI electrodes (N=5). Lastly, we measured excitatory and inhibitory postsynaptic currents (E/IPSCs) in rat auditory cortex (A1) neurons (N=12), evoked by individual CI electrodes prior to and after 2AFC training.
Results: ***(Human***) We found significant improvements in spectral acuity (QSMD) in 2 of 3 CI users. Improvements in measured speech perception were found in only the users with improved spectral acuity. ***(Rodent)*** Acute: iEEG recordings revealed clear tone and CI-evoked responses. After reducing responses to preferred stimulus maps, we found cochleotopic encoding of both tone-evoked and CI-evoked stimuli. We also found a greater trial-by-trail variability in the temporal but not the topographical aspects of CI-evoked compared to tone-evoked responses. Chronic: rats completed 2AFC training task with high discrimination (d'>1) after ~3 weeks of acoustic training and ~1 week of CI training. Whole cell recordings revealed that CI-evoked E/IPSCs in A1 were significantly less correlated in untrained compared to 2AFC trained rats.
Conclusion: In human studies, we found that spectral acuity is related to speech perception in

early CI use. In rodent studies, we found initially degraded encoding of CI stimuli (iEEG and E:I balance) and that behavioral training with CI (2AFC) related to improved encoding of CI stimuli (E:I balance).

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Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.06/AA13

Topic: D.05. Auditory & Vestibular Systems

Support: NRF-2020R1A6A1A03043283
NRF-2023K1A4A3A02057280
RS-2023-00220408

Title: The central auditory alteration and change in cognitive function in animal models induced by noise-induced hearing loss (NIHL) and photothrombotic hearing loss model (PT)

Authors: *J. PARK[1], N. HONG[2], J.-C. AHN[2,3], M. LEE[4,5];
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Abstract: Sound stimuli are converted into electrical signals by hair cells and transmitted to the central nervous system (CNS). Irreversible damage to the hair cells in the auditory peripheral organ, which is prone to permanent hearing loss, is considered the main cause of PNS damage. Damage to the peripheral nervous system (PNS) in the auditory area induces secondary degeneration in the neuronal cells in the auditory pathway in CNS. The alternation of the auditory cortex neuronal cell population after auditory sensory deprivation has been reported. However, there are many different pathomechanisms of hearing loss such as ototoxicity, excitotoxicity, or ischemic toxicity. It is not clear whether these different types of PNS damage are resulting in CNS alteration. And hearing impairment is known to be associated with deterioration of cognitive function. This study aims to verify and compare changes in the central auditory pathway and cognitive behavioral change in animal models induced by noise-induced hearing loss (NIHL) and photothrombotic hearing loss model (PT). We used male Sprague-Dawley rats (7 weeks old) for NIHL and PT models. All NIHL rats were exposed to a narrow band of noise (16kHz) for 5hr at a sound pressure level (SPL) of 105dB. The rats of the PT model were injected with RB in the femoral vein. After an intravenous injection of RB solution, the vessel was occluded with a photochemically induced thrombus within laser irradiation

(532nm, 175mW, 15 min). Brains were sampled after noise exposure and surgery 1 week. Immunohistochemistry (IHC) was performed for neuronal apoptosis and neuroinflammation. In the NIHL model, mean values of the threshold measured by auditory brainstem response (ABR) were increased after noise exposure. After noise exposure, mean values were increased before noise exposure. Anti-Neuronal Nuclear (NeuN) recognized matured neurons, immunofluorescence intensity, and anti-NeuN positive cell number decreased compared to the control group. However, anti-Glial Fibrillary Acidic Protein (GFAP) that recognized astrocyte immunofluorescence intensity, and anti-GFAP positive cell numbers tended to be increased compared to the control group. These results show an increase in neuronal cell death and an increase in neuroinflammation in the NIHL model's auditory cortex region. In the PT model, hearing loss was confirmed using ABR. After surgery, mean values were increased before surgery. Deterioration of cochlear structure was observed. Additional alternation of synaptic connection in the cochlear nucleus was observed.

Disclosures: **J. Park:** None. **N. Hong:** None. **J. Ahn:** None. **M. Lee:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

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Topic: D.05. Auditory & Vestibular Systems

Support: N66001-17-2-4010

Title: Vagus nerve stimulation recruits the central cholinergic system to enhance auditory perceptual learning

Authors: *K. A. MARTIN[1], E. S. PAPADOYANNIS[2], J. K. SCHIAVO[1], S. SHOKAT FADAEI[1], S. ORREY VALENCIA[1], R. C. FROEMKE[3];
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Abstract: Recent studies suggest the utility of peripheral nerve stimulation - specifically vagus nerve stimulation (VNS) - for numerous clinical applications in humans, including treating epilepsy and motor deficits after stroke. Despite its well-established benefits, less is known about the central mechanisms recruited by VNS. It's thought to activate neuromodulatory areas and subsequently alter neural activity and augment experience-dependent plasticity.
We sought to further expand both the understanding of central mechanisms involved in VNS and the applications for which VNS can be used. We focused on VNS applications in sensory perceptual learning since perception can only be refined by experience up to certain limits. These perceptual constraints are often variable across individuals, resulting in variable performance. It is unclear if the perceptual limitations are absolute or could be partially overcome via enhanced neuromodulation and/or plasticity.

To explore the potential benefits of VNS on sensory perceptual learning, we developed auditory discrimination task for mice. Mice were progressively trained to classify frequencies as a single, center frequency (ranging from 11-16 kHz) or non-center by licking left and right, respectively, for a water reward. Discrimination between center and non-center frequencies improved over 10-45 days through multiple phases of learning (N=72 animals). After reaching stable performance, there was significant across-individual variability in performance. We subsequently implanted a VNS cuff electrode on the left vagus nerve.
VNS occurring during behavior gradually improved discrimination abilities beyond the level achieved by training alone in sham-implanted control animals (N=7 experimental mice, N=10 sham-implanted control mice). Using two-photon imaging, we identified VNS induced changes to auditory cortical responses - namely a shift in representation to the frequency paired with VNS (N=7 animals). We next explored what neuromodulatory areas could be activated by VNS by doing fiber photometry of cholinergic cell bodies (N=3) and two-photon imaging cholinergic axons in auditory cortex with VNS (N=6). Anatomical and optogenetic experiments indicated that VNS could enhance task performance via activation of the central cholinergic system. Finally, we inhibited the cholinergic system during VNS and did not see the VNS-mediated improvements in behavior. These results highlight the importance of cholinergic modulation for the efficacy of VNS, perhaps enabling further refinement of VNS methodology for clinical conditions.

Disclosures: **K.A. Martin:** None. **E.S. Papadoyannis:** None. **J.K. Schiavo:** None. **S. Shokat Fadaei:** None. **S. Orrey Valencia:** None. **R.C. Froemke:** None.

Poster

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Program #/Poster #: PSTR342.08/AA15

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD R01 DC008343

Title: Auditory cortex is necessary for learning and expressing a socially rewarded auditory behavior

Authors: *K. T. Y. WONG, T. Y. SHI, L. ZHOU, J. YANG, M. HAN, A. LU, K. LU, R. C. LIU;
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Abstract: While socially-significant sounds that evoke natural behavioral responses in animals are usually assumed to be fixed and species-specific, they often require experience and learning to become meaningful for a listener. One example of social sound learning comes from the mouse maternal model of communication, in which mothers or virgin co-carers use distal cues to locate and retrieve their pups back to the nest. Traditionally, ultrasonic vocalizations emitted by

pups serve as a localizing signal, but mothers can also learn to associate synthetic sounds with pup retrieval. How and where this association is formed between novel auditory stimuli and social communicative significance is still poorly understood. While much research has implicated auditory cortex (ACx) in associative sound learning that guides behavioral actions in a variety of paradigms, not all auditory tasks have been found to require ACx - either to learn or express the behavior. Here, we tested how silencing ACx alters an animal's ability both to learn a new sound that reliably predicts where pups will be found, and to express the auditory behavior after it has been learned. We first asked whether ACx is necessary for expression of the learned behavior using chemogenetic inhibition. ACx of naïve, female virgin mice (N=11) were bilaterally injected with an adeno-associated virus carrying a silencing DREADD (i.e., designer receptor exclusively activated by designer drugs). After three weeks of expression, those animals were trained in a T-maze to enter one of the two arms cued by an amplitude-modulated band-pass noise and rewarded with pups, which were then retrieved back to the nest in the main stem. Within 8 days, most animals learned to use the sound to locate pups. After the task was learned, we temporarily inactivated the ACx by injecting clozapine-n-oxide (CNO) and found the performance significantly decreased ($p < 0.05$). Next we tested the effect of ACx inactivation during learning. CNO or saline was injected 30 min prior to each daily training session. Our data show that after eight sessions, the CNO animal group (N=14) showed a significant impairment in performance ($p < 0.05$) compared to the saline group (N=15) and the control virus group (N=10). Together, our results suggest that ACx activity is necessary for both learning a socially rewarded acoustic cue and expressing recognition for the sound in the behavior soon after learning. Continuing studies are examining how this ACx activity is being dynamically used for learning and guiding approach to a pup during behavior, ultimately helping to inform us about sensory cortical function during communication behaviors.

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Title: Serotonin release dynamics in auditory cortex during associative learning

Authors: *C. SWEENEY[1], C. LIU[2], K. SMITH[1], A. STEWART[2], A. TAKESIAN[1];
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Abstract: Auditory learning can induce plasticity in the auditory cortex, but the underlying neural mechanisms driving this plasticity require deeper understanding. While neuromodulators such as acetylcholine and norepinephrine are known to promote learning and plasticity, the role of serotonin (5-HT) remains unclear. Previous research shows that augmenting 5-HT signaling facilitates plasticity in the auditory cortex during associative fear learning. However, the dynamics of 5-HT release during auditory learning has not been studied. In this study, we investigated 5-HT signaling in mouse auditory cortex during associative learning. To monitor 5-HT release across auditory learning, we recorded the activity of the fluorescent serotonin sensor (GRAB-5HT, Yulong Li Laboratory) using fiber photometry in the auditory cortex of behaving mice. We trained animals on an appetitive, Go/No-go auditory associative learning task. Water-restricted mice underwent training to discriminate two pure tones that differed by one octave in frequency. Mice received water rewards following a correct response to the target tone ('Hit'), and received a punitive time-out period when incorrectly licking in response to the non-target tone ('False Alarm'). During the early training stage, as mice showed rapid improvements in behavioral performance, 5-HT signals during Hit trials exhibited a prominent increase. Moreover, single trial fluorescent 5-HT transients could discriminate 'Hit' from 'False Alarm' trials, suggesting the emergence of outcome-specific 5-HT responses during learning. Our ongoing work implicates 5-HT as a key neuromodulator released in the auditory cortex during learning and an intriguing therapeutic target for promoting sensory learning.

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH R00DC016046.

Title: Perineuronal Nets in Layer 2/3 of the Auditory Cortex Are Dynamically Regulated During Auditory Learning

Authors: *J. WINNE, K. CURE, M. L. CARAS;
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Abstract: Introduction: The perineuronal net (PNN) is an extracellular matrix structure that envelops parvalbumin-positive (PV+) interneurons. PNN reduction has been associated with decreased GABAergic signaling and heightened cortical plasticity. We hypothesize that the PNN is dynamically regulated and has implications for neural plasticity during auditory learning. **Method:** Male and female Mongolian gerbils were trained on an aversive go/nogo amplitude modulation (AM) detection task, and the expression of PNNs surrounding PV+ cells in the

primary auditory cortex was assessed using a combination of Wisteria Floribunda Agglutinin and PV antibody immunohistochemistry. Training was divided into two stages. During procedural training, animals learned to drink water during non-AM noise and to stop drinking during a highly salient (0 dB re: 100% depth) AM noise to avoid a shock. After animals achieved a d' > 2 for two consecutive sessions, they progressed to the perceptual training stage, during which weaker AM depths were presented over the course of several days. PNN expression was assessed in four groups of animals: naïve (untrained) controls, animals that completed procedural training, and animals that completed either two or seven days of perceptual training. **Results:** Auditory training had a significant effect on the proportion of PV+ cells surrounded by PNNs in layer (L) 2/3 ($F(3,8)=16.78$, $p=0.0008$, $n=3$ per group). A smaller proportion of L2/3 PV+ cells were surrounded by PNNs after procedural training compared to untrained controls (53.82 ± 2.50% vs. 64.49 ± 1.33%, respectively; $p=0.01$). In contrast, the proportion of PV+/PNN+ cells was significantly higher after seven days of perceptual training (70.94 ± 2.18%) compared to both procedural training ($p=0.0005$), and two days of perceptual training (61.58 ± 4.8%; $p=0.02$). Furthermore, the proportion of PV+/PNN+ cells in animals that underwent perceptual training was positively correlated with the degree of threshold improvement ($r=0.8636$, $p=0.0266$). These effects appeared to be specific to L2/3, as training had no effect on the proportion of PV+ /PNN+ cells in L5 ($F(3,8)=0.2650$, $p=0.8489$, $n=3$ per group). **Conclusion:** Our results suggest that auditory training transiently reduces PNN expression around PV+ cells in L2/3 of the auditory cortex, opening a window for neural plasticity and learning. Renormalization of PNN expression after extended training may stabilize network modifications and newly-acquired auditory expertise.

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH RO1DC017785 (POK)
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Title: Learning auditory discrimination tasks increases representation of task-related stimuli in dorsal auditory fields

Authors: *M. WANG, P. O. KANOLD;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Plasticity in the auditory cortex (ACtx) exists in various circumstances, including adaptation, learning, and memory. To identify where in ACtx changes emerge, we used an automated head-fixation training and wide-field imaging system to chronically track the neural

activity of all mouse ACtx sub-regions during the learning of an auditory discrimination task. Decoding models were then trained to predict the presented tone frequencies and behavior results. The performances of the models were used to interpret the representation of task-related stimuli or behavioral outcomes in the cortical responses. Using a forced-choice task with a well-separated decision and choice phase in each trial, 6 mice (4 females and 2 males, F1 generation of Thy1-GCaMP6s cross C57BL/6-Cdh23$^{Ahl/Ahl}$) were able to well discriminate tones of low or high frequency. Wide-field imaging of calcium fluorescence signals showed that responses in the primary auditory cortex (A1) adapt to task-related stimuli during the early training stage. A1 also showed an increasing predictability of behavioral outcomes after the decision phase. A high predictability of task-independent behavioral outcomes emerged in the dorsal posterior auditory field during the intermediate training stage. Meanwhile, in the dorsal anterior auditory field (DA), an increase in tone-related response areas, magnitudes, and the representation of task-related stimuli during the choice phase of correct trials emerged. We hence speculate that DA concurrently integrates stimulus-evoked responses with behavioral outcome-dependent responses. Altogether, our results indicate that learning forced-choice auditory discrimination tasks recruits higher-order auditory fields to establish a structured response patterns in behavioral context.

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH R21DC017829

Title: Repetition plasticity in primary auditory cortex occurs across long timescales for randomized sounds

Authors: N. K. GILL, **J. W. PUTNAM**, *N. A. FRANCIS;
Biol. Dept., Univ. of Maryland, College Park, MD

Abstract: Our ability to monitor the recurrence of sensory events across different timescales is essential for adapting to dynamic environments, optimizing neural resource management, and identifying behaviorally-meaningful stimuli. One way that the central nervous system accomplishes this task is by repetition plasticity, in which neural activity is modulated up or down by repetitive sensation. Timescales of repetition plasticity typically span milliseconds to tens of seconds, with longer durations for cortical vs subcortical regions. Here, we used 2-photon (2P) imaging to study repetition plasticity in mouse primary auditory cortex (A1) layer 2/3 (L2/3) during the presentation of spectrotemporally randomized pure-tones. Our study revealed neurons with both repetition enhancement and suppression for equiprobable pure-tone

frequencies spaced minutes apart, over a period lasting tens of minutes. Each neuron showed repetition plasticity for 1-2 frequencies near the neuron's best frequency. Our results highlight cortical specialization for pattern recognition over long timescales in complex acoustic sequences.

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Support: NIH R01 DC018650
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BBRF NARSAD

Title: The cumulative number of reinforced actions independent of trial spacing fails to explain learning rate

Authors: *R. MONGA, C. DRIEU, K. KUCHIBHOTLA;
Johns Hopkins Univ., Baltimore, MD

Abstract: How are associations between stimuli, actions and outcomes, the three elements of goal-directed sensorimotor behaviors, learned? Most models addressing this question make the implicit assumption that a 'trial' (i.e., individual reinforced pairing) is a fundamental unit of learning. Here we tested the validity of this assumption by training and comparing learning performance in 4 groups of mice with different numbers of trials per day. In addition, we exploited the use of non-reinforced (probe) trials to dissociate between 'acquisition' of task contingencies (measured in probe trials) from behavioral 'expression' (measured in reinforced trials). We reasoned that if the cumulative reinforced pairings ('practice') dominates these two processes, then reducing the number of reinforced trials to half in a session would double the number of days taken by mice to reach expert performance. Water-restricted mice were trained on auditory Go/No-Go task where they learned to lick to a S+ to receive a water reward and withhold licking to a S- to avoid a timeout. We evaluated performance of mice receiving either 140 (n=6) or 70 (n=12) reinforced trials interleaved with 20 probe trials in a session per day. Mice that received 140 reinforced trials per session took on average 4.3 ± 2.2 sessions to acquire the task contingencies and 11.6 ± 3.0 sessions to express those contingencies. Surprisingly, mice receiving 70 reinforced trials in a session acquired and expressed the task at the same rate (5.0 ± 1.4 and 13 ± 4.9 sessions respectively, $p>0.05$). These results suggest that a trial-based account fails to explain learning trajectories. To further test the limits of this effect, we reduced the number of trials per session to 35 (n=10). All mice acquired the task contingencies in more

sessions (7.8 ± 1.9, p=0.002). Interestingly, these mice rarely expressed the task contingencies even after 50 sessions (only 3/10 reached expert performance) and those that did took far longer (33.3±7.6, p~3.1e-8). To understand the contribution of motivation on learning in these short sessions, mice were trained for 35 reinforced trials per session but the reward was either quadrupled or devalued. In these two conditions, although all mice acquired the task contingencies, only 3/10 and 2/10 mice expressed the task knowledge. Overall, these results suggest that goal-directed sensorimotor learning is not just dependent on the 'practice' and is influenced by factors that operate orthogonal to such an account (e.g., between-session consolidation). Further, our data demonstrate that 'acquisition' and 'expression' are differentially affected, arguing that they rely on distinct neuronal computations.

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Topic: D.05. Auditory & Vestibular Systems

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Centre for Research on Brain, Language and Music
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Title: Acute psilocybin administration impairs stimulus specific adaptation in murine auditory cortex

Authors: *C. LANE, V. TARKA, E. DE VILLERS-SIDANI, E. HAMEL;
Neurol. & Neurosurg., McGill Univ., Montreal, QC, Canada

Abstract: Psilocybin - a psychoactive compound found mostly in the psilocybe genus of mushroom - has long been employed by various peoples for spiritual and therapeutic purposes. Promising clinical studies have created a surge of interest in the potential of psilocybin as a treatment for neuropsychiatric conditions, such as addiction and treatment resistant depression. It is increasingly believed that the serotonin 2A receptor agonist's therapeutic properties are exerted through facilitating structural and functional neuroplasticity, such as increasing dendritic spine formation and changes in whole-brain functional connectivity. Knowledge of the direct effects of psilocybin on neuronal activity, particularly with single-neuron resolution, remains sparse. Moreover, despite observed changes in human auditory perception, no study of psilocybin's effects on auditory cortex (ACx) sensory processing has been undertaken. We hypothesised that psilocybin may exert acute effects on ACx information processing through changes in stimulus-specific adaptation (SSA), the mechanism by which neurons become less sensitive to repeated stimuli over time, whilst remaining sensitive to novel or unexpected stimuli. This is vital for encoding of behaviourally relevant information and helps to fine-tune neuronal

circuitry through changes to synaptic structure and function. We used in vivo two-photon microscopy combined with a fluorescent (Thy1-GCaMP6s) neuronal calcium indicator mouse to examine the acute effects of 1 mg/kg psilocybin administration on ACx responses to pure tone stimulation. Awake mice (N = 7) were exposed to batteries of randomly presented 100 ms pure tones ranging from 4-64 kHz before and after drug administration. Administration of saline saw a 12.4% ± 5.2 (SE) mean reduction in sound-responsive cells between recordings, whereas psilocybin saw 1.2% ± 4.2 (paired t-test, p= 0.0036) reduction. The mean relative spiking activity of responsive cells was reduced 21.2% ± 3.3 following saline and psilocybin, 10.6% ± 3.6 (paired t-test, p= 0.037). The cumulative probability of a cell responding to the lowest intensity of sound (35 dB) was 35.5% for saline and 40.0% for psilocybin. These results indicate that psilocybin impairs SSA in excitatory ACx neurons, reducing the characteristic decrease in sensitivity to repeated stimuli. Interference with SSA suggests that psilocybin may enhance the brain's ability to adapt to new stimuli, and facilitate neuroplastic changes. With further research, this could be used to support recovery and rehabilitation from brain injuries or in neuropsychiatric conditions.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant DC016746
NIH Grant DC017470

Title: Role of striatal neurovascular coupling in an auditory Task Learning

Authors: *L. CHEN, Q. XIONG;
Stony Brook University, Neurobio. & Behavior, Stony Brook, NY

Abstract: The auditory striatum, the caudal tail region of the dorsal striatum, integrates inputs from primary auditory cortex and medial geniculate body to drive auditory decisions. Its plasticity is critical for task learning. Despite accumulating knowledge focusing on the neuronal network, however, little is known about how learning modulates the local striatal network which consists of glia, lymphocyte, and microvasculature, and how the local striatal network influences the learning. Here we used an in vivo deep brain imaging approach in freely moving mice to examine the neurovascular coupling in the auditory striatum during learning and assess its role in learning. We found that the auditory task learning induced non-linear dynamics in neurovascular

coupling, and pharmacological decoupling of the neurovascular unit impaired the learning process. Our findings reveal potential new network mechanisms underlying auditory learning.

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Topic: D.05. Auditory & Vestibular Systems

Support: BBSRC research grant (BB/X013103/1)

Title: The influence of sound statistics on auditory decisions in ferrets

Authors: *K. S. BOCHTLER[1], F. DICK[2], L. L. HOLT[3], A. J. KING[1], K. M. M. WALKER[1]; [1]Dept. of Physiology, Anat. and Genet., Univ. of Oxford, OXFORD, United Kingdom; [2]UCL Ctr. For NeuroImaging, Univ. Col. London, London, United Kingdom; [3]Dept. of Psychology, Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The ability to direct our attention towards a single sound source such as a friend's voice in a crowded room is necessary in our acoustical world. This process is thought to rely, in part, on directing attention to different sound dimensions, such as frequency. Previous investigations have shown task-dependent changes in the frequency tuning of auditory cortical neurons when ferrets actively detect or discriminate a particular frequency of sound (e.g. Fritz et al. 2010). However, questions remain about how attentional gain can arise based on sound statistics. Specifically, to what extent can this modulation occur even if frequency is not a necessary component of the task demands? Mondor & Bregman (1994) demonstrated that human listeners' reaction times on a tone duration task were slower when the presented tone frequency was unexpected (i.e. low probability). Here, we test the hypothesis that the statistical likelihood of sound frequencies alone can also affect animals' behavioural decisions on orthogonal dimensions of sounds. We trained ferrets on either a go/no-go threshold detection or 2-alternative forced choice tone duration discrimination probe signal task in which we manipulated the statistical likelihood of tone frequencies. Our results show that, similar to humans, ferrets' reaction times increased for low-probability frequencies but only if pure tones were presented near individual threshold for the go/no-go variant. As with humans, accuracy remained stable across frequencies for the duration discrimination variant while greater accuracy is seen for the expected frequency in the threshold detection variant. These results suggest that attentional filters are employed during listening, even for an acoustical dimension (frequency) that is orthogonal to the task demands (duration). Our future experiments will use this task in combination with microelectrode recordings to investigate the neurophysiological basis of statistical-based attentional filtering in the auditory cortex.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: JHU Discovery Award

Title: Continual Learning in a Self-Paced, Multi-Task Mouse Playground

Authors: ***A. BAL**, S. SOTO, A. SANTI, P. H. JANAK, K. KUCHIBHOTLA;
Johns Hopkins Univ., Baltimore, MD

Abstract: Animals possess a wide repertoire of cognitive abilities that enable learning of a diverse range of tasks and skills throughout the lifespan. However, this learning process is rarely isolated, and the ability to leverage past information significantly impacts the capacity to acquire new knowledge efficiently. Transfer learning, multi-task learning, and continual learning are three key neurobehavioral mechanisms that facilitate faster acquisition and retention of new knowledge by capitalizing on prior knowledge and task similarities. To investigate the neural mechanisms underlying multi-task and continual learning, we sought to develop an approach that allows mice to learn a diverse and large range of tasks that vary along different dimensions, such as stimulus perceptual dimensions, task rules, and motor outputs. If successful, dissecting the neural substrates of continual learning becomes more tractable due to the wide range of physiological, optical, and molecular tools available in the mouse. But how can we teach mice to learn many tasks? Most rodent literature requires animals to learn one or 2-3 tasks at most. We reasoned that this low number of tasks is heavily constrained by the method of training rather than a mouse's true cognitive abilities. To overcome these limitations, we created a self-paced, multi-task mouse playground, for automated training of cognitive tasks. Mice (n= 4-6) reside in a home-cage connected to a dedicated behavioral arena via a gating mechanism which only permits one mouse in at a time. Inside the behavioral arena, mice have the option to perform tasks to receive water reward, their only source of water. As a result, mice complete a variety of experimenter-administered tasks in a fully volitional manner. To test whether mice could learn many tasks, we trained mice (n=4) on 5 auditory Go-NoGo tasks in which mice had to learn a discrimination problem along distinct perceptual dimensions (frequency, spatial localization, stimulus duration, stimulus rate, and amplitude modulation). We found that mice learned all 5 tasks, reaching lesser than 30% in false alarm rates, within 2 weeks of training time. Additionally, we noticed that on average, mice required a fewer number of trials to reach an 90% accuracy level on task 4, which was later in the sequence, compared to task 1 which was early in learning (paired t-test; t=4.255, p=0.024), suggesting that mice had likely generalized the fundamental structure of a Go-NoGo task and were simply applying it to a newly presented

perceptual dimension. These data provide a foundation for training mice on a wide variety of tasks to study the neural mechanisms of continual and multi-task learning.

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PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.18/AA25

Topic: D.05. Auditory & Vestibular Systems

Title: Behavioral and neural responses in zebrafish to complex sounds presented within an oddball paradigm

Authors: *A. PEARSON, C. ALRAWASHDEH, J. MILLARD, J. S. KANWAL; Georgetown Univ., Washington, DC

Abstract: The neural mechanisms underlying deviant detection and the "cocktail party effect" remain important unresolved issues in auditory neuroscience. These phenomena typically involve a subject's attention to sound streams presented within a quiet vs. a cluttered acoustic background. Yet, in contrast to visual attention, auditory attention remains understudied at behavioral and neural levels because tracking shifts in auditory attention is more difficult than tracking visual attention. Quantitative tracking of behavioral parameters (swim direction, speed and turns) in free-swimming fish species can facilitate studies of overt auditory attention. We recently showed that adult zebrafish (n = 12) can be conditioned to swim/go towards or away from an LCD screen in response to different sounds (Millard et al., 2023). Here, we conditioned juvenile zebrafish, Danio rerio, (n = 48; 8 to 12 weeks post-fertilization) to attend to sound sequences within this Go/Go paradigm. One sound sequence (conditioned stimulus) was paired with the presentation of a reward (video of a shoal of zebrafish) and another with an aversive stimulus (video of an Indian bullfrog). Sound sequences consisted either of six repetitions of a multiharmonic tonal sound pulse or of a sequence where the fifth sound pulse (deviant) contained frequency- and amplitude-modulation. Markerless tracking was successfully accomplished with idTracker.ai (Romero-Ferrero et al., 2019). To identify auditory vs. attention-driven auditory responses of single neurons to the same complex sounds, we imaged the brain of awake gCaMP6-labeled zebrafish larvae (5 to 7 days post-fertilization; n = 8 to single sounds and n = 2 for sound sequences) embedded in agar using a two-photon microscope. We observed that neurons (n > 100; in 10 animals) at multiple locations responded robustly to the presentation of complex sounds (> 70 dB SPL). Analysis showed an on- and an off-response component in the auditory response. Our studies of complex sound perception in zebrafish have the potential to localize brain regions containing complex sound-responsive and attention-specific neurons, and provide new insights on the neural mechanisms and brain regions involved in auditory attention.

Disclosures: **A. Pearson:** None. **C. Alrawashdeh:** None. **J. Millard:** None. **J.S. Kanwal:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.19/AA26

Topic: D.05. Auditory & Vestibular Systems

Support: CIHR Operating Grant
CIFAR Senior Fellow
FRQNT Postdoctoral Fellowship

Title: Different cortical and subcortical responses to violations of top-down and bottom-up auditory predictions measured with 7T functional MRI

Authors: A. ARA[1], V. PROVIAS[2], K. SITEK[4], E. COFFEY[3], *R. ZATORRE[5];
[1]Montreal Neurolog. Inst., Montreal, QC, Canada; [2]Concordia Univ., Montreal, QC, Canada; [3]Concordia Univ., Montréal, QC, Canada; [4]Univ. of Pittsburgh, Pittsburgh, PA; [5]Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada

Abstract: Perception integrates both sensory inputs and internal models of the environment. Our sensory areas do not only encode incoming stimuli, but also predictions about future events, and their resolution or violation. In the auditory domain, this top-down predictive coding is often attributed to auditory cortical activity. The sub-cortical areas of the auditory pathway had been assumed not to encode top-down predictions. High-field imaging has revealed that top-down auditory predictions are also encoded by subcortical areas such as the Inferior Colliculus (IC) and the Medial Geniculate Body (MGB). The question remains, however, as to whether these dynamics are propagated from cortical areas, such as Heschl Gyrus (HG) and belt/para-belt area (PB). In addition, it is unclear what areas along this pathway encode lower-level auditory predictions and violations, such as changes in a tone's periodicity. To answer these questions, we studied manipulations that preferentially engage higher-order or lower-order mechanisms in both cortical and subcortical brain structures, with 7T fMRI.
We presented sequences of eight tones in an oddball paradigm where the position of the deviant stimulus varied from least to most predictable (4th through 6th positions). In addition to this top-down manipulation, a bottom-up manipulation (low-level prediction) was orthogonally implemented by assigning sound sequences to blocks with no disruption (most predictable); tones with an embedded noise disruption, returning in phase (less predictable); and tones with the same disruption, but returning in antiphase (least predictable). Average BOLD signals were recorded with a Siemens 7T MRI scanner from ROIs (HG, PB, IC and MGB) in native space and submitted to first-level statistical estimation of the conditions. Second-level effects were evaluated meta-analytically.
Data (N=10 healthy listeners) show that both cortical and subcortical areas show a strong

response to the first item in the sequence; but brain activity for the deviant stimuli scaled down with predictability (i.e. position, dev4 > dev5 > dev6). The presence of noise elicits a greater signal in all structures, but no difference was observed between in- and antiphase signals. We conclude that top-down predictions and their associated certainty are encoded in subcortical areas of the auditory pathway and we show that these trends are like those in cortical areas. This finding could be interpreted as the result of top-down predictions encoded in higher-order areas being propagated onto lower-level areas based on internal models. fMRI BOLD may not be sensitive to phase disruptions which require higher temporal resolution.

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Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.20/AA27

Topic: D.05. Auditory & Vestibular Systems

Support: DC015543
DC021067
NARSAD Young Investigator Grant to MNI

Title: Dynamic gating of perceptual flexibility by diverse cortical responses

Authors: ***T. HOU**[1], B. SIDLECK[1], J. TOTH[1], O. LOMBARDI[1], A. ELDO[1], P. AGARWAL[1], D. SAEED[1], D. LEONARD[1], L. ANDRINO[2], M. KERLIN[1], X. ZENG[3], M. INSANALLY[1]; [1]Otolaryngology, Neurobio., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA; [2]Neurosci. Inst., [3]Dept. of Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: The ability to flexibly respond to sensory cues in dynamic environments is essential to adaptive auditory-guided behaviors such as navigation and communication. How do neural circuits flexibly gate sensory information to select appropriate behavioral strategies based on sensory input and context? Auditory neural responses during behavior are diverse, ranging from highly-reliable ‘classical’ responses (i.e. robust, frequency-tuned cells) to irregular or seemingly random ‘non-classically responsive’ firing patterns (i.e., nominally non-responsive cells) that fail to demonstrate any significant trial-averaged responses to sensory inputs or other behavioral factors. While classically responsive cells have been extensively studied for decades, the contribution of non-classically responsive cells to behavior has remained underexplored despite their prevalence. Recent work has shown that non-classically responsive cells in auditory cortex (AC) and secondary motor cortex (M2) contain significant stimulus and choice information and encode flexible task rules. While it has been shown that both classically and non-classically responsive units are essential for asymptotic task performance their role during learning is unknown. Here, we explore how diverse cortical responses emerge and evolve during flexible

behavior. We recorded single-unit responses from AC while mice performed a reversal learning task. Cortical response profiles during learning were highly heterogeneous spanning the continuum from classically to non-classically responsive. Strikingly, we found that the proportion of task-encoding non-classically responsive neurons significantly increased during late learning when the largest behavioral improvements occur demonstrating that non-classically responsive neurons are preferentially recruited during learning. To identify the role of top-down feedback on AC circuits during key learning phases we optogenetically silenced M2→AC projection neurons while recording AC spiking responses. Remarkably, silencing M2 inputs selectively modulated non-classically responsive cells and impaired behavioral performance during post-reversal learning. Our findings demonstrate that task-encoding non-classically responsive cells are preferentially recruited during learning by top-down inputs enabling neural and behavioral flexibility.

Disclosures: **T. Hou:** None. **B. Sidleck:** None. **J. Toth:** None. **O. Lombardi:** None. **A. Eldo:** None. **P. Agarwal:** None. **D. Saeed:** None. **D. Leonard:** None. **L. Andrino:** None. **M. Kerlin:** None. **X. Zeng:** None. **M. Insanally:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.21/AA28

Topic: D.05. Auditory & Vestibular Systems

Support: JST Moonshot R&D Grant Number JPMJMS2292
The Encouraging Grant for Graduate Students at NIPS

Title: Neural selective consistency as a mechanism of implicit perceptual learning.

Authors: *Y. GOTO[1,2], K. KITAJO[1,2];
[1]Natl. Inst. for Physiological Sci., Okazaki, Japan; [2]The Grad. Univ. for Advanced Studies, SOKENDAI, Okazaki, Japan

Abstract: Understanding the mechanism by which the brain overcomes its inherent inconsistency in activity to achieve consistent information processing is one of the major challenges in neuroscience. The inconsistency of neural activity to an identical input is known to be caused by variabilities at various spatial scales from the single-cell spikes to scalp electroencephalograms and these variabilities can have various causes (Faisal et al., 2008). Nevertheless, despite these inherent inconsistencies in the nervous system, our perceptual and motor experiences are relatively consistent. This raises questions about how the brain, a system with both deterministic and stochastic fluctuations, achieves consistent experiences, and how variabilities affect neuronal information processing. Recently, it has been reported that the consistency of neural responses is enhanced implicitly and unsupervised to stimuli that are presented repeatedly, and results in improved perceptual consistency (Agus et al., 2010, Luo et

al., 2013). Here, we propose the term "selective consistency" to describe this input-dependent consistency and hypothesize that it will be acquired in a self-organizing manner by plasticity within the nervous system. To test this, we investigated whether a reservoir-based plastic neural network model could acquire selective consistency to repeated stimuli. The task we used is based on a human auditory experimental task, the noise-repetition detection (NRD) task. We used white noise sequences randomly generated every trial and referenced white noise presented multiple times. Then we evaluated whether the network obtained selective consistency only to the referenced white noise. The results showed that the plastic network was capable of acquiring selective consistency rapidly, with as little as five exposures to stimuli, even for white noise. This is similar to the results of behavioral and neural data of the NRD task. The acquisition of selective consistency could occur independently of performance optimization, as the network's time-series prediction accuracy for referenced stimuli did not improve with repeated exposure and optimization. Furthermore, the network could only achieve selective consistency when in the region between order and chaos. These findings suggest that the neural system can acquire selective consistency in a self-organizing manner, and that this may serve as a mechanism for certain types of learning.

Disclosures: **Y. Goto:** None. **K. Kitajo:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.22/BB1

Topic: D.05. Auditory & Vestibular Systems

Support: NSFC

Title: Whole-brain analysis of structure and function of raphe 5-HT neurons uncover a novel mechanism regulating behavior habituation

Authors: *N. N. GUAN, **C.-X. HUANG**, J. SONG;
Tongji Univ., Shanghai, China

Abstract: Facing with repetitive stimulations, animals constantly adapt their behaviors to accommodate with the environment. Serotonin released by raphe 5-HT neurons modulates the habituation of acoustic startle response (ASR) (a form of non-associative learning) through an unidentified mechanism due to the complexity of these neurons in terms of neuronal diversity and broad axon projection. In this study, we took advantage of larva zebrafish and investigated the mechanism allowing 5-HT neurons regulating the habituation of ASR at synaptic and circuit levels in both *in vivo* and *ex vivo* preparation. First, we found that the raphe 5-HT neurons were functionally and topographically diverse and further identified as the rostral, intermediate and caudal subclasses. Repetitive stimulation of auditory nerve in short interval induced a persistent calcium increase in the 5-HT neurons of the intermediate subclass, while a fast depression of

calcium dynamics in those belonging to the rostral and caudal subclasses. This phenomenon was not observed in the animals without habituation of ASR. The single neuron labelling reveals the whole-brain axon distribution of raphe 5-HT neurons, suggesting that the intense axon of 5-HT neurons of the intermediate subclass caudally project to the synapse formed by auditory nerve and Mauthner cell in a close proximity. However, axonal distribution of the 5-HT neurons in the other two subclasses were far away from this synapse. Patch-seq analysis also showed different genetic signatures of three subclasses of 5-HT neurons. Laser ablation of 5-HT neurons of the intermediate subclass, not the other two subclasses, prevented the habituation of ASR in both *in vivo* and *ex vivo* preparation. The modulatory effect of intermediate Raphe 5-HT neurons to the habituation of ASR was shown via Htr1aa/1ab receptors by both pharmacological and knock-out experiments. Thus, our data discovered a previously unappreciated mechanism governing the behavior habituation with a subclass of raphe 5-HT neurons constantly releasing serotonin and facilitating AST habituation.

Disclosures: **N.N. Guan:** None. **C. Huang:** None. **J. Song:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

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Program #/Poster #: PSTR342.23/BB2

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant F31-DA047068
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NARSAD 2017 YI-26050

Title: Early auditory processing is modified after experiences that associate sounds with cocaine

Authors: *M. A. PRESKER[1], K. M. BIESZCZAD[2];
[1]Psychology, Rutgers - The State Univ. of New Jersey, Piscataway, NJ; [2]Psychology, Behavioral and Systems Neurosci., Rutgers The State Univ. of New Jersey, Piscataway, NJ

Abstract: Drug-induced neural plasticity in primary sensory systems may underlie altered reactivity to drug-cues in cocaine addiction. Enhanced behavioral reactivity to and altered neural encoding of drug-associated cues is a primary feature of addiction that contributes to relapse vulnerability, a primary therapeutic target in addiction treatment. Substantial evidence supports a central role for mesolimbic system plasticity in addiction but much less is known about the involvement of sensory systems. In recent years, the centrality of sensory systems in learning and memory has emerged and it may be that the effects of drug taking on sensory systems lies at the heart of altered reactivity to drug cues in addiction. Experience-dependent plasticity in the auditory brainstem occurs over a lifetime of experiences and these early processing changes are prime candidate mechanisms for adaptive processes such as focused attention, or maladaptive disease states, like addiction. Here, we focus on the auditory brainstem response (ABR) to

investigate the possibility that basic sensory processing can be affected by a learning process that associates sound cues with the rewarding effects of drugs of abuse. The ABR is a transient, sound-evoked neural potential arising from synchronous activity in brainstem neurons and characterized by five distinctive peaks or waves (I, II, III, IV, V) arising from sequential nodes in the early auditory pathway. We hypothesized that a learning paradigm that pairs a tone with cocaine will induce sound-specific auditory brainstem plasticity that can be revealed in changes to the ABR. An auditory-cocaine conditioning paradigm for rats was used to obtain sound-evoked ABR recordings before and after conditioning (Experiment 1; N=19). Rats (n=14) underwent six daily 30min conditioning sessions to associate a pure tone stimulus with cocaine (20mg/kg; intraperitoneal). To serve as a control for cocaine exposure, a subset of rats were conditioned instead with saline (n=5). We report sound-specific changes in ABR that correlate with behavioral measures of stimulus control. A modified version of this paradigm employed an operant test of stimulus control in addition to ABR recordings before and after cocaine (n=16) or saline (n=6) conditioning (Experiment 2; N=22) to show operant effects of prior drug-cue exposure. These data support that drug addiction transforms the sensory systems, which are now prime candidate targets of drug addiction processes and recovery.

Disclosures: **M.A. Presker:** None. **K.M. Bieszczad:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

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Klingenstein-Simons Fellowship Award in Neuroscience

Title: Longitudinal imaging reveals distinct learning-dependent changes between layer 5 excitatory subtypes

Authors: ***N. SCHNEIDER**[1], M. MALINA[2], R. KRALL[1], R. S. WILLIAMSON[1];
[1]Univ. of Pittsburgh, Pittsburgh, PA; [2]Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Auditory-guided behavior is a fundamental aspect of our daily lives, as we rely on auditory information to guide our decisions and actions. The primary route for auditory information to propagate from the ACtx is through intratelencephalic (IT) and extratelencephalic (ET) neurons in layer (L) 5. These neurons form the major output of the ACtx and, as a result,

are in a privileged position to readily influence auditory-guided behavior. To investigate the behavioral role of IT and ET neurons, we devised a head-fixed choice task where mice categorized the rate of sinusoidal amplitude-modulated (sAM) noise bursts as either fast or slow to receive a water reward. To ascertain the necessity of ACtx, we conducted bilateral optogenetic inhibition with GtACR2 and observed a significant decrease in hit rate during inhibition trials. We then used two-photon calcium imaging alongside selective GCaMP8s expression to monitor the activity of L5 IT and ET populations.
Clustering analyses of these populations revealed heterogenous response motifs that correlated with various stimulus and task variables. Of particular interest was a distinct motif primarily present in ET neurons, characterized by "categorical" firing patterns that indicated a preference for either slow or fast sAM rates. This categorical selectivity was not initially present, but was revealed through longitudinal recordings, illustrating dynamic alterations in the responses of ET neurons across learning to align with distinct perceptual categories. Critically, this categorical selectivity in ET neurons did not manifest during passive exposure to identical stimuli. This suggests that learned categorical selectivity is shaped via top-down inputs that act as a flexible, task-dependent filter. Moreover, ET activity reflected behavioral choices independently of stimulus identity or reward outcome, with choice selectivity increasing throughout learning.
In contrast, L5 IT neurons initially exhibited category information which then degraded as mice acquired task proficiency. Furthermore, the ability to decode both stimulus identity and behavioral choice from IT activity decreased across learning. This suggests a tradeoff of information between these two distinct populations within L5, with IT projections playing a role in initial task acquisition, while ET projections are recruited and reinforced throughout the learning process. Collectively, these findings underscore the differential roles of L5 neurons and contribute to our understanding of how auditory information is processed and utilized to guide decision-making and action.

Disclosures: **N. Schneider:** None. **M. Malina:** None. **R. Krall:** None. **R.S. Williamson:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

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Program #/Poster #: PSTR342.25/BB4

Topic: D.05. Auditory & Vestibular Systems

Support: NIH T32 Training Grant
NIH R01 DC018650-03

Title: Cortical-subcortical dynamics in the auditory system during learning and overtraining

Authors: *S. KIM, K. KUCHIBHOTLA;
Johns Hopkins Univ., Baltimore, MD

Abstract: Sensorimotor learning requires linking sensory input with an action and an outcome. How a new sensorimotor pairing engages and evolves along a sensory hierarchy, from the midbrain to thalamus to cortex, remains unknown. Interestingly, recent evidence suggests that the auditory cortex (AC) is the default pathway for audiomotor discrimination learning but then becomes dispensable at expert levels. The mammalian auditory system is organized in a feedforward fashion with auditory stimuli being progressively processed by the cochlea, brainstem, the midbrain (IC, inferior colliculus), the thalamus (MGB, medial geniculate bodies), and finally the AC. In particular, the IC-MGB-AC circuit exhibits rich feedforward and feedback projections that, to date, have not been monitored simultaneously during a learning process. To do this, we trained mice to lick to a pure tone for a water reward (S+) and withhold licking to another tone (S-) to avoid a timeout. We developed a new surgical preparation which allows the implantation of a single cranial window over the IC and the AC. We then used two-photon mesoscopic calcium imaging throughout goal-directed learning to monitor cell bodies (jRGECO1a) in the AC and the IC , and the feedforward projections (axon-GCaMP6s) from the MGB to the AC (n=4). By tracking the same cell bodies and axons across a month of training, we examine the sequence of stimulus-related and non-stimulus related plasticity and the nature of how learning and overtraining impact these processes across the sensory hierarchy.

Disclosures: S. Kim: None. **K. Kuchibhotla:** None.

Poster

PSTR342. Auditory Processing: Adaptation, Learning, and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR342.26/BB5

Topic: D.05. Auditory & Vestibular Systems

Support: DFG

Title: Stimulus-specific adaptation in the bat's frontal and auditory cortex

Authors: *E. GONZALEZ PALOMARES[1], J. C. HECHAVARRIA[2];
[1]Inst. of Cell Biol. and Neurosci., Goethe-University Frankfurt, Frankfurt, Germany; [2]Goethe Univ. Frankfurt, Frankfurt am Main, Germany

Abstract: In humans, scream vocalizations have strong amplitude modulations (AM) at 30-150 Hz. These AM correspond to the acoustic correlate of perceptual roughness. In bats (species *Carollia perspicillata*), distress syllables also carry amplitude fluctuations at rates of approximately 1.7 kHz (more than 10 times faster than in humans). The distress calls with these modulations are used more prominently by males and might signal a greater urgency, since they elicit larger heart rate increments than their demodulated versions. In order to study the neural processing of these two sounds (the distress calls with fast AM and the demodulated versions), we simultaneously recorded in two brain areas from the bat neocortex, the auditory cortex and the frontal auditory field , a frontal area responsive to sounds. We searched for stimulus-specific

adaptation (SSA), which is described as the neuronal adaptation to a frequently presented stimulus (standard) yet responding strongly to an infrequent sound (deviant). The amplitude modulated natural calls and their demodulated forms were used as stimuli pairs. Our results show the existence of stimulus-specific adaptation in response to natural distress sounds produced by the bats. In addition, we describe that the dynamics of stimulus specific adaptation differs between frontal and auditory areas within the bat brain.

Disclosures: E. Gonzalez Palomares: None. **J.C. Hechavarria:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

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Program #/Poster #: PSTR343.01/BB6

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant NS114682
NIH Grant NS129695

Title: Sex differences in vocal learning ability in songbirds are linked with differences in flexible rhythm pattern perception

Authors: A. A. ROUSE[1], A. D. PATEL[1], S. WAINAPEL[2], ***M. KAO**[2];
[1]Psychology Dept., [2]Biol. Dept., Tufts Univ., Medford, MA

Abstract: Humans readily recognize rhythmic patterns, such as that of a favorite song, independent of the tempo at which they occur. This reflects a facility with perceiving the relative timing of events, not just absolute interval durations. Several lines of evidence suggest that this ability is supported by precise temporal predictions arising from forebrain auditory-motor interactions: 1) neuroimaging studies have shown that a complex auditory-motor cortical network is strongly engaged during musical rhythm perception; and 2) transient disruption of auditory-motor connections can disrupt beat perception in humans without affecting perception of the timing of absolute intervals. Given that vocal learning species often communicate using rhythmically patterned sequences and have evolved neural adaptations for auditory-motor processing, we hypothesize that such species share our facility for flexible rhythm pattern perception. Using a sequential training paradigm with naturalistic sound sequences and multiple tempi, we previously showed that a vocal learning songbird (male zebra finches) can recognize a fundamental rhythmic pattern - equal timing between events (isochrony) - based on the relative timing of events, rather than on absolute durations (Rouse et al., 2021). Here, we take advantage of sex differences in zebra finches to further test the hypothesis that differences in vocal learning abilities correlate with differences in auditory rhythm pattern perception. Auditory sensitivity is similar across sexes in this species, but only males learn to imitate songs and the neural circuitry subserving vocal learning is greatly reduced in females. Using the same behavioral training paradigm as in our study with males, we find the female zebra finches can recognize isochrony

across a wide range of rates, but perform slightly worse than males on average. Our findings are consistent with recent work showing that while female zebra finches have reduced forebrain song regions, the overall network connectivity of vocal premotor regions is similar to males and may support predictions of upcoming events. Comparative studies of male and female songbirds thus offer an opportunity to study how individual differences in auditory-motor connectivity influence perception of relative timing, a hallmark of human music perception that underlies the positive effect of music-based therapies on speech and movement disorders. In ongoing work, we are investigating the contribution of a motor planning region that is reciprocally connected with auditory regions to flexible rhythmic pattern perception.

Disclosures: **A.A. Rouse:** None. **A.D. Patel:** None. **S. Wainapel:** None. **M. Kao:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.02/BB7

Topic: D.05. Auditory & Vestibular Systems

Title: Different effects of visual and auditory stimuli on the neural activity in the auditory cortex.

Authors: *S. NOGUCHI[1], M. IWASAKI[1], M. INDA[2], K. HOTTA[1], K. OKA[1,3];
[1]Keio Univ., yokohama, Japan; [2]Fakultät für Psychologie, Ruhr-Universität Bochum, Fakultät für Psychologie, AE Biopsychologie, Bochum, Germany; [3]Kitasato Univ., Sagamihara, Japan

Abstract: Male zebra finches (*Taeniopygia guttata*) sing to females, and females choose their partners based on the quality of the song and social context. This social context includes visual, postural, and motor elements. Previous studies suggest that visual information influenced other sensory modalities (Campbell and Hauber 2009; Carouso-Peck and Goldstein 2019; Varkevisser et al., 2022), and song-induced immediate early gene expression in the higher auditory cortex NCM (caudal medial nidopallium) and CMM ((caudal medial mesopallium) of females is modulated by visual stimulation from courting males (Avey et al., 2005). Furthermore, the higher brain region NCL (nidopallium caudolateral) is connected to the visual pathway (Hsiao et al. 2020) and auditory cortex L1 (Field L subdivision 1, Stacho et al., 2020). These findings suggest that there is a functional connection between the visual and auditory cortex. We hypothesized that visual information affects the auditory cortex and examined this hypothesis by recording neural activities in free-moving birds.First, we recorded the neural activities of the NCM in female birds when presented with (1) video and song of a male bird directly singing to a female, (2) song only, (3) video only, and (4) no song and no video with only background pictures. The results showed that (1), (2) and (3) had significantly greater mean firing rates than ones from (4). In order to investigate the differences in the effects of visual and auditory stimuli on the neural activity in the higher auditory cortex, we estimated the latency of the neural activities to (1), (2), and (3) by taking the median time of the first spike for each trial after the onset of stimulation. The results showed that the latency of neural activity in the higher auditory

cortex is longer when visual stimuli are presented than when auditory stimuli are presented. Furthermore, to examine whether there are similarities in the timing of spikes between each stimulus, the correlation of temporal neural responses between each stimulus was examined using Time Series Correlation (Inda et al., 2020). The results showed that visual stimuli elicit similar temporal neural responses to auditory stimuli in the higher auditory cortex.

Disclosures: **S. Noguchi:** None. **M. Iwasaki:** None. **M. Inda:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.03/BB8

Topic: D.05. Auditory & Vestibular Systems

Title: Reflex-based estimation of auditory thresholds in rodents: How modulation of the acoustic startle response can efficiently be used to determine limits of perception

Authors: L. KOCH, K. CRAMER, T. KUTTNER, ***B. H. GAESE**;
Goethe-University Frankfurt, Frankfurt am Main, Germany

Abstract: Testing animals for the ability to discriminate between stimulus levels or frequencies at the behavioral level mostly requires time-consuming training. Here we explore the possibilities of applying a reflex-based behavioral method for a more time-efficient measurement of discrimination abilities. The startle response to acoustic stimuli is a fast, reflexive contraction of skeletal muscles. A preceding weaker stimulus can reduce the startle response, a phenomenon called prepulse inhibition (PPI). This procedure does not require training of animals.
We tested rats and mice in a modified behavioral paradigm combining a continuous background stimulus with a shift in frequency or stimulus level acting as a startle-modifying prepulse (= shift-prepulse). A thorough characterization of the effects of shift-prepulses was performed by systematically changing the following three stimulation parameters: i) frequency of background stimulation, ii) step size and direction of shifts and iii) timing of the shift-prepulse. For investigating level changes, step sizes up to ±15 dB were tested, starting from different background levels. Frequency shifts were tested in the range of ±1% up to ±30% around background frequencies of 8 and 16 kHz.
Change-induced PPI increased with change size for both, frequency and stimulus level shifts. Maximal inhibition depended in both cases on background frequency, with strongest inhibition occurring at the lowest frequency tested. Inhibition increased with background level and with shift amplitude. Level increases led to significantly higher inhibition values than level decreases. Frequency shifts were tested with different timings of the shift-prepulse. Highest inhibition values were found for shift-prepulses clearly separated from the startle stimulus (80 ms shift duration, starting 130 ms before startle pulse) compared to shift-prepulses lasting until the start of the startle pulse. Dependent on timing and background frequency, different thresholds for

eliciting significant inhibition were found with lowest thresholds as small as ±2% around 8 kHz for shift-prepulses starting 130 ms before the startle pulse.
In summary, the prepulse inhibition paradigm optimized this way allows for a fast and reliable characterization of auditory discrimination and detection thresholds. Even small changes in background stimulation parameters can induce strong PPI. This can be applied to determine suprathreshold hearing deficits after acoustic trauma. In addition, these paradigms are well suited to investigate the neuronal basis of prepulse processing by combining behavioral with electrophysiological measurements.

Disclosures: **L. Koch:** None. **K. Cramer:** None. **T. Kuttner:** None. **B.H. Gaese:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.04/BB9

Topic: D.05. Auditory & Vestibular Systems

Support: NRF Grant funded by the Ministry of Science and ICT NRF-2021R1A2C3012159
ETRI Grant 22RB1100
ETRI Grant EA20230930

Title: Separation of task-relevant information encoding during auditory reversal learning in top-down projections from the mouse posterior parietal cortex.

Authors: *J. LEE, E. JUNG, S.-H. LEE;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Reversal learning tasks have been widely used in mammals to study cognitive flexibility, the ability to change one's behavioral response to different circumstances. The task involves subjects rapidly adapting to changes in stimulus-outcome or response-outcome contingencies and therefore requires both sensory and motor regions of the brain to work in tandem. Previous research has demonstrated the importance of the posterior parietal cortex (PPC), the auditory cortex (AC), and the inferior colliculus (IC) in auditory tasks. In this study, we examined how these three regions encode task-relevant information, such as auditory stimuli identity, contingency (stimulus-outcome association), reward, reward history, and licking behavior, at the level of individual neurons using a statistical approach based on generalized linear models. *In-vivo* single-unit recordings before, during, and after stimulus-contingency reversal revealed key differences between the three regions in both which and when different task variables are encoded. We next explored how these regions worked together by conducting *in-vivo* calcium imaging of two unique top-down projections from the PPC to the AC (PPC_{AC}) and the IC (PPC_{IC}). Using the same approach as above, we showed that PPC_{AC} neurons encode stimulus contingency and update reward history in the next trial, whereas PPC_{IC} neurons encode

"Go" stimuli and reward feedback, which may facilitate fast licking responses to the relevant stimuli. Taken together, our findings demonstrate that PPC plays a central role in flexible decision making, with cortico-cortical and cortico-collicular circuits to AC and IC, respectively, playing separate but crucial roles in encoding changes in stimulus-outcome associations.

Disclosures: **J. Lee:** None. **E. Jung:** None. **S. Lee:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.05/BB10

Topic: D.05. Auditory & Vestibular Systems

Title: Precise extraction of neural activity motifs encoding stimulus and choice information in mice primary auditory cortex

Authors: *L. XIANG, P. KANOLD, A. CHARLES;
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Abstract: Navigating and interacting with the external world requires that organisms process massive amounts of sensory information to guide behavioral choices. It has been hypothesized that cortical circuits communicate by generating stereotypical and recurring neural firing patterns, i.e., neural motifs, including synfire chains, sequences, packets, assemblies, etc. These motifs capture the dynamic flow of neural activities which may reflect how cortical circuits transform sensory information into behavior. The discovery of stimulus-evoked motifs in early sensory cortices has exposed the complex processing of sensory information by the neural circuits. However, it remains to be tested whether, in the sensory cortices, there exist stereotypical motifs encoding complex behavioral information such as choices. To test the hypothesis, we analyzed 2-photon calcium imaging (CI) data from A1 layer 2/3 of mice that were trained to perform a sound discrimination task (Francis et al., 2022) with an unsupervised motif discovery algorithm: SeqNMF (Mackevicius et al., 2019). We found that the multiplication updating rules in SeqNMF caused low temporal precision of motifs, preventing exact identification of motifs. We thus devised a new updating rule based on Split Bregman Iterations, which provided more precise temporal identification of motif occurrences. We show on simulated data that our adapted algorithm is robust to various noise conditions and could capture motifs when the motifs only occurred very few times (about 4) in the data. When applied to 10 sessions from the CI dataset from Francis et al. 2022, our approach was able to extract repeated neural activity patterns that only appear during certain stimuli frequency. Our method further detected motifs that are dominant when the animal made a specific decision. These results indicate that there do exist task-driven motifs in early auditory cortex. Moreover, these motifs overlap in their neural representation, indicating the reuse of neurons across contexts.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.06/BB11

Topic: D.05. Auditory & Vestibular Systems

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Mice Exhibit an Intralaminar Thalamic Awareness Potential

Authors: *S. H. MCGILL[1], M. GUHA[2], C. W. ZHAO[4], L.-A. SIEU[2], T. NGUYEN[2], Q. PERRENOUD[3], J. A. CARDIN[3], H. BLUMENFELD[2];
[1]Yale Univ., New Haven, CT; [2]Neurol., [3]Neurosci., Yale Univ. Sch. of Med., New Haven, CT; [4]Massachusetts Gen. Hosp., Boston, MA

Abstract: Animals from mice to humans live within complex worlds profuse with detail. Amidst this sensory expanse, at any moment any feature may be essential for them to note and consider to generate useful behaviors. However, only some of the stimuli transduced and processed by the nervous system can enter conscious perception at once. Studies conducted by our lab have demonstrated that perceived stimuli evoke a rapid, stereotyped series of cortical and subcortical responses in human subjects that identical unperceived stimuli do not. We hypothesize that these responses determine which stimuli enter conscious perception. Among these responses is the thalamic awareness potential (TAP), a biphasic event related potential found in the intralaminar thalamus. This analog has not yet been identified within mice. Here we report that the mouse intralaminar thalamus exhibits a response during auditory perception. Adult c57bl6 mice were surgically implanted with twisted-pair electrodes targeting the frontal association region, visual cortex, auditory cortex, and/or the central lateral nucleus of the thalamus. In the same procedure, steel headplates allowing for head fixation were applied to their skulls. The mice were then either subjected to a passive listening task or trained in a go/no-go auditory discrimination task. In the passive listening task, auditory stimuli of varying intensity were presented to the mice without training the mice to respond. In the go/no-go auditory discrimination task, the mice were conditioned to lick a spout in response to presentation of a stimulus and withhold licking otherwise. Access to the lickport was mechanically restricted to a one-second period following a delay after presentation of the auditory stimulus to eliminate the effect of motor correlates. In both tasks, measurements the local field potentials of target brain areas were made using the implanted electrodes. We observed a selective response in the local field potentials of the central lateral nuclei within 100ms of stimulus presentation. This response achieved statistical significance compared to both the baseline of the same condition and of the no-stimulus condition (p<0.05, cluster-based time course permutation test). Similar responses were also observed within the auditory cortices of the animals, and in the frontal association regions of the mice but not in the visual cortices. These preliminary results suggest that mice, like humans,

exhibit a thalamic awareness potential contingent upon perception and point to a modulatory role of the thalamus in regulating auditory perception.

Disclosures: **S.H. McGill:** None. **M. Guha:** None. **C.W. Zhao:** None. **L. Sieu:** None. **T. Nguyen:** None. **Q. Perrenoud:** None. **J.A. Cardin:** None. **H. Blumenfeld:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.07/BB12

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC017480

Title: Utilizing VNS to ameliorate auditory processing deficits in a rodent model of ASD

Authors: *B. M. WILLIAMS[1,2], T. DANAPHONGSE[2], S. KROON[1], Y. TAMAOKI[1,2], J. R. RILEY[2], C. T. ENGINEER[1,2];
[1]Neurosci., The Univ. of Texas at Dallas, Richardson, TX; [2]Texas Biomed. Device Ctr., Richardson, TX

Abstract: The comprehension and identification of human speech is dependent on the health of the auditory pathway. In neurodevelopmental disorders like autism spectrum disorder (ASD), developmental alterations to the auditory pathway can disrupt typical function - causing a cascade of processing errors. These physiological deficits in how sound is processed are often linked to poor performance in clinical evaluations of language. Modest improvements are accessible through extensive speech therapy, but many individuals still report deficits following treatment. To improve therapeutic outcomes, adjunctive therapies are needed. One potential adjunct to traditional speech therapy is vagus nerve stimulation (VNS). When paired with a sound, VNS drives plasticity in the auditory cortex, improving neural response strength and latency to the paired sound. Utilizing in-vivo multi-unit electrophysiology and go/no-go behavioral discrimination tasks, this study assesses the physiological and functional discrimination ability of rats prenatally exposed to valproic acid (VPA). A subset of VPA-exposed rats will receive sound-paired VNS to determine whether VNS driven improvements in auditory processing can overcome the physiological and behavioral deficits previously reported. Preliminary data suggests that despite having significantly weaker mean response to the 40ms onset of speech sounds in the anterior auditory field (AAF), VPA-exposed rats exhibit no behavioral deficit in discriminating these sounds. Furthermore, for VPA-exposed rats who received VNS, no significant behavioral changes were observed. Ongoing experiments include further characterizing the behavioral discrimination abilities in VPA-exposed rats, and using VNS-sound pairing to restore AAF responses to sounds. The results of this research will contribute towards the characterization of the VPA-exposure model of autism, and our understanding of the relationship between AAF physiology and behavioral sound discrimination.

This research is one step towards our long-term goal of developing novel interventions which improve sound processing and language ability for individuals with neurodevelopmental disorders.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.08/BB13

Topic: D.05. Auditory & Vestibular Systems

Support: NIDCD Grant T32DC00046

Title: Subcortical contributions to auditory perceptual learning

Authors: *R. YING[1], D. STOLZBERG[1], M. L. CARAS[2];
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Abstract: Sensory perception is highly dynamic, capable of both rapid context-dependent shifts, as well as slower changes that emerge over time with extended training. Previous research has shown that these perceptual fluctuations are driven by corresponding changes in the sensitivity of auditory cortical neurons to sound. However, it is unclear whether these changes emerge in the ascending auditory pathway and are inherited by the auditory cortex, or arise in the cortex de novo. As a first step towards answering this question, we implanted Mongolian gerbils with chronic microelectrode arrays in either the central nucleus of the inferior colliculus (CIC) or the ventral medial geniculate nucleus (vMGN). We recorded single- and multi-unit activity as animals trained and improved on an aversive go/no-go amplitude modulation (AM) detection task, and during passive exposure to the same AM sounds. AM-evoked firing rates and vector strengths were calculated and transformed into the signal detection metric d'. Neural thresholds were obtained for each training day by fitting d' values across AM depths and determining the depth at which d' = 1. Thresholds were compared between periods of task performance and passive sound exposure across several days of training to determine whether there were learning-related and/or context-dependent changes in activity. Neural thresholds obtained from CIC and vMGN during task performance and during passive sound exposure improved across days of training, suggesting that both regions display learning-related plasticity independent of context. While both regions also exhibited a context-dependent change in coding strategy, such that AM stimuli were better encoded by vector strength during passive exposure and by firing rate during task performance, only the vMGN exhibited lower (better) AM thresholds during the task context. These findings raise the possibility that extended perceptual training improves neural sensitivity by acting at or below the level of the auditory midbrain, whereas rapid, context-

dependent sensitivity enhancements first emerge in the auditory thalamus. Our results contribute to a deeper understanding of the circuits supporting perceptual flexibility, and may ultimately inform strategies for improving sound perception in hearing-impaired individuals.

Disclosures: R. Ying: None. **D. Stolzberg:** None. **M.L. Caras:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.09/BB14

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01 DC018650
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NSF CAREER 2145247
Kavli NDI Distinguished Fellows Program

Title: Rapid emergence of latent knowledge in the sensory cortex drives learning

Authors: *C. DRIEU[1,2], Z. ZHU[1], Z. WANG[1], K. FULLER[1], A. WANG[1], S. ELNOZAHY[1,3], K. KUCHIBHOTLA[1,2];
[1]Johns Hopkins Univ., Baltimore, MD; [2]Johns Hopkins Kavli Neurosci. Discovery Inst., Baltimore, MD; [3]Sainsbury Wellcome Ctr. for Neural Circuits and Behaviour, London, United Kingdom

Abstract: Goal-directed learning is traditionally considered a slow and gradual process. An alternative view suggests that animals, including humans, experience insightful moments with rapid, step-like changes during learning. Recent research reconciles these views, arguing that latent task knowledge can emerge rapidly even though behavioral performance improves gradually. The neural mechanisms that drive these parallel learning processes, however, remain unknown. The sensory cortex, given its brain-wide inputs from feedforward sensory, ascending neuromodulatory, and top-down frontal and motor regions, is a promising candidate for identifying these neural dynamics. Here, we trained mice on an auditory go/no-go task, employing optogenetic suppression and two-photon calcium imaging to investigate the role of the auditory cortex (AC) during learning. We found that optogenetic suppression during the stimulus or reward period respectively delayed learning. Complete trial suppression led to even greater delays. These effects waned during learning until vanishing when the animals reached expert performance, indicating a transient, associative, and instructional role for the AC. We then longitudinally tracked the same large excitatory network in layer II/III (n=4,643 neurons) using two-photon calcium imaging in mice performing the task (n=5) or listening passively to the same pure tones (n=3) over 15 days. Using unsupervised low-rank tensor decomposition, we identified distinct neural ensembles that encoded task contingencies, including reward prediction and behavioral inhibition. Remarkably, these contingency-related signals emerged within just 20-40

trials exhibiting ‘insight-like’ properties, strengthened during task acquisition, and eventually receded during extended training. The contingency-specific ensembles were organized into spatial domains separate from underlying stimulus representations, indicating a higher-order functional segregation within the AC. Therefore, latent task knowledge manifested early and rapidly in cortical networks in the form of contingency-related signals. These data challenge the classical view of goal-directed learning as slow and gradual and suggest the sensory cortex serves as an associative engine, directly linking stimuli with actions that produce desirable outcomes.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.10/BB16

Topic: D.05. Auditory & Vestibular Systems

Title: Project tiny dancer: mice can be trained to synchronize movements with auditory beat patterns

Authors: J. ROZELLS, C. JENSEN, S. KNUDSTRUP, ***J. GAVORNIK**;
Boston Univ., Boston, MA

Abstract: Humans can easily synchronize self-generated movements to match auditory beat patterns. This requires recognizing a beat, extracting its temporal structure, and integrating this temporal information into planned motor movements. It is unknown how the brain performs these tasks, and there is currently no good animal model in which to study and characterize the underlying circuitry, largely because most animals either can’t or won’t “dance.” With very limited exceptions (cockatoos, notably), animal models have resisted attempts to train them to “dance” and it is unknown whether this is because their brains lack required perceptual or sensory-integrative mechanisms or because they do not find the behavior intrinsically rewarding enough to demonstrate the desired behavior. Hypothesizing that the latter explanation is more likely correct, we developed a system to use optogenetic manipulation of the dopamine system to provide “rewards” when mice performed characteristic movements that randomly coincided with auditory beeps. Specifically, we expressed channelrhodopsin in dopaminergic cells in the VTA (ventral tegmental area) and used implanted light fibers to excite these cells when mice moved their bodies up/down within a “reward window” surrounding auditory events. We developed a real-time video analysis system to track animal location and body height, automatically triggering laser pulses when the desired behavior occurred. In an initial training period, both sound and laser signals were triggered by natural rearing movements as mice freely explored an open field. Animals rapidly shifted their behavior to increase the number of laser stimulations by spontaneously rearing more often, at which point we shifted to a training paradigm where the

laser was triggered only when rearing coincided with a temporally consistent beat pattern. By analyzing animal height as a function of time, we show that the temporal distribution of rearing events shifts with training to match the beat pattern and that animals successfully earn laser rewards at a higher rate than would be expected if their movements were random. These results demonstrate that mice are capable of synchronizing movements to auditory patterns and can be used as a model system to study the circuits responsible for this ability.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

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Topic: D.05. Auditory & Vestibular Systems

Support: RNID – International Project Grant
BrainsCAN Accelerator Grant

Title: Investigating the effect of hearing loss as a risk factor for Alzheimer's disease-related neuropathology and cognitive impairment in a rodent model

Authors: *S. V. PATEL, S. J. MYERS, A. L. SCHORMANS, L. AL MALOUF, S. H. HAYES, S. N. WHITEHEAD, B. L. ALLMAN;
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Abstract: Dementia affects 55 million people worldwide; a staggering statistic which highlights the need to limit its risk factors. Importantly, epidemiological studies have reported that hearing loss significantly increases the risk of developing dementia, including Alzheimer's disease (AD). At present, however, the mechanisms underlying this relationship are not well explored. To investigate the effect of noise-induced hearing loss on AD-related neuropathology and cognitive impairment, we utilized transgenic Fischer 344 (TgAPP) rats that overexpress pathogenic human amyloid precursor protein, but do not spontaneously develop β-amyloid plaques. At 12 months of age, adult male and female, TgAPP and wildtype, Fischer 344 rats were noise- (100 dB SPL; 4 hours/day; 30 days) or sham-exposed. Afterward, hippocampal cognitive function was assessed with the Morris water maze task and striatal-dependent visuomotor associative learning was tested using an operant conditioning-based task. Lastly, microglial and cholinergic function post-noise/sham exposure were examined using immunohistochemical analysis of Iba1 and choline acetyltransferase (ChAT) expression, respectively. Consistent with hearing loss often experienced by humans, our protocol resulted in high-frequency hearing loss in the rats. As expected, TgAPP rats showed impaired reference memory, increased Iba1 cell density in the hippocampus, and decreased ChAT cell density in the medial septum; however, in contrast to our prediction, noise exposure did not exacerbate these results. Similar to our previous studies, there was also no evidence of microglial activation, as assessed by a soma/size ratio, following noise

exposure. We did, however, observe a differential effect between the sexes and genotypes for how noise exposure affected visuomotor associative learning and striatal Iba1 cell density. While noise-exposed wildtype male rats exhibited a *faster* reaction time and *decreased* striatal Iba1 cell density compared to their sham-exposed counterparts, noise-exposed TgAPP males showed a significantly *slower* reaction time and *increased* striatal Iba1 cell density. Interestingly, despite these differential effects of noise exposure and genotype on microglia expression, the striatal Iba1 cell density did not predict the reaction time of rats in the visuomotor associative learning task, as assessed with regression analyses of the various experimental groups. Overall, our collective findings point to a complex relationship between noise-induced hearing loss, visuomotor associative learning, and genetic susceptibility to AD.

Disclosures: S.V. Patel: None. **S.J. Myers:** None. **A.L. Schormans:** None. **L. Al Malouf:** None. **S.H. Hayes:** None. **S.N. Whitehead:** None. **B.L. Allman:** None.

Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

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Program #/Poster #: PSTR343.12/BB18

Topic: D.05. Auditory & Vestibular Systems

Support: The Kady M. Gjessing and Rahna M. Davidson Distinguished Chair in Gerontology

Title: Voxelwise analysis of white matter in the central hearing pathway of senior dogs

Authors: C.-C. YANG, *N. OLBY;
North Carolina State Univ. Col. of Vet. Med., Raleigh, NC

Abstract: Presbycusis, or age-related hearing loss, is a common sensory impairment among the elderly population. It is primarily attributed to peripheral defects involving hair cells, the stria vascularis, and afferent spiral ganglion neurons. However, emerging evidence suggests that pathological changes in the central auditory system also contribute to presbycusis, which may develop independently of peripheral defects. Dogs also experience presbycusis as they age, negatively affecting their interaction with owners and cognitive status. While peripheral changes have been observed in dogs with presbycusis, there is limited research on central changes in dogs. Diffusion tensor imaging (DTI) can be used to detect and quantify white matter abnormalities in the brain. Various DTI scalars, including fractional anisotropy (FA), mean diffusivity (MD), axial diffusivity (AD), and radial diffusivity (RD), can be calculated from the DTI data and serve as indicators of different pathological changes. This prospective cross-sectional study aimed to investigate the DTI scalars of the central hearing pathway in senior dogs to enhance our understanding of presbycusis in dogs. Dogs aged above 75% of their expected lifespan were recruited for this study. In order to select dogs prior to development of severe hearing loss, brainstem auditory evoked response (BAER) was performed and only those with a

threshold ≤ 70 dB nHL were included. Fourteen dogs met the criteria and underwent scanning using a 3T magnetic resonance scanner. Tract-Based Spatial Statistics (TBSS) was utilized to examine age-related changes in DTI scalars. The streamlines connecting regions of the central hearing pathways (caudal colliculus, medial geniculate nucleus, and middle ectosylvian cortex) were extracted from whole-brain tractography and used as regions of interest in the TBSS. There was a significant negative correlation between FA and fractional lifespan ($p<0.05$) in the region connecting the medial geniculate nucleus and middle ectosylvian cortex. This finding suggests age-related white matter changes in the upper hearing pathway. These changes occur prior to the manifestation of severe hearing loss and may contribute to the development of central presbycusis. These findings underscore the importance of further studies to advance our knowledge of presbycusis in dogs.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

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Program #/Poster #: PSTR343.13/BB19

Topic: D.05. Auditory & Vestibular Systems

Support: P50 HD103536-7954
R21 EB033122

Title: Neurophysiological and neuropathological changes of auditory processing in a mouse knockout model of juvenile neuronal ceroid lipofuscinosis

Authors: *Y. DING[1], J. J. FOXE[2], V. PRIFTI[3], K. H. WANG[4], E. G. FREEDMAN[5]; [1]Neurosci., Univ. of Rochester Med. Ctr., Rochester, NY; [2]John Foxe, Univ. of Rochester, Bronx, NY; [3]Univ. Of Rochester, [4]Univ. of Rochester Med. Ctr., Univ. of Rochester, Rochester, NY; [5]Univ. of Rochester - Neurosci., Univ. of Rochester - Neurosci., Rochester, NY

Abstract: CLN3 disease is a prevalent form of Neuronal Ceroid Lipofuscinosis (NCL) due to mutations in the *CLN3* gene and characterized pathologically by intracellular accumulation of ceroid lipofuscin. Common CLN3 disease symptoms include early vision loss followed by cognitive decline. However, objective neurophysiological markers of disease progression and the underlying neural circuit mechanisms in the brain are not well established, in part because the early appearance of peripheral visual deficits precludes vision-based neurophysiological tests. Building on our pilot electroencephalography (EEG) measurements of auditory processing changes in CLN3 human subjects, we utilized a mouse knockout model (Cln3 -/-) to investigate neurophysiological deficits in auditory processing and the underlying cellular pathology in auditory pathways. With implanted high-density EEG electrode arrays, we repeatedly conducted auditory duration mismatch negativity (MMN) tests to probe auditory change detection and sound discrimination across ages. Our results show that wild-type (WT) mice displayed a robust

MMN response from 3 to 9 month of age. In contrast, Cln3-/- mice started with a normal MMN response at 3-month, then proceeded to a nearly complete absence of MMN response by 5-month. Additional recordings of auditory brainstem responses (ABR) confirmed that the robust reduction of MMN in Cln3-/- mice is not caused by peripheral hearing loss but of central origin. Interestingly, MMN responses reappeared in 7 to 9-month-old Cln3 -/- mice, while other waveform features of cortical auditory-evoked potentials remained different between WT and Cln3-/- mice, suggesting a compensatory process for MMN responses taking place in aged Cln3-/- mice. To further examine age-dependent progression of pathological changes, we used immunostaining and confocal microscopy to image the accumulation of Subunit C of Mitochondrial ATP Synthase (SCMAS), a common marker for ceroid lipofuscin, in auditory pathways. We found an early appearance of SCMAS+ ceroid lipofuscin in the auditory thalamus, particularly the reticular nucleus, followed by the auditory and frontal cortices. On the other hand, brainstem auditory areas were much less affected. Taken together, our studies reveal robust and age-related deficits in the central auditory processing pathways of Cln3-/- mice, which supports the use of auditory MMN as a clinically relevant neurophysiological biomarker for the progression of CLN3 disease and provides a foundation to uncover the underlying neuropathophysiological mechanisms.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

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Program #/Poster #: PSTR343.14/BB20

Topic: D.05. Auditory & Vestibular Systems

Support: NSF Award #2051105

Title: Subdermal Versus Epidural Long-Latency Auditory ERPs in Rats: A Validation Study

Authors: L. M. BREWER[1], R. J. RICCI[2], J. J. DAVIS[1], F. ANDRASIK[1], J. J. SABLE[2], *H. J. K. SABLE[1];
[1]Psychology, Univ. of Memphis, Memphis, TN; [2]Behavioral Sci., Christian Brothers Univ., Memphis, TN

Abstract: Event-related potentials (ERPs) are the summation of postsynaptic activity time locked to a stimulus which are recorded using electroencephalography (EEG). ERPs offer high temporal resolution and reflect complex activation of neuronal networks in relation to particular events or cognitive processes. Notably, they also provide a phenotypic measure that is analogous between humans and animal models, and thus, are well suited for studying mental processes and improving the validity of existing psychiatric animal models. Except for the measurement of auditory brainstem responses, ERP research in rodents has predominantly utilized relatively

invasive EEG recording procedures such as epidural electrodes or microarrays. Although these methods have the advantage of improved signal quality (e.g., lower impedance and less susceptibility to artifact contamination), they also have a higher risk of infection, injury, or loss of the animal. In line with the 3Rs of animal research, it is imperative researchers take steps to introduce or improve upon methods that reduce the discomfort or harm to research animals. Thus, the purpose of this study was to validate the use of less invasive subdermal methods for recording ERPs that we have previously utilized in our own work. Twenty adult, male Wistar rats were implanted with subdermal needle electrodes while anesthetized with continuous isoflurane. Once fully awake, they were then presented with a passive auditory paradigm consisting of a sequence of 5-tone trains with either 1- or 5-s inter-train intervals (ITIs) presented at a frequency of either 8 kHz (i.e., high) or 500 Hz (i.e., low). To assess temporal reliability, subdermal needle recordings were conducted in the same animals with 2-3 months between recordings. Approximately 1-2 weeks following the second round of subdermal needle recordings, rats underwent a craniotomy to implant three skull screw electrodes for invasive recordings using a placement similar to the needle recordings. Qualitatively, the ERP waveforms demonstrated the anticipated auditory P1-N1-P2-N2 complex, with definable peaks present for both the subdermal needle and screw electrode recordings. However, noticeable quantitative differences were present between these methods that should be considered when making comparisons across studies or when deciding which method to use. Overall, this work demonstrates the applicability of a semi-invasive approach for recording ERPs in awake rats that is in line with the principles of "refinement" and "reduction" as described by the 3Rs of animal research.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

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Program #/Poster #: PSTR343.15/BB21

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC012947

Title: Investigating the cellular and circuit mechanisms of EEG biomarkers associated with schizophrenia using a multiscale model of auditory thalamocortical circuits

Authors: *S. MCELROY[1], A. THIEME[3], P. GHOSH[3], J. CHEN[2], I. BERNARDI[4], E. GRIFFITH[5], S. NEYMOTIN[6], D. D'SOUZA[7], P. SKOSNIK[8], R. RADHAKRISHNAN[9], C. METZNEER[3], S. DURA-BERNAL[10];
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Haven, CT; [9]Yale Univ., New Haven, CT; [10]Physiol. and Pharmacol., SUNY Downstate, Brooklyn, NY

Abstract: Individuals with schizophrenia exhibit a deficit in sensory processing, which researchers have extensively investigated in primary auditory cortex (A1) using EEG techniques. These deficits manifest as abnormalities in event related potentials and cortical oscillations. To investigate these phenomena, we used our previously developed model of auditory thalamocortical circuits to reproduce schizophrenia-related EEG biomarkers. The A1 model simulates a cortical column with a depth of 2000 μm and 200 μm diameter, containing over 12k neurons and 30M synapses. Neuron densities, laminar locations, classes, morphology and biophysics, and connectivity at the long-range, local and dendritic scale were derived from published experimental data. Auditory stimulus-related inputs to thalamus were simulated using phenomenological models of the cochlear/auditory nerve and the inferior colliculus. The model reproduced in vivo cell type and layer-specific firing rates, local field potentials (LFPs) and electroencephalogram (EEG) signals. We leveraged this validated A1 model to gain insights into mechanisms responsible for observed EEG changes in schizophrenia patients. First, we looked at signal-to-noise ratio (SNR) in response to pure tones and auditory steady-state responses (ASSRs) in the gamma band range, examining the effect of 4 different schizophrenia-associated changes of the excitatory-inhibitory (E-I) balance: 1) reduced output of parvalbumine-positive (PV) interneurons, 2) reduced output of somatostatin-positive (SOM) interneurons, 3) increased GABAergic inhibitory postsynaptic current (IPSC) decay times, and 4) reduced glutamatergic activation of PV interneurons, due to N-methyl-D-aspartate (NMDA) receptor hypofunction. We found that except for reduced PV output, all parameter changes, and combinations of changes, reduced the SNR in superficial excitatory cells, in line with previous findings. Currently, we are employing the model to elucidate the roles of NMDA receptors and SOM interneurons in modulating EEG-measured ASSR, and to characterize the impact of CB1 and GABA receptor modifications on stimulus-specific adaptation (SSA) and EEG responses. This work aims to shed light on the cellular and circuit mechanisms underlying EEG changes in schizophrenia.

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Poster

PSTR343. Auditory Processing-Perception, Cognition, and Action I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR343.16/BB22

Topic: D.05. Auditory & Vestibular Systems

Support: KAKENHI 21H05176
AMED 17dm0207004h0004

Title: Differential effects of ketamine on gamma oscillations in the frontal and temporal cortices in awake monkey

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Abstract: Gamma oscillation has attracted attention for its relationship with cognition and neuropsychiatric disorders. Recent studies have revealed that altered gamma oscillation in patients with psychiatric disorders and wide spread of gamma oscillation is observed in the several regions of the brain. However, the reasons or mechanisms why such distribution of gamma oscillation are not well understood. Using high density electrocorticography (ECoG) examinations of both the auditory and frontal cortices in monkeys (n=3), we employed auditory steady-state response (ASSR), one of the most robust measures of evoked oscillations, to investigate the cortical distribution and vulnerability to low-dose administration of ketamine, an NMDA receptor antagonist that induces psychotic symptoms in humans. We found that ketamine reduces gamma-band oscillatory responses in the temporal cortex, but increases them in the lateral prefrontal cortex. These opposite results reveal a differential cortical profile of oscillatory responses in the monkey, implying that the local circuits that generates gamma oscillation differ in components according to the cortical area involved.

Disclosures: **M. Tada:** None. **K. Kasai:** None. **T. Uka:** None.

Poster

PSTR344. Subcortical Visual Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR344.01/BB23

Topic: D.06. Vision

Support: NIH Grant EY033528
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Title: Retinal input is required for the maintenance of neuronal laminae in the ventral lateral geniculate nucleus

Authors: *K. STEBBINS[1], R. SOMAIYA[2], U. SABBAGH[3], Y. LIANG[1], J. SU[1], M. A. FOX[1];
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Abstract: In the visual system, retinal ganglion cell (RGC) axons provide direct input into visual thalamus. Two major retinorecipient regions innervated by retinal axons are the dorsal lateral geniculate nucleus (dLGN), which is important for classic image-forming vision, and the ventral lateral geniculate nucleus (vLGN), which is associated with non-image-forming vision. Through both activity-dependent and morphogen-dependent mechanisms, retinal inputs play important

roles in the development of cells and circuits in dLGN, including the refinement of retinal projections, morphological development of thalamocortical relay cells, and migration of inhibitory interneurons from progenitor zones. In vLGN, retinal inputs are known to direct the recruitment of inhibitory interneurons, but their role in the formation and maintenance of cell-specific laminae remains unknown. Grossly, vLGN is divided into retinorecipient external vLGN (vLGNe) and non-retinorecipient internal vLGN (vLGNi). We previously found that vLGNe consists of transcriptionally distinct subtypes of GABAergic cells that are distributed into at least four adjacent laminae. In this study, we elucidated the developmental timeline for the formation and maintenance of GABAergic laminae in the mouse vLGNe. Our results indicate that these subtype-specific laminae in the neonatal vLGN are specified before the emergence of experience-dependent visual activity. We observed that mutant mice without retinal inputs have vLGNe with a laminar distribution of GABAergic cells at birth, suggesting that they do not depend on RGC axons for their formation. However, after the first week of postnatal development, these mutants exhibited a dramatic disruption in the laminar organization of inhibitory neurons and the separation of vLGNe and vLGNi. Taken together, our results show that the subtype-specific laminar distribution of retinorecipient cells in vLGNe is determined during embryonic development. While their formation does not depend on retinal inputs, retinal signals are critical for the maintenance of these GABAergic laminae.

Disclosures: **K. Stebbins:** None. **R. Somaiya:** None. **U. Sabbagh:** None. **Y. Liang:** None. **J. Su:** None. **M.A. Fox:** None.

Poster

PSTR344. Subcortical Visual Circuits

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Topic: D.06. Vision

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Title: Encoding visual stimuli by striatal neurons

Authors: *J. PÉREZ-BECERRA, L. CARRILLO-REID;
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Abstract: The interaction between cortical areas and basal ganglia structures is essential for perceptual integration. The striatum is the main input of the basal ganglia, and it has been recently shown that its dorsomedial region (DMS) responds to visual stimuli. In mice, neurons

from primary visual cortex (V1) send direct projections to DMS. It has been shown that such cortico-striatal interaction is necessary for visual discrimination tasks. However, it is still unknown if different features of visual stimuli are represented in the striatum. It has been described that groups of neurons in V1 encode visual-oriented drifting-gratings; nevertheless, it has not been reported if DMS preserves such code. We aimed to investigate how visual-oriented drifting-gratings are represented in the electrical activity of DMS neurons during stationary and movement states. We performed single-unit activity recordings in DMS populations with tetrode arrays in awake mice during stationary and movement trials. We calculated the Orientation Selectivity Index (OSI) for DMS neurons responding to 4 different oriented drifting-gratings (head-fixed). In stationary trials, we observed a high proportion of DMS neurons that show responsiveness to visual stimuli (~60%), but just a subpopulation of neurons (15%) that showed a high OSI>0.5. Interestingly, orientation coding is preferentially represented in putative medium spiny neurons (MSNs) but not in putative Fast spiking interneurons. In trials in which visual stimuli and mice movement were both present, visual evoked activity in MSNs increased, as well as the OSI. Finally, demixed Principal Component Analysis (dPCA) showed that population coding for the orientation of visual stimuli is preserved in stationary and movement states. We conclude that DMS populations encode visual-oriented drifting-gratings during stationary and movement states, suggesting that the basal ganglia have a fundamental role in the formation of visual percepts.

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Poster

PSTR344. Subcortical Visual Circuits

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR344.03/BB25

Topic: D.06. Vision

Support: Research grant by Dopavision GmbH

Title: Regional blue light stimulation of the blind spot gains access to pupillomotor control in the human brainstem

Authors: P. ADHIKARI[1], T. SCHILLING[2], B. FEIGL[1], H. BAHMANI[2], A. J. ZELE[1], ***J. ELLRICH**[3,2];
[1]Queensland Univ. of Technol., Brisbane, Australia; [2]Dopavision GmbH, Berlin, Germany; [3]Friedrich-Alexander-University, Erlangen, Germany

Abstract: Melanopsin is expressed by axons of intrinsically photosensitive retinal ganglion cells (ipRGC) that pass through the optic disc, corresponding to the blind spot. Blue light stimulation of the blind spot has been demonstrated to activate melanopsin and to modulate retinal function by retrograde signaling in ipRGC axons. Besides the centrifugal pathway to the retina, an afferent impact of blind spot stimulation on the brainstem is hypothesized. The experimental

study investigated the potential modulation of the pupillary light response (PLR) by blue light stimulation of the blind spot in humans. Five stimulus variations including the central, superior, inferior, nasal, and temporal regions of the blind spot were applied in six healthy volunteers. In all experiments, a 2.2° radius, 1 s, 10 Hz, 464 nm blue light stimulus of 13.4 log quanta.cm^{-2}.s^{-1} corneal irradiance was used. An irradiance-matched 630 nm central red light stimulus served as control. To desensitize any PLR evoked by rod stimulation due to inadvertent intraocular scatter, the effect of a constant, low-irradiance, 508 nm cyan adapting background was evaluated. PLR recording and analysis were performed under strict fixation monitoring and in accordance with the 'Standards in Pupillography'. All regional blind spot stimulations evoked measurable pupil responses. A larger PLR was produced with both the blue and red central stimuli without the cyan background. With the cyan background, the peak PLR amplitude evoked by the central blind spot stimulation was 21.0±3.4% (mean±SD) for blue light and 8.2±4.8% for red light (paired t-test, $t=9.4$, $p<0.001$). With blue stimulation, the peak PLR amplitude was 18.7±4.5% for superior, 20.5±4.5% for inferior, 17.7±3.9% for nasal, and 22.2±4.4% for temporal blind spot stimulation. Across the stimulus positions, the variation in the peak PLR amplitude was up to 4.6% but was not significant (RM-ANOVA, $F=2.8$, $p<0.1$). Blue light stimulation of the blind spot consistently evoked a PLR in all volunteers. Inclusion of a cyan background, to which rods are maximally sensitive, minimizes the effect of intraocular scatter and resultant rod intrusions to access melanopsin contributions to the PLR with the blind spot stimulation. Blue light stimulation of the cardinal blind spot regions evoked slightly different PLR amplitudes indicating subtle regional variations in melanopsin photopigment density across the blind spot, especially in horizontal direction. The experimental data indicate that blue light stimulation of the blind spot gains access to pupillomotor control in the human brainstem.

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Poster

PSTR344. Subcortical Visual Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR344.04/CC1

Topic: D.06. Vision

Support: R01-EY023591
R01-EY023581
R01-EY023603
R01-EY023603-S1
P30-EY003039
AFOSR-2020-0002

Title: Photoreceptor resolved temporal contrast encoding in LGN neurons during ongoing stimulation

Authors: *K. RAMSEY[1,2], P. TELLERS[1], C. NYANKERH[1], A. MEADWAY[1], L. SINCICH[1]; [2]Neuroengineering Program, [1]Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: The response properties of neurons in the lateral geniculate nucleus (LGN) are typically studied with large full-field stimuli that do not probe vision at the scale of cone photoreceptors or in the presence of other stimuli. To understand how LGN cells respond to stimuli covering small portions of the receptive field during ongoing stimulation, we examined spike activity changes resulting from contrast steps occurring in fine-grained noise stimuli. We recorded extracellularly from 29 LGN cells in 2 anesthetized macaques with receptive fields located 0.5°-4° from the fovea. An adaptive optics scanning laser ophthalmoscope was used with an infrared (842±25 nm) channel to image the retina, while red (711±12 nm) and green (543±11 nm) channels delivered binarized white noise movies. Each color was modulated independently over a 0.32° stimulus field. A movie pixel subtended 0.6 arcmin (~3 μm), just smaller than a single cone photoreceptor. Spike-triggered averaging was used to map receptive fields and classify cells by color and ON or OFF preference. To examine response characteristics, we determined a cell's baseline average spike probability across all stimulus movie frames. We then computed spike probability changes during specific conditions for the pixel located at the receptive field center's peak. Conditions included single frame stimuli (ON or OFF), temporal contrast stimuli (ON-to-OFF or OFF-to-ON), and recovery in the subsequent frame for all these scenarios. Of the cells recorded, 13 OFF and 7 ON cells showed a change in spike probability from baseline. In these cases, the single frame pattern of the preferred stimulus increased the mean spike probability by 18% (±13% SD). Notably, for both cell types the single frame patterns led to spike probability changes that were equal in magnitude, but opposite in polarity, for preferred and non-preferred stimuli. Temporal contrast patterns produced higher changes in spike probability than single frame patterns, but differed in magnitude for preferred (28±19%) versus non-preferred (24±17%) stimuli. During the recovery frame, spike probability also differed from baseline with equal magnitude for the single frame condition regardless of preference (6.5±5.9%), while the temporal contrast conditions changed probability differentially (5.9±8.2% for preferred, 1.1±5.8% for non-preferred). Our results suggest that single frame conditions lead to symmetric changes in spike rates in ON and OFF conditions, while temporal contrast patterns drive asymmetric responses weighted toward preferred stimuli. Thus, contrast encoding in time shows a history dependence detectable even at a cone resolved level in LGN neurons.

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Poster

PSTR344. Subcortical Visual Circuits

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Program #/Poster #: PSTR344.05/CC2

Topic: D.06. Vision

Support: R01NS109978
F31EY033691

Title: Thalamus dynamically adjusts visual responses to context

Authors: *K. PEELMAN, B. HAIDER;
Emory Univ. & Georgia Inst. of Technol., Atlanta, GA

Abstract: The physical context of an environment shapes and constrains perception and behavior. It is well-known that different behaviors in mice modulate several aspects of visual processing, likely due to changes in arousal. While many studies measure these effects of arousal on visual neural responses in contexts that permit locomotion, others do not. It is thus not known how physical context itself may drive changes in neural processing of the same stimuli. Even when brain state and arousal are identical, does physical context afford distinct brain dynamics that appropriately sculpt visual processing? We addressed this by recording in awake head-fixed mice exposed to two different physical contexts: sitting stationary in a tube (N=9) or free to run on a circular treadmill (N=5). We measured local field potential (LFP) in visual cortex simultaneously with single unit activity in dLGN using Neuropixels. We used low frequency LFP power to categorize arousal level, and also quantified changes in pupil size and facial movements. Black or white sparse noise stimuli (7-degree squares) were shown in one visual hemifield. We examined single unit spatiotemporal receptive fields as a function of pre-stimulus arousal level, movement level, and physical context. We found that even when examining trials with identical arousal levels, physical context had unique consequences for visual processing. In the wheel context, spontaneous activity was elevated, and evoked responses were faster than in the tube context, but at the cost of reduced signal-to noise in the receptive field, with no change in receptive field size. We next examined how changes in arousal exerted effects within each context. In the tube, mice could not run but as arousal spontaneously increased, so did spontaneous firing rates ($r=0.90$, $p<0.05$), while baseline-subtracted visual response amplitude remained unchanged. However, in the wheel context, baseline-subtracted visual response amplitude increased with arousal in the absence of running ($r = 0.90$, $p=<0.05$). On trials with running, spontaneous firing rates strongly increased with arousal ($r=0.98$, $p<0.01$) which leads to diminished baseline-subtracted visual response amplitude with increasing arousal during running ($r=-0.97$, $p<0.01$). Taken together, these results show that even during moments where arousal level is identical, physical context changes the relationship between arousal and the modulation of sensory responses. Physical context may thus inherently constrain the way arousal shapes the dynamic range of visual response properties in the early visual system.

Disclosures: K. Peelman: None. **B. Haider:** None.

Poster

PSTR344. Subcortical Visual Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR344.06/CC3

Topic: D.06. Vision

Support: NIH grant DP2MH125812
Institutional Startup support provided by the University of Maryland

Title: Activity-dependent synapse clustering contributes to eye-specific refinement in the mouse

Authors: *C. ZHANG[1], C. SPEER[2];
[1]Dept. of Biol., Univ. of Maryland, Col. Park, College Park, MD; [2]Dept. of Biol., Univ. of Maryland, College Park, MD

Abstract: The development of synaptic connections in the dorsal lateral geniculate nucleus (dLGN) of the thalamus has been studied as a model for activity-dependent synaptic plasticity. During the first postnatal week in mice, retinogeniculate inputs from both eyes initially intermix and then segregate into distinct eye-specific regions. The underlying mechanisms by which neural activity influences the stabilization of axons and synapses from the same eye and the elimination of inputs from the opposite eye remain unclear. To explore the relationship between synapse maturation and eye-specific axon development, we applied volumetric STochastic Optical Reconstruction Microscopy (STORM) together with synaptic and axonal labeling techniques in wild-type and mutant mice with disrupted cholinergic retinal waves during the first postnatal week. Within the projections from each eye, we identified a subset of complex synapses characterized by larger vesicle pools and multiple active zones (AZs) which provided foundation for local synapse clustering and competitive refinement. Eye-specific synaptic clustering was distance-dependent and complex synapses from both eyes helped to increase the formation of nearby synapses from the like-type eye. During synaptic competition, dominant-eye complex synapses in the correct future eye-specific territory eliminated synaptic clustering around complex synapses from the non-dominant-eye. In mutant mice with abnormal cholinergic retinal waves, complex synapses from both eyes were still present, but distance-dependent eye-specific stabilization and elimination effects were disrupted. Our findings demonstrate the early onset of retinogeniculate synaptic clustering prior to eye-opening and provide super-resolution anatomical evidence consistent with non-cell-autonomous synaptic stabilization and punishment signals underlying eye-specific competition.

Disclosures: C. Zhang: None. **C. Speer:** None.

Poster

PSTR344. Subcortical Visual Circuits

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Topic: D.06. Vision

Support: NIH Grant 5R01EY009593

Title: Comparing visual responses in the thalamic reticular nucleus versus dorsal lateral geniculate nucleus of mouse

Authors: *Y. MIAO[1], U. M. CIFTCIOGLU[2], A. GORIN[3], S. AHN[2], J. A. HIRSCH[1];
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Abstract: All visual information that the dorsal lateral geniculate nucleus (dLGN) transmits from retina to cortex is processed by two separate sources of inhibition: local interneurons in dLGN and cells in the thalamic reticular nucleus (TRN), a network of GABAergic neurons that forms reciprocal connections with primary sensory nuclei across modalities. Unlike local interneurons, whose strongest synaptic drive derives from retina, visual TRN receives its dominant input from dLGN. Historically, TRN was considered a source of non-selective gain control to the thalamus, as formalized by Francis Crick's "thermostat" hypothesis. However, increasing evidence supports Crick's alternative "searchlight" hypothesis-that TRN contributes to spatial attention. This idea suggests that TRN supplies focal and feature-selective inhibition to dLGN. Our previous studies in carnivore showed that visual receptive fields of TRN are, indeed, localized and have similar sizes to those of dLGN. To determine if this finding is conserved across species and to explore further the complexity of TRN visual properties, we adopted a comparative approach and conducted companion experiments in mouse. For our initial studies, we made *in vivo* electrophysiological recordings from optogenetically identified cells in murine TRN during the presentation of sparse and dense visual noise. As for carnivore, the spatial scales of receptive fields in murine TRN were similar to those in dLGN. Here, we extended our initial work by sampling TRN cells across the visual field to address regional bias. We then quantified the neural preference for bright and/or dark stimuli using an index we developed for this purpose; luminance preference in TRN was as diverse as in dLGN. Further, we compared receptive field shapes qualitatively; while almost half of relay cells in dLGN have receptive fields with a stereotyped center-surround profile, neurons in TRN typically had irregular and patchy receptive fields, presumably reflecting convergent input from different types of relay cells. To determine if TRN captures how responses of relay cells change with varying stimulus conditions, we displayed a constant visual stimulus on backgrounds of different light intensities. These manipulations had robust effects on the envelopes of responses in both TRN and dLGN, suggesting that feedback inhibition is continuously updated by dLGN. These results contribute to our ongoing studies of how inhibition from TRN and local interneurons reshapes the signals that travel from the periphery to cortex.

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Poster

PSTR344. Subcortical Visual Circuits

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Program #/Poster #: PSTR344.08/CC5

Topic: D.06. Vision

Support: NIH Grant EY013588

Title: Oscillation Dynamics and Visual Processing: Insights from LGN:V1 Network Analysis.

Authors: *H. J. ALITTO[1], A. N. SANCHEZ[2], W. M. USREY[1,2];
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Abstract: Visual information is conveyed from the retina to the primary visual cortex (V1) through the lateral geniculate nucleus (LGN) in the thalamus. In the process of relaying signals to the cortex, the LGN dynamically regulates signal transmission based on the characteristics of the visual stimulus and the current behavioral state. Oscillations within specific frequency bands, which can vary depending on the species, presence of anesthesia, and arousal level, have been observed in the LGN-V1 network. These oscillations are thought to play a significant role in the transmission of retinal signals to V1.
To investigate the influence of oscillations on visual processing in the LGN, we analyzed a large dataset of paired LGN and V1 recordings obtained from animals engaged in a passive fixation with visual stimulation or a more active task involving spatial attention. Consistent with our previous findings, there were pronounced oscillations in the alpha-beta range that exhibited coherence between the LGN and V1. However, despite this coherence, the alpha-beta oscillations had minimal to no impact on LGN spiking activity, as assessed with LGN spike-LGN field pairwise phase consistency. Furthermore, the phase consistency of oscillations was coherent across retinotopically distant recording sites. Although oscillation magnitude increased as arousal levels decreased, as evidenced by changes in pupil size, oscillations were not modulated by the strength of the visual signal.
Based on these results, we conclude that alpha-beta oscillations are unlikely to exert a significant influence on the transmission of visual information to the cortex under the conditions in which our data was recorded. We hypothesize that these oscillations may increase in strength and coherence as behavioral arousal further decreases, perhaps acting in part to disconnect the cortex from the periphery during periods of quiescence.

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Poster

PSTR344. Subcortical Visual Circuits

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Program #/Poster #: PSTR344.09/CC6

Topic: D.06. Vision

Title: Ultra-high resolution images improve modeling of neural activity evoked by natural scenes

Authors: *N. MÜLLER, I. I. A. GROEN, H. S. SCHOLTE;
Univ. of Amsterdam, Amsterdam, Netherlands

Abstract: The vast majority of studies investigating early processing of information during visual scene perception uses compressed, limited-resolution images (e.g, < 500 pixels) both when measuring and when modeling human brain responses.
The human retina is capable of processing visual inputs at several times higher resolution than used in these typical lab settings. To better approximate real-world visual processing, we here use uncompressed, ultra-high resolution images (20 megapixels) in combination with precortical models of early visual processing and assess how image resolution affects downstream neural processing and the ability of these models to predict neural activity.
We collected EEG data from 28 participants while they were rapidly presented with high-resolution natural scenes (3 megapixels). We compare cross-validated explained variance (R2) between models of early visual processing in predicting ERP amplitude at occipital channels using a linear encoding model per subject, electrode and time point.
First, we systematically varied the image resolution of the input to the models and observed a number of changes in encoding performance by a model of summary statistics of local receptive field outputs. When matching the image resolution to that of previous work (Groen et al. 2013; 640x480 pixels) we find similar levels of explained variance (average R2 = 0.23 at Iz, t = 0.132 s). When matching the model's input resolution with that of the images shown to participants in the current experiment, explained variance increases (**R2 = 0.34** at Iz, t = 0.136 s). Drastically decreasing the input resolution of the model (10-, 20-, 40-fold) on the contrary results in a monotonic decrease in explained variance (range R2 = [0.08, 0.19] at Iz, t = 0.132 s). Second, we compare these model fits to a model that uses retinal ganglion cell sampling (GCS) prior to the computation of summary statistics. We find that this model exhibits higher explained variance across all reduced image resolution conditions (range **R2 = [0.14, 0.28]** at Iz, t = 0.136).
To summarize, we demonstrate an effect of decreasing resolution on the explainability of human EEG data using models of precortical visual processing. Moreover, we provide evidence that image sampling based on the distribution of retinal ganglion cells in the human retina leads to increased explainability of human EEG data as compared to previous models. Overall, these results highlight a benefit of using ultra-high resolution input for modeling neural responses to natural scenes.

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Poster

PSTR344. Subcortical Visual Circuits

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Topic: D.06. Vision

Support: NIMH MH002964
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NIMH MH002952

Title: The role of the vLGN in non-image-forming visual behaviors and its underlying circuit organization

Authors: *C. BEIER[1], Z. ZHANG[1], M. YURGEL[2], M. THURMAN[4], S. HATTAR[3]; [1]Natl. Inst. of Mental Hlth., Bethesda, MD; [2]Natl. Inst. of Mental Hlth., Rockville, MD; [3]Section on Light and Circadian Rhythms, Natl. Inst. of Mental Hlth., Bethesda, MD; [4]Rockefeller Univ., New York, NY

Abstract: The mouse ventral lateral geniculate nucleus (vLGN) receives dense retinal ganglion cell (RGC) innervation but its role in vision remains unclear. We recently showed that the caudal vLGN segregates the visual input of RGCs that drive image-forming vision from the intrinsically photosensitive RGCs (ipRGCs) that drive non-image-forming behaviors. Here, in the context of this newly understood circuit organization, we investigate cell type-specific retina-to-vLGN pathways that drive and modulate non-image-forming behaviors (such as the pupillary light response, circadian photoentrainment, and light-induced sleep). Immunohistochemistry of *cFos* induction was paired with markers for vLGN cell types (*Penk* and *Nos1*) following circadian phase shifting light pulses (phase delay, ZT14, or phase advance, ZT22). We mapped afferents of vLGN $Penk^{Cre}$ and $Nos1^{Cre}$ cell types with Cre-dependent helper viruses and monosynaptic G-deleted rabies virus. Calcium imaging of Penk cells in vivo in response to light at different times of revealed a circadian-time dependent light response within the vLGN. We used viral chemogenetic strategies to activate (Gq DREADD) vLGN $Penk^{Cre}$ cells to investigate their role in modulating pupil constriction. *cFos* induction in the vLGN increases in *Penk* cells in response to a ZT22 light pulse but not to a ZT14 light pulse compared to dark controls. Light-induced *cFos* in *Nos1* cells is not time-dependent. $Penk^{Cre}$, but not $Nos1^{Cre}$, cells in the vLGN are post-synaptic to non-image-forming retinal ganglion cells. Activating vLGN $Penk^{Cre}$, but not $Nos1^{Cre}$, cells causes a deficit in pupil constriction during a light step. We find that *Penk* vLGN cells are light-responsive in a circadian time-dependent manner. In contrast, vLGN *Nos1* cells do not share these same features. In agreement with these findings, we show that vLGN *Penk* cells play a role in driving the pupillary light response during light steps.

Disclosures: C. Beier: None. **Z. Zhang:** None. **M. Yurgel:** None. **M. Thurman:** None. **S. Hattar:** None.

Poster

PSTR344. Subcortical Visual Circuits

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Program #/Poster #: PSTR344.11/CC8

Topic: D.06. Vision

Support: NIH U01NS122040

Title: The Synaptic Circuitry in Konio vs. Magno/Parvo-Input Recipient Laminae of the Tree Shrew LGN are Morphologically Distinct

Authors: *F. SCIACCOTTA[1,2], S. ZHANG[1,2], S. HOLTON[1,2], A. ERISIR[2];
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Abstract: In the primate visual system, 3 functionally distinct pathways extend from retina to thalamus: magnocellular (M), parvocellular (P), and koniocellular (K). A pre-primate species, tree shrew (Tupaia belangeri) is an advantageous model to study K-pathway in isolation because, while M- and P-pathways mix in laminae (L)1, 2, 4 and 5 of the lateral geniculate nucleus (LGN), L3 and L6 only receive K-axons. We used 3 datasets to reveal how synaptic circuitries in K vs. M/P-input laminae differ: 1) all synaptic terminals at the EM level, using mitochondria as a criteria to classify retinal and non-retinal inputs, 2) synaptic terminals at the EM level collected from tracer-injected or VGlut2-labeled tissue, and 3) volumetric renderings of retinal injection tissue dually labeled with VGlut2 at the confocal level. In each, retinal terminals in either both or one of the K laminae were significantly smaller compared to M/P-input laminae. Furthermore, L6 lacked substantial input from cortex, suggesting that M/P and each K laminae may contain distinct circuitries. Additionally, analysis of non-retinal terminal areas revealed that there were larger-sized boutons containing dark mitochondria in K laminae L3 and L6 vs. M/P lamina L1. To reveal how SC boutons contribute to these differences, we examined anterogradely labeled tectogeniculate (TG) terminals and found that they range in size, including a subpopulation of large-sized terminals that made perforated synapses onto multiple postsynaptic targets, similar to that of retinal terminals. Given the morphometric resemblance between TG, retinal, and VGlut2+ boutons, we then investigated if TG axons also utilize VGlut2 by creating 3D-renderings from confocal z-stacks of tracer-injected tissue. We found that the ratio of VGluT2+ terminals that colocalized with the tracer injected into the eye was not significantly different across M/P versus K-recipient laminae, providing evidence against the presence of VGluT2 in TG boutons. Together, these results provide evidence that the synaptic circuitry in K-laminae of the tree shrew LGN is morphologically distinct and that TG terminals constitute the larger-sized, VGluT2- boutons containing dark mitochondria in these laminae.

Disclosures: F. Sciaccotta: None. **S. Zhang:** None. **S. Holton:** None. **A. Erisir:** None.

Poster

PSTR344. Subcortical Visual Circuits

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Program #/Poster #: PSTR344.12/CC9

Topic: D.06. Vision

Support: NIH Grant EY030507
Research to Prevent Blindness/The Glaucoma Foundation Career Advancement Award

Title: Strengthening of feed-forward inhibitory synapses in the visual thalamus in a mouse model of inherited glaucoma

Authors: *M. VAN HOOK, J. C. SMITH;
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Abstract: Elevated intraocular pressure (IOP) in glaucoma damages retinal ganglion cells (RGCs), leading to dysfunction and degeneration of excitatory retinogeniculate (RG) output synapses in the dorsolateral geniculate nucleus (dLGN). It is unclear whether high IOP impacts the dLGN network beyond RG synapses. To test this, we used a mouse model of inherited glaucoma (DBA/2J; D2) to determine whether glaucoma impacts the function of feed-forward inhibitory synapses in the dLGN that arise from local inhibitory interneurons. For these experiments, we used D2 mice with elevated IOP, measured monthly with a rebound tonometer, while controls were a strain-matched line (DBA/2J-gpnmb+; D2c) that do not develop high IOP. Mice of both sexes were used, and ex vivo slice electrophysiology experiments were performed at 10-12 months of age. In voltage clamp recordings from dLGN TC neurons, optic tract stimulation evoked excitatory post-synaptic currents (EPSCs) that had smaller amplitude in slices from D2 mice (612$\pm$210 pA; mean+SEM; n = 10 cells, 3 mice) relative to D2c (1569$\pm$190 pA; n=13 cells, 6 mice; p=0.001, nested t-test). EPSCs from D2 mice showed a higher paired pulse ratio (PPR; 200 ms interval), consistent with reduced vesicle release probability. Feed-forward inhibitory post-synaptic currents (IPSCs), evoked by optic tract stimulation while voltage-clamping TC neurons at the cationic reversal potential, were not significantly different between D2 and D2c mice (D2: 588$\pm$125 pA; D2c 947$\pm$193 pA; p=0.082). Feed-forward IPSCs also showed a higher PPR, likely the result of altered short-term plasticity at RG synapses. When IPSC amplitude was normalized to the RG EPSC amplitude (IPSC/EPSC), the relative strength of feed-forward inhibition was higher in D2 mice (D2: 4.2$\pm$2.2; D2c: 0.59$\pm$0.06; p=0.025). Measurements of miniature inhibitory post-synaptic currents (mIPSCs; recorded with CNQX, D-AP5, and TTX) showed a higher mIPSC amplitude (D2c: 12$\pm$1 pA, n=12 cells, 5 mice; D2: 20$\pm$1 pA, n = 9 cells, 4 mice; p=0.0063) without a change in frequency. Peak-scaled nonstationary fluctuation analysis of mIPSCs indicated an increase in the number of post-synaptic GABA receptor channels at the synapse (D2: 16.8$\pm$1.7; D2-control: 25.3$\pm$3.2; p=0.024) without change in the unitary conductance. These results indicate that feed-forward inhibition from local inhibitory interneurons in the dLGN of mice with glaucoma is enhanced due to changes in post-synaptic GABA receptors. Thus, effects of glaucoma appear to reach beyond RG synapses to impact other members of the dLGN network in ways likely to influence TC neuron response properties and encoding of visual signals.

Disclosures: M. Van Hook: None. **J.C. Smith:** None.

Poster

PSTR344. Subcortical Visual Circuits

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Topic: D.06. Vision

Support: Vada Kinman Oldfield Fund
T32 AG076407

Title: Visual pathway function in a mouse model of neurodegeneration

Authors: *S. MCCOOL[1,3], M. J. VAN HOOK[1,3,2], J. SMITH[1,3], K. ZHANG[1,3], A. SLADEK[1,3]; [1]Ophthalmology and Visual Sci., [2]Cell. and Integrative Physiol., Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Truhlsen Eye Inst., Omaha, NE

Abstract: Visual Pathway Function in a Mouse Model of Neurodegeneration
Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder worldwide. AD pathology is attributed to amyloid beta (Aβ) plaque formation, tau inclusions, & cerebral atrophy. Structural & functional changes are seen in the visual system of AD patients, but the mechanism behind these changes is not well grasped. The goal of our study was to determine the role of Aβ in visual system dysfunction, which we accomplished using 6- and 9-month (m) old 5xFAD mice, which develop Aβ plaques and AD-like cognitive deficits in an age-dependent manner. Age-matched C57BL/6J mice were used as controls, and mice of both sexes were used. Using dark-adapted in vivo electroretinogram (ERG) recordings we found a significant increase in A- and B-wave amplitudes at 6m (n = 15) compared to controls (n = 13) ($P < .0001$). The ERG A- wave amplitudes were lower at 9m (n = 17) compared to controls (n = 10) ($P < .0001$), and analysis of the B/A wave ratio indicated photoreceptor dysfunction ($P = .0004$). At 9m, males showed a decrease in A wave amplitude while females did not. Within the dorsolateral geniculate nucleus (dLGN), a retinal ganglion cell (RGC) projection target for conscious vision, thioflavin S staining revealed a high burden of Aβ plaques in the 5xFAD mice of both ages. To test for degeneration of retinal ganglion cell projections to the dLGN, we stained dLGN sections for vGlut2, finding a significant decrease in vGlut2 density between the 6m (n = 11) & 9m 5xFAD mice (n = 17) ($P = .0015$). Despite the loss of vGlut2 & presence of Aβ plaques, there was no detectable difference in the frequency of miniature excitatory post-synaptic currents (mEPSCs) recorded from retinorecipient thalamocortical (TC) neurons at 6m or 9m compared to controls. Additionally, Sholl analysis of TC neuron dendrite structure revealed no difference in dendritic complexity in TC neurons from 6m 5xFAD mice compared to controls. Finally, reflexive visual behavior was examined via optomotor response (OMR). Analysis masked to genotype indicated no detectable deficits in contrast sensitivity or spatial acuity of the 5xFAD mice compared to controls. Even with histopathological evidence of disease in the brain, there were few detectable effects on visual system structure and function in 5xFAD mice. This suggests that Aβ might have fairly modest influences on the visual system or point to adaptive mechanisms that preserve function of visual pathways in this mouse model.

Disclosures: S. McCool: None. **M.J. Van Hook:** None. **J. Smith:** None. **K. Zhang:** None. **A. Sladek:** None.

Poster

PSTR344. Subcortical Visual Circuits

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P30EY002162
Gilbert Vision Restoration Initiative
Knights Templar Eye Foundation Grant

Title: The role of postsynaptic neuronal activity in retinal axon regeneration

Authors: *S. VARADARAJAN[1], F. WANG[2], O. S. DHANDE[1], P. LE[1], X. DUAN[2], A. HUBERMAN[1];
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Abstract: The eye-to-brain pathway is the sole source through which visual information is relayed to the brain. A major milestone in the formation of this pathway occurs when retinal ganglion cell (RGC) axons connect with postsynaptic visual targets in the brain, i.e. the retinorecipient neurons. Like other mammalian CNS neurons, damaged RGCs fail to regenerate, leading to a loss of vision. Therefore, this eye-to-brain pathway represents a key bottleneck in conditions that cause blindness, such as glaucoma and eye injuries. One promising approach is to reapply developmental mechanisms that form visual circuits towards promoting repair. During development the retina, and the postsynaptic targets in the brain, provide necessary signals to wire visual circuits. Postsynaptic retinorecipient neurons play a crucial role by specifying target-derived signals that regulate RGC axon extension, target-selection and synapse formation during development. While many studies have focused on the cellular and molecular events occurring in RGCs to promote regeneration, far less is known about the potential of postsynaptic target neurons in the brain in promoting RGC axon regeneration and re-connectivity. Here we show that a distal injury to the optic tract can be used as a model to investigate the role of target-derived signals in reconnecting visual circuits. Using this distal injury model, we determined that increasing neural activity posterior to the lesion site can promote regeneration of RGC axons up to 2mm past the lesion, reinnervate target nuclei, and rescue deficits in optomotor function two weeks post-injury. Further, to understand the specific contribution of retinorecipient target cells in this model, we screened a new mouse line to precisely activate cells that reside in the nucleus of the optic tract (NOT), a subcortical retinorecipient target in the accessory optic system that is responsible for image stabilization. Using mouse genetics and viral tracing approaches, we show that selective activation of specific retinorecipient cells is sufficient to promote regeneration of RGC axons. These findings underscore the importance of post-synaptic cells in CNS repair and will have widespread relevance for treating blinding diseases, stroke, and traumatic brain injury.

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Poster

PSTR344. Subcortical Visual Circuits

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Topic: D.06. Vision

Support: NIH Grant R01 EY030507
Research to Prevent Bindness/The Glaucoma Foundation Career Advancement Award
NIH Grant T32 NS105594

Title: Microglia in the dorsolateral geniculate nucleus respond to ocular hypertension during glaucomatous neurodegeneration.

Authors: *J. L. THOMPSON[1], J. C. SMITH[2], S. MCCOOL[3], M. VAN HOOK[2];
[1]Dept. of Pharmacol. & Exptl. Neurosci., Univ. of Nebraska Med. Ctr. Dept. of Pharmacol. and Exptl. Neurosci., Omaha, NE; [2]Dept. of Ophthalmology and Visual Sciences, Truhlsen Eye Inst., [3]Dept. of Pharmacol. & Exptl. Neurosci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Glaucoma is often overlooked as a neurodegenerative disease because it presents, is diagnosed, and is treated in the eye, however, blindness is the result of retinal ganglion cell (RGC) dysfunction and degeneration, and visual brain regions experience synaptic changes well in advance of neuronal death. Here, we investigated the effects of elevated eye pressure (ocular hypertension; OHT) - glaucoma's only modifiable risk factor - on retinogeniculate synapses in the visual thalamus (dorsolateral geniculate nucleus; dLGN) and asked whether microglia-mediated synaptic pruning aberrantly reoccurs in the dLGN within the context of disease. We used the DBA/2J (D2) mouse model of inherited glaucoma and its strain-matched control (DBA/2J^{Gpnmb+}; D2-Control), which does not develop OHT. During the living phase, we took monthly intraocular pressure (IOP) measurements with a rebound tonometer, and mice were sacrificed at timepoints corresponding to pre- (4m), early- (9m), and progressive glaucoma (12m). Fixed tissue from central regions of the dLGN were immunolabeled for analysis. In order to investigate retinal innervation, we quantified RGC axon terminals that positively labeled for an antibody against vesicular glutamate transporter 2 (vGlut2+). The D2 dLGN had an IOP-dependent reduction in vGlut2+ density (R^2 = .313 ; p < 0.001; n = 39); no age- or IOP-dependent vGlut2+ loss was observed in controls (p = 0.703; p = 0.817; n = 36). A subset of D2 (n = 25) and D2-Control (n = 22) tissue was likewise stained for complement component C1q, which tags immature retinogeniculate synapses for removal during development and might imply the involvement presynaptic pruning. Intensity analysis revealed that C1q expression in D2s increased by 9- and 12m: this increase was IOP-dependent (R^2 = 0.384; p < .001) and associated with vGlut2+ loss (R^2 = .304; p = .0011). Pre-glaucoma C1q expression was consistent with the

low, unchanging levels that were detected at all timepoints in controls (p = .277). Importantly, we labeled microglia/macrophages with an antibody against ionized calcium-binding adaptor molecule 1 (Iba1) and analyzed the presence and morphology of Iba1+ cells. A global skeleton analysis revealed negative associations between OHT and features of Iba1+ cell ramification, with IOP explaining ~ 30% of arborization variability (R^2 = 0.298, p < .0001) and 26% of endpoint variability (R^2 = 0.263, p < .0001). We further report these features correlating with the loss of RGC terminals, and the increased C1q expression, suggesting that microglia/macrophages in the visual thalamus have a characteristic response to OHT that may include complement-mediated synapse elimination.

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Poster

PSTR344. Subcortical Visual Circuits

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR344.16/CC13

Topic: D.06. Vision

Support: NIH Grant U01NS122040

Title: 3d morphology of mouse geniculate relay cell dendrites and synaptic boutons before eye opening

Authors: S. HOLTON[1], M. MASON[1], T. CHOWDHURY[1], B. GUDURU[1], V. LI[1], S. ZHANG[1], M. A. FOX[2], *A. ERISIR[1];
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Abstract: Eye opening and subsequent patterned vision are essential for the maturation of postsynaptic responses mediated by retinal, corticothalamic, and inhibitory inputs and for initiating activity-dependent refinement of synaptic circuitry in the visual thalamus and cortex. To examine the morphological properties of developing neuropil, we reconstructed dendrites of relay cells and synaptic boutons in the dorsal lateral geniculate nucleus (dLGN) of mice at postnatal day 14 (P14), using image scans obtained on a Scanning Blockface Electron Microscope (SBEM). In P14 mice LGN, the retinal and cortical terminals and presynaptic appendages of interneuron dendrites are readily identifiable by morphological criteria. We recorded dendrite morphology and the volume, frequency, and target-selectivity of origin-sorted input terminals at P14 and compared their properties to that in the adult LGN, as observed in a recent study from our lab (Maher, Briegel, et al., 2023). Our results reveal that complex synaptic arrangements that are characteristic of the adult dLGN, including triads and glial ensheathment of glomeruli, are present at P14. Retinal bouton volumes were also larger than non-retinal boutons at P14, which is consistent with adult findings. However, the P14 retinal bouton

population did not contain a larger-sized subpopulation observed in adults, suggesting that visual activity may induce further growth of retinal bouton size. Cortical terminals exhibited selectivity for tertiary dendritic branches in the P14 and adult LGN, although their relative frequency was significantly lower at P14. This property suggests that corticothalamic axon innervation of the LGN to its adult-like pattern may require visual activity. The most striking morphological feature that is unique to the pre-eye opening LGN was observed in the geniculate relay dendrites: many filopodia, spines, and larger appendages gave the dendrites a "thorny" appearance. The thorny appendages were also frequently found at the end of dendritic segments and, at all sites, received synapses from multiple retinal terminal boutons. Retinal bouton/thorn complex location on distal dendrites that are specific for corticothalamic inputs in the adult LGN suggests that immature retinal terminals may guide developing cortical terminals at these early developmental ages, or they may represent ectopic retinal terminals that are destined for pruning.

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Poster

PSTR345. Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: 4VA-Foundation minigrant

Title: Characterizing the signaling pathway for intrinsic photoresponses in the mammalian iris

Authors: *M. WALKER, D. TOBIN;
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Abstract: The iris is a muscular tissue in the anterior of the eye that controls the amount of light entering the eye. Iris muscle activity such as pupillary light reflex (PLR) is driven by a neurological signal in response to changes in ambient light. This neurological signal is initiated in the retina and transmitted to the brain which directs the neuronal input to drive PLR via the oculomotor nerve. In non-primate vertebrates, iris tissue constriction can also be driven by an intrinsic light-response pathway in the iris tissue. Both retinal-driven and intrinsic light-responses are driven by photoreceptor cells that require the melanopsin photopigment for initiating constriction. It has also been shown that the intrinsic iris light-response pathway also requires the Gq G-protein, PLC beta, and the Trpm1 cation channel to drive light-dependent constriction. Currently it is still unclear how photoreceptive cells in the iris are transmitting light-dependent signals to the iridial muscle cells. Some studies investigating this intrinsic mechanism have indicated that cholinergic chemical synapses are not involved in signal transmission. Based on these studies, we hypothesize that connexin protein electrical synapses are responsible for the transmission of the intrinsic light signaling within the iris. We have designed tests to identify the

location and morphology of melanopsin expressing photoreceptors and to identify the expression of connexins in the iris tissue. We used RT-PCR to probe mouse iris tissue for the presence of 5 connexin proteins that have previously been demonstrated to be expressed in the eye. Our results show the presence of Connexin 43 and Connexin 45 in wild-type mouse iris tissue. Presence of these proteins suggests that connexins may be responsible for the light signaling transmission, and support the need for further studying the role of these proteins in the iris.

Disclosures: **M. Walker:** None. **D. Tobin:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

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Title: Discrete PFC subregions provide distinct feedback modulation to the visual cortex that differentially shape visual processing

Authors: *S. K. ÄHRLUND-RICHTER, Y. OSAKO, K. R. JENKS, M. SUR;
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Abstract: The prefrontal cortex (PFC) is a source of top-down feedback modulation for the visual cortex- guiding, biasing or modulating activity in the visual cortex to optimize visual processing. The visual cortex of the mouse receives monosynaptic input from two discrete PFC subregions, the anterior cingulate cortex (ACA) and the orbitofrontal cortex (ORB), which are implicated in distinct aspects of cognition. However, it is currently unknown if these discrete PFC subregions contribute to different aspects of visual attention and perception, and how this process relates to the behavioral state of the animal and previously experienced visual contexts. To better understand how discrete PFC subregions shape the activity of the visual cortex, we performed in-vivo calcium imaging of ACA and ORB axonal activity in the visual cortex of mice viewing visual stimuli. In parallel, behavioral variables such as running speed, pupil size and face movements were recorded, and unexpected air puffs were delivered to evoke behavioral state shifts. To be able to parcellate the discrete activity communicated uniquely to the visual cortex versus more global behavioral signals, the ACA and ORB axonal activity was also

recorded in the primary motor cortex to allow for comparison. We found that both ACA and ORB axonal activity in the visual cortex incorporate visual and behavioral variables. Using generalized linear models (GLMs) of axonal activity, we found that ACA axons encode visual information more strongly than ORB axons, and that their visual responses scale with contrast. Importantly, the observed PFC axons' activity was more aligned to its source region, rather than the cortical regions they terminated within (i.e., they conveyed activity globally across cortex). To directly evaluate the impact of distinct PFC inputs on the activity of the visual cortex, we also recorded the activity of visual cortex neurons with or without DREADD-mediated inhibition of ACA or ORB neurons projecting to the visual cortex. We are currently evaluating the effects of feedback modulation from the ACA or the ORB on the visual cortex at both population and single neuron levels. Our work so far suggests that even in a passive viewing context, the ACA and the ORB are actively conveying information in regards to the visual scene and behavioral state of the animal that shape the activity of the visual cortex.

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Poster

PSTR345. Visual System: Response Modulation and Adaptation

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Topic: D.06. Vision

Support: ARC Discovery Project DP210102107

Title: Trial-to-trial variations in neural latency correlate across cortical populations.

Authors: *I. R. COWIE-KENT[1,2], B. H. OAKLEY[2], M. A. HAGAN[2], Y. T. WONG[2,3], T. J. ALLISON-WALKER[2,4], S. J. MEIKLE[2,3], D. SHIMAOKA[2], E. ZAVITZ[2,3], N. S. C. PRICE[2]; [2]Dept. of Physiol. & Monash Biomedicine Discovery Inst., [3]Electrical and Computer Systems Engin., [1]Monash Univ., Melbourne, Australia; [4]Sch. of Engin., RMIT Univ., Melbourne, Australia

Abstract: In the visual system, the latencies of neural and behavioural responses are correlated when identical stimuli are repeated. Little is known about how widespread temporal variations in activity are throughout the visual system and whether they simply reflect global variations in cortical gain that affect firing rate. To explore the timing of responses throughout the visual system, we simultaneously recorded from populations of neurons in primary visual cortex and secondary visual cortex (V1, V2; 2 animals), and V1 and middle temporal area (MT; 4 animals) in sufentanil-anaesthetised marmosets (Callithrix jacchus). In total, we obtained the responses to moving stimuli in 392 V1, 34 V2 and 170 MT visually-responsive neurons. We found significant positive correlations in trial-to-trial latency variations. Correlations were higher for pairs of neurons in the same area (r_{V1} = 0.06, r_{MT} = 0.09, r_{V2} = 0.25) than for pairs of neurons in different

areas ($r_{V1\text{-}MT}$ = 0.03, $r_{V1\text{-}V2}$ = 0.17). Critically, correlation strength was still high when we controlled for firing rate variations. The degree of common inputs shared by neurons are closely related to similarity in their tuning and receptive field. Surprisingly, we found no systematic relationship between strength of correlation and direction tuning similarity. However, latency correlations were higher when neurons had greater receptive field overlap or were closer together, both laterally and across cortical layers. We conclude that characterisation of trial-to-trial latency variation propagation across populations of cortical neurons can serve as a proxy measure of shared input and functional connectivity. Our underlying model of information processing would then suggest that latency variations could accumulate between hierarchical levels in a network, so that trial-by-trial differences in brain state are reflected in behavioural outputs.

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Poster

PSTR345. Visual System: Response Modulation and Adaptation

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Title: Local V1 NMDA receptor blockade alters visual mismatch responses and augments prefrontal-V1 synchrony

Authors: *C. G. GALLIMORE[1], J. M. ROSS[1,2], J. P. HAMM[1,2,3];
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Abstract: The oddball paradigm is a hallmark psychophysiological assay used to study brain responses to predictable vs unexpected (“oddball”) stimuli. Human studies using this paradigm have shown that people in neuropsychiatric disease-states, such as schizophrenia, display reductions in evoked sensory cortical responses to oddballs, referred to as “mismatch negativity” (MMN). MMN responses depend on NMDA-receptor function, as shown by past work in humans, non-human primates, and rodents utilizing NMDAR blockade, but the underlying cell- and circuit-level mechanisms explaining MMN’s dependence on NMDAR function are unknown. Conceptualized as arising from predictive processing in cortical networks, MMN relies on the integration of top-down predictions (e.g. one stimulus is expected) with bottom-up

sensory data (e.g. the current stimulus is an oddball). Mice provide a powerful model system to tease out the cell and circuit roles of NMDARs in predictive processing, yet past work is inconclusive. The analogous brain response to MMN observed in individual neurons, termed "deviance detection" (DD), has been shown to be dose-dependently blocked in auditory circuits of anesthetized rats by NMDAR block. Awake rodent studies complicate the story, showing reductions in late-latency DD potentials—thought comparable to human MMN—yet augmentations of early-latency ERPs. Other rodent studies have examined global NMDAR hypofunction in higher cortical areas such as the anterior cingulate area (ACa), finding significant increases in top-down axonal activation which suppressed spontaneous and sensory-evoked activity in primary visual cortex (V1). These discrepancies leave open a critical question: does NMDAR blockade alter MMN by biasing cortical circuits towards top-down predictions, or bottom-up sensory input? Here, we sought to address this by simultaneously measuring intracranial local field potentials (16-channel multielectrode probes) in the V1 and ACa of mice (n=5) during visual oddball sequences of full-field oriented square-wave grating stimuli (redundant, p=.9; deviant, p=.1) both before and after V1 NMDAR block via local application of MK-801. As expected, NMDAR block reduced theta-band DD. Further, we found late-latency (200-500 ms post-stimulus) increases in deviant-induced high gamma-band activity after NMDAR block. Prior to NMDAR block, ACa-V1 phase coherence was strongest at 10-Hz, peaking in deep (L5) and supragranular (L2/3) V1 layers. Post-NMDA block, ACa-V1 coherence increased across all layers and shifted down to 4-8 Hz, suggesting NMDA block shifts cortical priority to internally-generated predictions over bottom-up inputs.

Disclosures: C.G. Gallimore: None. **J.M. Ross:** None. **J.P. Hamm:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.05/CC18

Topic: D.06. Vision

Support: Natural Sciences and Engineering Research Council of Canada (NSERC) #327588
Canada Foundation for Innovation (CFI) # 12807

Title: The effect of continuous theta burst stimulation to primary visual cortex on binocular rivalry

Authors: R. COHAN, ***S. S. MORO**, J. K. E. STEEVES;
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Abstract: Promoting plasticity in neural networks is one of the hallmarks of neuromodulation techniques. Continuous theta burst stimulation (cTBS) is a technique that has been shown to induce inhibitory responses when applied to the primary motor cortex. To evaluate the efficacy

of cTBS in the visual brain we applied 600-pulses of cTBS at 80% individual phosphene threshold (PT) to primary visual cortex (V1) before and after performing a binocular rivalry (BR) task. BR is a perceptual phenomenon that occurs when the two eyes are each presented with different images simultaneously. This leads to alternating dominance and suppression periods of encoding of visual information as the brain attempts to reconcile the conflicting information. BR involves an interplay between multiple levels of the visual system, from the eyes to V1, and higher-level cortical areas. Research has identified cortical columns in V1 that respond preferentially to one eye or the other. These columns play a role in the alternating perception observed in BR. Our study explored the effect cTBS to V1 on BR in right-handed, right eye-dominant subjects. Participants engaged in a BR task where they viewed orthogonal grey-scale gratings of fixed orientation (+/-45°) through a stereoscope. Subjects were instructed to indicate perceived visual dominance (leftward tilting grating, rightward tilting grating, or mixed percept) by pressing and holding designated buttons upon perception change. cTBS was applied to left V1 and BR was again measured. Our preliminary results indicate an increase in the alternation rate of BR post-cTBS. This suggests that cTBS to V1 induces an alteration in visual perceptual dominance and furthers our understanding of the neuromodulatory effects of cTBS on visual perception. Overall, these results shed light on the potential of cTBS as a research and therapeutic intervention for visual disorders with neural origins and contribute to the growing body of literature on neuromodulation, visual perception, and brain plasticity.

Disclosures: R. Cohan: None. **S.S. Moro:** None. **J.K.E. Steeves:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.06/CC19

Topic: D.06. Vision

Support: NIH Grant 1RF1NS127305-01

Title: Contributions of visual cortex to prey capture in the mouse

Authors: *I. RHIM, D. PISCOPO, Y. FAN, E. ABE, C. NIELL;
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Abstract: The ability to effectively process the visual scene in order to parse targets from backgrounds in complex environments is pivotal to an animal's survival. Prey capture is an ethological behavior in the mouse that provides a paradigm to investigate such computations. While previous studies have demonstrated the role of SC cell types in prey capture, the role of visual cortex remains unknown. We hypothesized that the cortex would be necessary for complex computations such as segmenting prey from the background or discriminating prey from distractors. To determine the contributions of V1 for successfully detecting, pursuing, and capturing target prey, we bilaterally silenced binocular V1 areas optogenetically during freely

moving prey capture. In our experiment, we tested the impact of optogenetic inactivation while increasing task complexity through introduction of visual patterns and distractors. Following a simple 2-dimensional pose estimation from the data, we aimed to quantify the mouse's behavior using unsupervised machine learning methods to assess the mouse's behavior motifs. We found that optogenetic shutdown of V1 impairs prey capture behavior in mice particularly in the more complex environments. These results provide new insight into the relative role of cortical and subcortical processing in natural contexts.

Disclosures: I. Rhim: None. **D. Piscopo:** None. **Y. Fan:** None. **E. Abe:** None. **C. Niell:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.07/CC20

Topic: D.06. Vision

Support: National Science and Technology Innovation 2030 Major Program (2022ZD0204600)

Title: Population response associated with contour integration in macaque V1 and V4

Authors: *D. JIANG, C. CHEN, S. TANG, C. YU;
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Abstract: Following the Gestalt rule of good continuation, discrete but aligned stimuli can be integrated and perceived by the brain as continuous contours. Although early psychophysical and computational models assume the roles of V1 neurons in contour integration through "association field" or orientation-specific horizontal connections, later studies tend to support that V4 neurons with large RFs may detect contours first and top-down modulate V1 responses later. Here we used two-photon calcium imaging to study population responses of large neuron samples mostly triggered by contour stimuli in V1 and V4 of awake, fixating macaques. We imaged neural responses to a curved contour comprised of nine high-contrast Gabors (for V1 recording) and circular hard-edged gratings (for V4 recording). The contour elements had a drifting speed 4 cycle/sec, and a spatial frequency varies among FOVs according to their preferred frequency band. The contour was imbedded in a field of randomly oriented stimuli that were otherwise identical to the contour elements. Data analysis identified a total of 78.7% and 45.7% orientation-tuned V1 and V4 neurons, respectively. The average responses and population orientation tuning functions of V1 and V4 neurons to a grating stimulus were substantially and similarly suppressed when the grating stimulus was either part of the curved contour in a random field, or was in a random field without the contour, which revealed no contour information. However, contour information carried by V1 and V4 population responses can be decoded. Specifically, we used PCA to reduce the dimensions of neurons and trained a linear decoder to decode contour information using PCA-transformed neural responses. The results indicated that

less than 10 principle components were required to decode contour information with an accuracy of 70% in both V1 and V4. These population coding results suggest that, when no contour detection task, and thus no task-specific top-down modulation, is involved, V1 and V4 can still encode contour information in terms of population response patterns, which may be linearly read out by downstream brain areas for decision making.

Disclosures: **D. Jiang:** None. **C. Chen:** None. **S. Tang:** None. **C. Yu:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.08/DD1

Topic: D.06. Vision

Support: Canadian Foundation of Innovation and Ontario Research Fund (CFI/ORF project no. 37597)
NSERC (RGPIN-2019-06479)
CIHR (Project Grant 437007)
Connaught New Researcher Awards

Title: A midbrain inhibitory pathway mediates the contextual modulation of visual processing in the superior colliculus

Authors: *X. CHOU, M. RUSSO, B. LIU;
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Abstract: Our visual perception of objects is malleable and subject to the modulatory influences by their contexts, a fundamental attribute of sensory processing. For instance, the visual percept of a foreground object and related visual activity can be profoundly impacted by the background motion which could occur when we turn our heads, or the environment moves. Previous studies mainly focused on the retinal circuits and the local inhibition within a single visual structure. However, the neural mechanisms underlying this process remain incomplete. Here, we examined the role of a non-retinal projection pathway in the contextual modulation of visual processing in the superior colliculus (SC), a midbrain structure essential for visual perception. We found that the visual activity evoked by a transiently appearing foreground stimulus was substantially suppressed by background motions. To understand the underlying mechanism, we aimed at the nucleus of optic tract and dorsal terminal nucleus (NOT-DTN), since it preferentially responds to the background motion. With anatomical tracing and slice electrophysiology, we showed that inhibitory neurons in the NOT-DTN directly innervated SC neurons. Next, with in vivo extracellular recording we demonstrated that inhibitory NOT-DTN neurons which project to the SC were indeed strongly responsive to background motion, but not to transiently flashing foreground stimuli. Last, with optogenetic circuit perturbation we found that this inhibitory projection contributed significantly to the contextual modulation of SC neurons and could impact

visual guided behaviors. In conclusion, we identified a novel inhibitory midbrain circuit from NOT-DTN to SC which underlies the modulation of visual processing and perception by the background motion.

Disclosures: **X. Chou:** None. **M. Russo:** None. **B. Liu:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.09/Web Only

Topic: D.06. Vision

Support: TÜBİTAK 1001 Grant 217K163

Title: A parsimonious recurrent cortical model can explain behavioral and neural effects of expectation on perception

Authors: B. M. URGEN[1], ***H. BASTÜRK**[2], H. BOYACI[3];
[1]Psychology, TED Univ., Ankara, Turkey; [2]Neurosci., Bilkent Univ. Neurosci. Grad. Program, Ankara, Turkey; [3]Neurosci. Dept., Bilkent Univ., Ankara, Turkey

Abstract: Prior knowledge and expectations can shape perceptual processes. Although the behavioral effects of expectations are relatively well-established, the underlying neural mechanisms associated with these effects remain controversial. Specifically, some neuroimaging studies have shown facilitatory while others have shown suppressive effects of expectations on neural activity. Predictive processing accounts of brain function may offer a generic framework for understanding the behavioral and neural effects of expectations on perceptual processes. Yet the computational mechanisms underlying these effects have been largely unclear. To this end, we implemented a recurrent cortical model (Heeger, 2017) which does not include subpopulations of neural units, e.g. for error or prediction computation. First, we modeled the behavioral data of Urgen & Boyaci (2021). Next, using three previous fMRI studies (Egner et al. 2010, Kok et al. 2011, Aitken et al. 2020) and using the optimized parameters obtained in the first step, we simulated fMRI BOLD responses. The responses were compared across conditions with a 2 (trial type: expected, unexpected) x 2 (validity: 75, 50) repeated measures ANOVA. Overall, our results showed that the cortical model successfully predicts the behavioral effects of expectation. Specifically, the results revealed a main effect of expectation ($F(1,7) = 18.511$, $p = 0.004$), implying that the sensory process needs to be completed with additional, and consequently longer, computations in unexpected trials when the actual input does not match the expectations ($t(7) = 3.220$, $p = 0.015$). The simulated BOLD responses largely agreed with the empirical data, as well. In line with previous studies on V1 (Kok et al.(2011) & Aitken et al. (2020)), our model predicted an expectation facilitation effect in lower layers ($F(1,7) = 5.811$, $p = 0.047$; $F(1,7) = 6.019$, $p = 0.044$). Our model results on higher-order layers yielded a trend towards expectation facilitation for both stimuli (houses and faces) ($F(1,7) = 4.973$, $p = 0.061$),

which is consistent with the empirical findings of Egner et al. (2010). The only discrepancy between the empirical data and our results was observed in responses to the preferred stimulus type (e.g. faces in FFA), where our results on higher-order levels showed an increase in responses to the preferred stimulus despite a decrease in the empirical findings. Overall, our findings show that a parsimonious recurrent cortical model can elucidate the behavioral and neural effects of expectations on perceptual processes. This model provides a biologically plausible mechanism that offers a link between behavioral and neural responses.

Disclosures: B.M. Urgen: None. **H. Bastürk:** None. **H. Boyaci:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.10/DD2

Topic: D.06. Vision

Support: EY23384

Title: The Effect of Natural Scene Image Contrast on Visual iEEG Recordings and Pupil Diameter

Authors: *M. MONTOYA[1,2], H. HUANG[2], Z. QADIR[2], G. OJEDA VALENCIA[2], K. J. MILLER[3], K. KAY[4], D. HERMES[2];
[2]Physiol. and Biomed. Engin., [3]Neurosurg., [1]Mayo Clin., Rochester, MN; [4]Univ. of Minnesota, Minneapolis, MN

Abstract: Neural responses in human visual cortex can be measured with intracranial EEG and these responses may be modulated by task parameters and brain state. One potential measurement that may capture some state related changes is pupil diameter, which has been suggested to modulate neural responses to visual stimuli. Here, we measure stereo EEG (sEEG) broadband responses from early visual areas (V1-V4) simultaneously with pupil diameter during a natural scenes viewing task, and test how image contrast and luminance modulate pupil diameter and broadband responses. Two subjects (18 years old, 1 female) viewed 1000 select images from the Natural Scenes Data (NSD), while collecting pupillometry data from a Tobii Pro Spectrum eye tracker. Images were shown 800ms on, 800ms off and were displayed in 10 separate runs recorded over multiple days. At the end of each run, participants did a brief memory task with 3 images shown and were asked whether the images were old or new. We calculated mean luminance and local Root Mean Square (RMS) contrast for each image. We extracted broadband changes from electrodes in early visual areas. Broadband power was increased with RMS contrast, but not with luminance. Changes in pupil diameter, on the other hand, showed increased pupil light reflexes in response to increases in luminance. We further found that variance in the broadband responses to natural scenes in the early visual cortex was largely explained by RMS contrast, and pupil diameter did not significantly explain additional

variance in sEEG broadband responses. While pupil diameter may affect neural signals related to cognitive responses, our recordings do not provide evidence that pupil diameter has large effects on the iEEG broadband signals in early visual cortex.

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Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant R01 DA042855
NIH Grant R01 AG039283

Title: Propranolol during decision tasks reduces tonic pupil size in a dose-dependent manner

Authors: *H. FAN[1], E. DIAL[1], K. M. LEMPERT[2], D. SAMBRANO[1], E. A. PHELPS[1];
[1]Harvard Univ., Cambridge, MA; [2]Dept. of Psychology, Adelphi Univ., Garden City, NY

Abstract: Although the beta-adrenergic receptor antagonist propranolol is often used to manipulate physiological arousal, and pupil size has been argued to be a real-time proxy of physiological arousal, the literature on the effect of propranolol on pupil size is mixed. Here, we reanalyze two existing dataset with a placebo-controlled, double-blinded within-subjects design in order to investigate the relationship between propranolol and pupil size. In both studies, participants received a propranolol pill (80mg) at one session and a matched placebo at the other, with the order counterbalanced, before they completed a decision-making task. Each trial comprised a fixation phase, a stimulus phase (showing different options), and a feedback phase (confirming the subject's chosen option). We focused on investigating the effect of propranolol on tonic pupil size (average over 1 second before stimulus phase onset) and baseline-corrected stimulus-evoked phasic pupil size (average over the interval of 1 to 3 seconds after stimulus phase onset), including effective dose based on body weight (mg/BMI) as a covariate (mean ± SD: 3.03 ± 0.45).
For tonic pupil size, we found a significant effect of propranolol when accounting for effective dose (propranolol × effective dose interaction: $F(1,4421) = 214.96$, $p < .001$). Post-hoc comparison reveals that when the effective dose was high, the propranolol group had a smaller tonic pupil size (propranolol – placebo: $M = -119.7$, $p < .001$) while when the effective dose was low, the propranolol group had a larger tonic pupil size ($M=176.6$, $p < .001$) than the placebo group. We found an opposite relationship between propranolol and phasic pupil size modulated by effective dose (interaction: $F(1, 4421) = 19$, $p < .001$). Specifically, propranolol elevated phasic pupil response in the high effective dose group ($M = 21.92$, $p = .03$) while suppressing the phasic pupil response in the low effective dose group ($M = -37.1$, $p < .001$). The results remained

the same using other proxies of effective dose, including heart rate difference and systolic blood pressure difference between propranolol and placebo conditions.
Our results provide evidence that the downstream effect of propranolol on pupil size during decision-making tasks is modulated by a variety of factors, including effective dose and tonic/phasic phase.

Disclosures: H. Fan: None. **E. Dial:** None. **K.M. Lempert:** None. **D. Sambrano:** None. **E.A. Phelps:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

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Program #/Poster #: PSTR345.12/DD3

Topic: D.06. Vision

Support: Sheryl and Dan Tishman Postdoctoral Fellowship
Simons foundation
Philippe Foundation

Title: Role of GABAergic inhibitory interneurons in the postnatal emergence of sensory processing in the visual cortex

Authors: *M. SALMI, E. SABRI, R. BATISTA-BRITO;
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Abstract: Sensory perception allows us to receive and integrate information from the environment, a process that starts right from birth. Indeed, newborns can interact with their environment and perform sensorimotor tasks soon after birth, without any previous experience of patterned sensory input. Understanding how neurons assemble into circuits that developmentally prepare to encode visual information accordingly to behavioral states is still poorly understood and is likely to provide critical insight not only into how these circuits function, but also how they malfunction in various neurological conditions. Parvalbumin (PV) fast-spiking expressing inhibitory interneurons (INs) are the main source of cortical GABAergic inhibition. The maturation of PV INs has been shown to drive the timing of the critical period for visual plasticity, however their contribution towards *in vivo* sculpting of early sensory network activity remains largely unknown. More specifically how PV INs shape the network to support the emergent visual processing in V1 throughout the visual onset period (eye opening) has not been previously addressed. Here, we selectively disrupt PV INs function during embryonic development by removing *Mef2c*, an activity-dependent transcriptional factor, from PV INs progenitors. Longitudinal *in vivo* recordings in the murine V1 around the time of eye-opening (from P10 to P17), revealed that embryonic removal of *Mef2c* from PV INs leads to (1) an increase of spindle burst activity and network synchrony, as well as to (2) a decrease of visual responses and orientation selectivity in an arousal state-dependent manner. Taken together our

data show that early on PV INs progenitors shape the development and maturation of the V1 network activity and as consequences the proper development and maturation of the emergent visual sensory processing.

Disclosures: **M. Salmi:** None. **E. Sabri:** None. **R. Batista-Brito:** None.

Poster

PSTR345. Visual System: Response Modulation and Adaptation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR345.13/DD4

Topic: D.06. Vision

Support: NIMH R00MH116100

Title: Late Hierarchical Emergence of Global Prediction Error Encoding in the Macaque Cortex

Authors: ***Y. XIONG**[1], A. V. MAIER[2], J. A. WESTERBERG[5], A. BASTOS[3], H. NEJAT[2], K. GABHART[1], P. D. MENG[4];
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Abstract: Prediction is a fundamental function of the cortex. Predictive routing is a leading theory in the implementation of predictive coding. It proposes that lower order cortex feeds forward prediction error signals in gamma frequency band (>40Hz) to higher order cortex, which in turn feeds back prediction signals in alpha/beta (8-30Hz). Alpha/beta is theorized to inhibit gamma which implements selective suppression to predictable inputs. According to predictive coding/routing theories, prediction is not dependent on stimulus repetition and/or neuronal adaptation. However, much of the current evidence for predictive processing uses paradigms that implement prediction with simple repetition. In this experiment, we used a global/local oddball paradigm to create predictions that do not depend on stimulus repetition. We hypothesize that local and global oddballs are encoded in areas at different levels of cortical hierarchy.Over several training sessions, we habituated macaque monkeys to a visual oddball pattern with drifting gratings (e.g. AAAB), creating a local-level oddball but global-level prediction. During high-density multi-area laminar intracranial recording sessions, monkeys were presented with habituated patterns (e.g. AAAB) on 80% of trials and with global oddball patterns with local repeats (e.g. AAAA) on 20% of trials. We hypothesize that areas lower in the cortical hierarchy will encode local oddballs but not global oddballs, and that areas higher in the cortical hierarchy will encode both local and global oddballs. Neuronal spiking results show that visually responsive multi-unit firing rates in areas V1, V2, V3/V3a, V4, TEO, MT, MST, FEF, and PFC all encode local oddballs. In contrast, global oddball encoding only occurs in areas higher in the functional hierarchy, including areas V3/V3a, TEO, FEF and PFC. Furthermore, the significant encoding time onset of local oddballs precedes the encoding time onset of global oddballs in

areas that exhibit both. Ongoing analyses aim to resolve the oscillatory roles of alpha/beta and gamma. Together, these findings demonstrate a late hierarchical emergence of global oddball processing in the cortex.

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Poster

PSTR345. Visual System: Response Modulation and Adaptation

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Program #/Poster #: PSTR345.14/DD5

Topic: D.06. Vision

Support: CIHR grant MOP-119498
FRQS doctoral fellowship

Title: Cortical State Effects Vary Across Different Neuronal Subclasses in the Primary Visual cortex.

Authors: *J. SOORIYAARACHCHI[1], C. ZHAN[3], C. L. BAKER[2];
[1]Dept. of Physiol., [2]Dept. of Ophthalmology, McGill Univ., Montreal, QC, Canada; [3]Sch. of Biomed. Engin., Southern Med. Univ., Guangzhou, China

Abstract: Primary visual cortex neurons respond selectively to features of visual stimuli such as spatial frequency and orientation. Linear-nonlinear models containing a single receptive field filter can be used to model phase-sensitive simple cells. However, more elaborate models having a combination of nonlinear subunits are required to model phase-invariant complex cells. Further, cortical neurons' trial-to-trial response variability can make the parameter estimation of these models more difficult. This variability is thought to be caused by cortical state fluctuations and its effects are poorly understood, potentially compromising RF estimation. Here we utilized extracellular recordings from the primary visual cortex of anesthetized, paralyzed cats with 32-channel NeuroNexus probes in response to rapid sequences of natural images (Talebi and Baker, 2012). We developed a simple compact convolutional neural network method to estimate receptive field models for both simple and complex visual cortex cells from their responses to natural images (Nguyen et al, bioRxiv 2023). A single model parameter from a parameterized rectifier unit in the model determined the simple vs. complex nature of the receptive field. We further analyzed the interaction between a neuron's spiking response and cortical state inferred from local field potentials (LFPs) and multi-unit activities (MUAs), to obtain better quantitative estimates of neuronal RFs. For this purpose, we extended our model architecture by incorporating LFP and MUA-driven filtering pathways in parallel with the aforementioned stimulus-driven receptive field pathway. The model parameters were estimated with an iterative regression algorithm with L2 regularization, using TensorFlow and Keras on a training dataset, along with a validation dataset for regularization. The variance accounted for (VAF) was used to

evaluate the model's predictive performance on a hold-back test dataset. Incorporating brain state effects provided significant improvements in VAF measures of predictive performance despite the additional model parameters. These improvements were dependent on the type of neuron, e.g. occuring more in complex than in simple cells, more in those with oriented than non-oriented RFs. These findings highlight the importance of incorporating the trial-to-trial variability due to brain state variations in studies of sensory coding at the single neuron level, and indicate that the nature of brain state variations may vary substantially across different types of neurons.

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Poster

PSTR346. Visual Attention, Learning, and Categorization

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR346.01/DD6

Topic: D.06. Vision

Support: DFG CRC1436 Grant TP B05

Title: Mechanisms of adjusting the resolution of color selectivity in human visual cortex

Authors: M.-C. SCHULZ[1], M. V. BARTSCH[1], C. MERKEL[2], H. STRUMPF[1], M. A. SCHOENFELD[1,3], *J.-M. HOPF[1,4];
[1]Univ. of Magdeburg, Magdeburg, Germany; [2]Otto-von-Guericke Univ., Magdeburg, Germany; [3]Kliniken Schmieder, Heidelberg, Germany; [4]Leibniz Inst. for Neurobio., Magdeburg, Germany

Abstract: The ability to distinguish between colors defines human behavior at a very fundamental level. The degree to which color needs to be resolved can vary substantially: Selecting a banana among cucumbers requires a coarse separation of yellow from green, judging the ripeness of banana involves a fine discrimination among greenish yellows. Here, we use magnetoencephalography to investigate the cortical processes underlying the flexible tuning of color selectivity to different resolution contexts. To this end, we recorded the event-related magnetic field (ERMF) response while participants judged whether the color of a probe patch in one visual field (VF) matched the color of a target patch in the opposite VF. As a control, participants were to ignore color and judge the vertical alignment of the patches. In three experiments we biased participants to set their resolution of color selectivity to a coarse, medium, or fine level by varying probe relative to the target color in large, medium, or small (equidistant) steps in color space, respectively. We thereby doubled distances such that step 1, 3, and 5 of the finer resolution condition corresponded with step 1, 2, and 3 of the next coarser resolution condition, permitting us to assess the ERMF response to a given color under the different resolution contexts. In line with previous observations, we find that for all resolution contexts, color attention modulates cortical activity in ventral extrastriate cortex in two phases between 160-250 and 250-300ms after stimulus onset. Under coarse conditions, a broad response

enhancement is seen for the target and neighbouring color. In contrast, for the medium and fine conditions, an initial response maximum appears for a more distant color (off-target enhancement), followed by a response attenuation of all non-target colours. Importantly, under fine resolution conditions, the initial off-target enhancement comes closer to the target than under medium resolution conditions. These observations are taken to suggest that the visual cortex adjusts color selectivity to resolution context by an initial phase of optimal tuning, that is followed by a phase non-target attenuation as proposed by the selective tuning model of visual attention.

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Poster

PSTR346. Visual Attention, Learning, and Categorization

Location: WCC Halls A-C

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Program #/Poster #: PSTR346.02/DD7

Topic: D.06. Vision

Support: NIH R01-EY029666

Title: Inverted feature preference in visual cortical neurons during feature attention and working memory

Authors: *D. MENDOZA-HALLIDAY[1], H. XU[1], R. DESIMONE[2];
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Abstract: Visual feature attention and working memory, two interrelated by distinct cognitive functions, are thought to act through mechanisms that depend on the similarity of neurons' feature preference and the attended or memorized feature. One dominant model of attention proposes that the modulation of visual cortex neuronal responses by feature attention is governed by a feature-similarity gain principle, whereby the strength of attentional modulation of each neuron positively correlates with the similarity between the attended feature and the neuron's preferred feature. Similarly, major models of working memory are based on the notion that the maintenance of feature representations in working memory is subserved by the persistence of activity of neurons whose preferred feature matches the memorized feature. Here we show that in motion direction-selective visual cortical areas (middle temporal or MT and medial superior temporal or MST) of two macaques performing a working memory-guided feature attention task, the feature preference of nearly half of the neurons during attention (MT and MST) and working memory (MST) was opposite to that predicted by these models: during the sustained attention period of the task, the responses of these MT and MST neurons were significantly decreased by attention to the preferred motion direction compared to the antipreferred direction. Similarly, during the working memory delay period, the preferred memorized motion direction of nearly half of these MST neurons was opposite to the preferred motion direction during the cue

presentation. Interestingly, along the visual processing stream, the percentage of neurons with these inversions of feature preference was highest in earlier processing stages, decreased downstream, and was lowest in later processing stages (lateral intraparietal area LIP and lateral prefrontal cortex LPFC). In an independent study in two additional macaques performing a delayed match-to-sample task, we replicated the above results by showing the occurrence of inverted feature preference during working memory in a large fraction of neurons in MST and a lower fraction in LPFC. Our results challenge the generality of the feature-similarity gain model of attention and persistent activity models of working memory, showing that these models can account for the activity patterns of neurons in downstream areas in prefrontal and parietal cortex but not in early visual and visual association cortex.

Disclosures: D. Mendoza-Halliday: None. **H. Xu:** None. **R. Desimone:** None.

Poster

PSTR346. Visual Attention, Learning, and Categorization

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR346.03/DD8

Topic: D.06. Vision

Support: NIH Grant SC2GM099626
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Title: The impact of task demands on spatial and feature based attention

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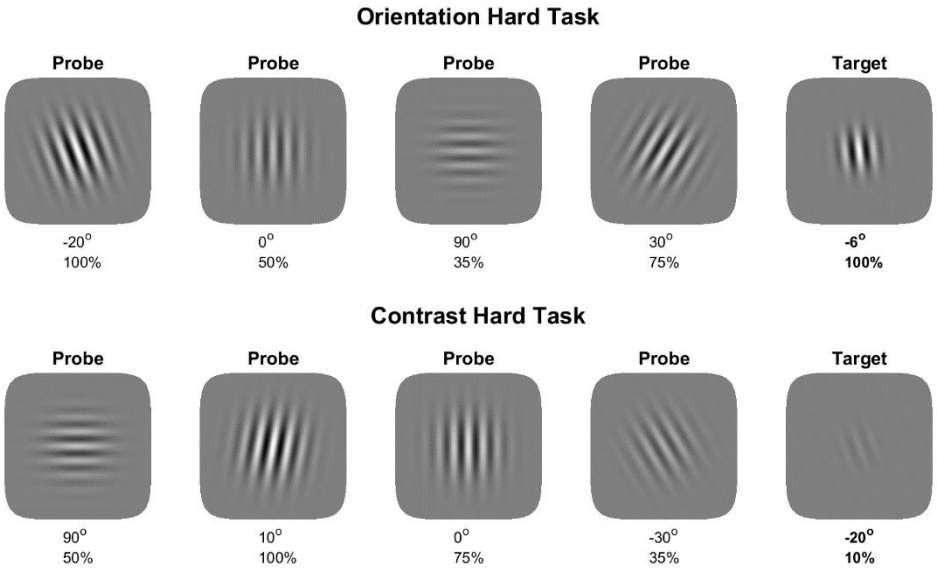
Abstract: Spatial and feature-based attention (FBA) are often considered as separate systems, with spatial attention (SA) boosting all features at a specific location and FBA boosting a specific feature at all locations. However, theoretical, behavioural and neurophysiological studies are increasingly showing that attentional allocation can be strategically adapted based on task demands. This begs the question of how task demands might affect the conjoint effects of SA and FBA. To address this, we recruited 21 participants to take part in an EEG experiment in which they performed a spatially cued orientation judgement (left/right tilt from vertical) on Gabor stimuli where difficulty was driven either by fine orientation differences or by low target contrast (Fig 1). We hypothesized that attentional allocation across orientation, contrast and space may depend on the locus of task difficulty. Attentional modulations were captured via visual evoked potential components elicited by larger "probe" Gabors presented at a range of orientations, contrasts and both locations. Targets (15% of stimuli) were either high contrast and low tilt or low contrast and high tilt, and were randomly interspersed amongst probes, appearing at the cued location 83% of the time.Both tasks elicited similar contrast functions of attention (response gain) as well as an off-target boosting effect whereby the probed orientations that

received the highest gain were more extreme than the targets, an effect which was greater in the orientation-hard task. In the contrast-hard task, a unique interaction between SA and FBA was observed in the P1-N1 complex whereby SA was entirely absent for the non-informative vertical orientation, despite being robust at other orientations. These results showcase some alteration and some consistency in the allocation of attention depending on the nature of task demands. Moreover, they demonstrate that in some circumstances SA and FBA can act in a mutually dependent manner.

Figure 1: Two example sequences of five stimuli, one for each task, culminating in a target.



Disclosures: **K.S. Mohr:** None. **M.I. Vanegas:** None. **S.P. Kelly:** None.

Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR346.04/DD9

Topic: D.06. Vision

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Title: Selective communication of feature information through the visual hierarchy during attention

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Abstract: Visual attention is known to improve behavioral performance pertaining to the attended spatial location, feature, or object. The underlying mechanistic principles governed at the level of neural populations are yet to be fully understood. Research to date has largely associated the improvement in behavioral performance with two cortical processes, generally thought to be distinct: 1) a more faithful representation of the attended stimuli, 2) a higher degree of information transmission from areas at lower levels in the cortical hierarchy e.g. visual areas V1, V2, etc. to areas at a higher level, e.g. V4, PFC, etc. In this study, we present a unifying framework of how selective attention achieves both of these processes and how both may serve each other with the goal to facilitate behavior. We simultaneously recorded neural populations in four visual areas i.e. V1, V2, V4, and MT, while the rhesus monkey was engaged in a 4-AFC feature-based attention task. The monkey was required to detect a change in color or motion direction of the moving dot stimuli. Attention was manipulated by making one type of feature change more probable than the other within a block. We observed an improvement in accuracy for the attended feature compared to a neutral cue condition and a decrement in accuracy for the changes in the unattended feature. To estimate the strength of the stimulus signal in the neural activity in a time-resolved manner, we designed the stimuli in such a way that the motion coherence changed from low to high at one frequency and the color of the dots changed from red to gray at another frequency. This evoked two distinct SSVEPs associated with the two features. Using dimensionality reduction and rank regression methods, we estimated distinct dimensions in the neural subspace that either encoded the stimulus with the highest SNR or communicated maximum information from lower-order areas i.e. V1/V2 to higher-order areas V4 and MT. Our preliminary results showed that behaviorally relevant information gets communicated better in the cortical hierarchy. In the future, we aim to find the relationship between the strength in communication and the likelihood of correct performance.

Disclosures: B. Sahoo: None. **A. Snyder:** None.

Poster

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Topic: D.06. Vision

Support: NSF DBI 2015317

Title: Neural network application of bio-inspired stereoscopic vision system

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Abstract: When attempting to gauge depth through binocular vision, animals and robots alike utilize the technique of stereopsis. This process triangulates depth based on the location of an object on the retina of each eye and the pupillary distance. However, a major challenge to this approach is determining which features in each retina's image represent the same object in the visual field. Solving this 'correspondence problem' can be computationally intensive and thus excludes some applications where reading depth is necessary. One model insect in which to study stereopsis is the praying mantis, which is effective in perceiving the location of predators and prey around them. Although the precise details of the mechanism by which depth is perceived is unclear, the characteristics of mantis stereopsis behavior has inspired a stereoscopic vision system that does not rely on solving the correspondence problem. We show the implementation and testing of a mantis based neural network model to demonstrate stereopsis without correspondence in a robotic platform.
The model, developed in prior work, is a winner-take-all neural network that reports the azimuth and elevation of the nearest object in the visual scene. The network is trained on computer-generated scenes of spherical targets. To test this model in a less idealized setting, a robotic chassis with two cameras has been created. The implemented model is shown various scenes with multiple visual targets of varying ocular size, distance from the chassis, and height relative to the horizon. The model perceives each frame independently, enabling the use of static or dynamic scenes. From the scene, the model attempts to determine the azimuth angle of the closest object. The model output is then compared to ground truth data to gauge accuracy. Preliminary results have shown that this implementation has comparable accuracy to the computer model (Model: 70% success rate; Robot: 51% success rate within 7.5°, 75% success rate within 15°). Through further testing, the properties of the model in the real-world will demonstrate the possibility of binocular depth perception without solving the correspondence problem.

Disclosures: D. McDonald: None. **V. Nityananda:** None. **J.C.A. Read:** None. **N. Szczecinski:** None.

Poster

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Topic: D.06. Vision

Support: NIH Grant R03DA050962

Title: Larva in the loop, utilizing optokinetic response of Zebrafish larvae as a control schema for a line following robot through a closed-loop 2D virtual reality system

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Abstract: The optokinetic response (OKR) in Zebrafish (Danio Rerio) had been characterized for its robust response to visual stimuli. Many works had displayed this responsiveness through various means ranging from simple rotating drum systems to complex 3-dimensional spherical LED systems. Expanding on these works, we developed a novel closed loop control schema to drive a robot utilizing the OKR of Zebrafish larvae. Our system keeps a larva's body constrained via an agarose mold while allowing eye rotation and vision. The larva is then put under a microscope camera to apply real time computer vision in order to track its eye orientation. Relative eye angle data is then parsed through an algorithm and used to send movement signals to a robot on a lined track. The robot returns its relative position to the line as an OKR animation which is displayed on an LCD screen in the ventral plane of the larva, thus closing the loop. Through this work we show the capability of larvae OKR to keep a robot on track and complete multiple laps. This novel control schema has potential in studying fundamental behaviors of Zebrafish larvae cognition noninvasively.

Disclosures: J. Jutoy: None. **E. Jung:** None.

Poster

PSTR346. Visual Attention, Learning, and Categorization

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Program #/Poster #: PSTR346.07/DD12

Topic: D.06. Vision

Support: CIHR Project Grant
NSERC Discovery Grant

Title: Reverse correlation reveals visual attention to features and space in mice

Authors: *J. LEHNERT[1], K. CHA[2], J. HALPERIN[1], K. YANG[1], D. ZHENG[1], A. KHADRA[2], E. P. COOK[2], A. KRISHNASWAMY[2];
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Abstract: Visual attention allows the brain to evoke behaviors based on the most important visual features. Mouse models offer immense potential to gain a circuit-level understanding of this phenomenon, yet, how mice distribute attention across features and locations is not well understood. Here, we describe a new approach to address this limitation, by training mice to detect weak vertical bars in a background of dynamic noise while spatial cues manipulated their attention. By adapting a reverse correlation method from human studies, we linked behavioral decisions to stimulus features and locations. We show that mice deployed attention to a small rostral region of the visual field. Within this region, mice attended to multiple features (orientation, spatial frequency, contrast) that indicated the presence of the weak vertical bars. This attentional tuning grew with training, multiplicatively scaled behavioral sensitivity, approached that of an ideal observer, and resembled the effects of attention in humans. Taken

together, we demonstrate that mice can simultaneously attend to multiple features and locations of a visual stimulus.

Disclosures: **J. Lehnert:** None. **K. Cha:** None. **J. Halperin:** None. **K. Yang:** None. **D. Zheng:** None. **A. Khadra:** None. **E.P. Cook:** None. **A. Krishnaswamy:** None.

Poster

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Program #/Poster #: PSTR346.08/DD13

Topic: D.06. Vision

Support: SFB 1372 Neu06 - Compass and map neurons in the avian brain

Title: Investigating neural correlates of spatial cognition in the pigeon hippocampus: resizable arena and chronic neural recording

Authors: *M. INDA, G. H. GADEA, C. S. SEVINCIK, R. PUSCH, O. GUNTURKUN; Dept. of Biopsychology, Fac. of Psychology, Ruhr Univ. Bochum, Bochum, Germany

Abstract: Spatial cognition, a fundamental aspect of navigation and survival, has been extensively studied in mammals, revealing the existence of specialized cell types such as place cells, grid cells, and head direction cells. Some of these cell types have also been found in avian species like titmice, quails, barn owls, streaked shearwaters, and black-capped chickadees. Pigeons are capable of navigation and command excellent spatial cognition, but the neurons underlying their neural basis are not well known. Previous research has demonstrated that neurons involved in spatial codings, such as place cells, can be influenced by factors like distances from walls or the shape of the surrounding environment. These findings have been consistently supported by experimental studies, which have shown that place cells are indeed influenced by these spatial features. Additionally, several studies have highlighted the significance of encoding geometry and topology in hippocampal coding, as these aspects enable the representation of continuity and sequence. However, this particular aspect of spatial coding has often been overlooked, despite its potential to offer important computational advantages. To validate this hypothesis within the cognitive framework of pigeons, we developed an experimental arena that could be resized. We conducted experiments in pigeons to investigate the neurons associated with spatial cognition in the hippocampus. We built an enclosed space referred to as the "arena", 5m x 2m x 2m, where the pigeons could freely move. We installed 18 food feeders on the ceiling of this arena, enabling us to drop food onto the floor randomly. The long axis direction of the arena can be freely resized, and recordings were conducted at half size (2.5 m x 2 m x 2 m) and one-third size (1.67 m x 2 m x 2 m). An 8-channel, 4-shank linear silicon probe was used to record neural responses. The probe was inserted into the pigeon hippocampus and the microdrive was used to periodically change the position of the electrodes, resulting in chronic neural response recordings for two months or longer. The pigeons were

allowed to move freely in the arena and neural responses were recorded wirelessly with neural loggers for 30 minutes to 1 hour. Furthermore, we have succeeded in extracting behavioral parameters such as the moving trajectory, head direction, and speed, by using markerless pose extraction with a machine-learning approach. Through this comprehensive approach, we successfully obtained chronic neural responses from the pigeon hippocampus.

Disclosures: M. Inda: None. **G.H. Gadea:** None. **C.S. Sevincik:** None. **R. Pusch:** None. **O. Gunturkun:** None.

Poster

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Program #/Poster #: PSTR346.09/DD14

Topic: D.06. Vision

Title: An Orientation-Change Detection Task for Measuring the Scale of Spatial Attention Effects on Neural Firing in Primate Visual Cortex

Authors: *J. AMODEO, A. DISNEY;
Duke Univ., Durham, NC

Abstract: An Orientation-Change Detection Task for Measuring the Scale of Spatial Attention Effects on Neural Firing in Primate Visual Cortex
Attention is a stupendously complicated term often applied to describe the perceptual effects of increased discriminability between a sensory signal of interest and a 'noisy' background. Physiologically, this sensory signal equates to a neural firing pattern representing a component (e.g., tree) of the physical world; attention then amplifies and sharpens the tuning of neural firing elicited by a physical object attribute of interest (feature attention) and/or a specific physical location (spatial attention). The mechanism(s) accomplishing attention are unclear, but the spatial scale of their action is remarkably fine, with visual cortical area V4 neurons showing distinct responses when attention is directed to different stimuli in the same receptive field (RF) (Reynolds et al. 1999). Just how fine the spatial scale(s) of attention might be remains an open question, the answer(s) to which will be key to filtering hypotheses on biological mechanisms. To measure the spatial scale of attention effects on neural firing in visual cortex, we developed a four-stimulus, change-detection task, inspired by a two-stimulus task used by Cohen and Maunsell (2010). We find that adult macaque monkeys can perform this task both with (spatially focused attention) and without (spatially diffuse attention) spatial cues, and present preliminary data from electrophysiological recordings in the primary visual cortex (V1) during task performance.

Disclosures: J. Amodeo: None. **A. Disney:** None.

Poster

PSTR346. Visual Attention, Learning, and Categorization

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Program #/Poster #: PSTR346.10/DD15

Topic: D.06. Vision

Title: Generalization from one exemplar in mice

Authors: *M. NUÑEZ, L. ZHONG, F. DU, C. STRINGER, M. PACHITARIU;
Mechanistic Cognitive Neurosci. / Computation & Theory, Janelia Res. Campus, Ashburn, VA

Abstract: Learning general principles based on a single example is a characteristic of advanced cognitive abilities. While this type of learning poses computational challenges for artificial agents, many animals can effortlessly accomplish it. To examine the phenomenon of generalizing from a single example in a laboratory setting, it would be advantageous to create appropriate behavioral tasks using model organisms like rodents. In our study, we demonstrate that mice can exhibit one-exemplar generalization when presented with visually complex stimuli such as "leaves" and "rocks". We trained mice to differentiate between individual images from two texture categories, using water rewards as positive reinforcement for one of the images. After learning, we introduced new images from the same texture categories, but without any reward association. Mice responded to the new image from the previously rewarded category, indicating a generalization effect, whereas they did not respond to the new image from the unrewarded category.
To investigate the neural properties underlying generalization, we simultaneously recorded activity from large populations of neurons in the primary and higher-order visual cortex of naive mice. During these recordings, we presented several textures, including the same set of images used in the training phase. By employing simple linear decoders trained on neural responses to individual examples, we observed that the decoders were able to generalize to new examples, similar to the behavior of the mice. We also identified variations in generalization properties across different depths and visual areas.
Furthermore, we are currently analyzing data obtained from recordings of large neural populations in trained mice to explore potential changes in neural coding resulting from the learning process. These findings establish mice as a viable model for studying generalization from a single example, opening up possibilities for employing various neuroscience techniques to unravel the algorithms employed by the brain to generalize.

Disclosures: M. Nuñez: None. **L. Zhong:** None. **F. Du:** None. **C. Stringer:** None. **M. Pachitariu:** None.

Poster

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Program #/Poster #: PSTR346.11/DD16

Topic: D.06. Vision

Support: EY022951

Title: Plasticity of cortical networks during visual associative learning

Authors: *H. BATCHELOR[1], C. LI[2], J. A. CARDIN[3];
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Abstract: Processing reward-associated cues is disrupted in several psychiatric disorders, including schizophrenia and addiction. However, the cortical dynamics that support cue-reward learning remain poorly understood.
Little is known about how cortical activity changes during associative learning, where one stimulus is paired with reward (CS+), and another is not (CS-). Plasticity has been observed in distinct cortical regions after associative learning in animal models, but learning is not a unitary process. Indeed, previous work has found dynamic changes in pyramidal neuron activity in the primary visual cortex at different times throughout the learning process. Until recently, it has not been possible to longitudinally track neural activity in several cortical regions simultaneously throughout all learning stages. To address this, we utilized widefield 'mesoscopic' calcium imaging to record neural activity across the dorsal cortex of mice performing a visual associative learning task over several weeks. Notably, our task design allows us to distinguish early, stimulus non-specific learning from late, stimulus-specific learning and reversal learning.
We have identified broad activation across several cortical regions in response to visual associative cues. During early stages of learning, visually evoked cortical activity decreases to both the CS+ and the CS-. However, after animals reach behavioral proficiency, cortical responses increase for CS+ and decrease for CS-. Reversal learning is associated with a decrease in cortical responses for the new CS- and an increase for the new CS+. These patterns remain after regressing out confounding variables associated with behavioral arousal, such as running and facial motion, which are known to independently modulate cortical activity. These data suggest that visual responses emerge during learning, exhibiting selectivity and sensitivity to stimulus value. Using correlational analysis, we have further uncovered changes in the spatiotemporal structure of activity across cortico-cortical networks, suggesting a remapping of cortical functional connectivity during learning. Together, these data provide new insight into cortical plasticity and lasting changes in corticocortical functional connectivity throughout learning.

Disclosures: H. Batchelor: None. **C. Li:** None. **J.A. Cardin:** None.

Poster

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Israel Science Foundation (ISF 526/17)
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Ariane de Rothschild Women's Doctoral Program

Title: Improving the efficiency of repetition-based learning with brief memory reactivations engages distinct neural mechanisms

Authors: *T. KONDAT[1], H. SHARON[4], I. TAVOR[1,2], N. CENSOR[1,3];
[1]Sagol Sch. of Neurosci., [2]Sackler Fac. of Med., [3]The Sch. of Psychological Sci., Tel Aviv Univ., Tel Aviv, Israel; [4]Tel Aviv Sourasky Med. Ctr., Tel Aviv, Israel

Abstract: The adult human brain shows remarkable plasticity following perceptual learning, resulting in improved visual sensitivity. However, such improvements in visual perception commonly require extensive practice and hours of training. Therefore, an ongoing challenge has been to improve the efficiency of such prolonged repetition-based learning. Here we aimed to uncover whether improving the efficiency of repetition-based learning with brief memory reactivations engages distinct neural mechanisms. To address this question, 40 participants performed a standard texture discrimination task (TDT). The memory was encoded and consolidated on a Day1 standard session (252 trials), during which the discrimination threshold was measured. 20 participants then returned for three sessions on separate days, during which the encoded memory was reactivated with only five near-threshold memory reactivation trials (Reactivation group). The additional 20 participants performed full standard daily sessions (252 trials, Repetition group). A standard retest session was performed on Day5 to measure the final discrimination threshold. Pre- and post-learning fMRI scans were performed on Day1 and Day5. Results show that the Reactivation group exhibited significant learning (mean learning gains = 24.7% ± 4.1% SE), comparable to the Repetition group (25.4% ± 3.9%, p=.90, BF_{01}=3.22), indicating remarkable efficiency by reducing training duration. Following reactivation-induced learning, activity relative to baseline in the bilateral intra-parietal sulcus (IPS) was greater compared to repetition-based learning. Furthermore, resting-state functional connectivity between the inferior parietal lobule and the middle and inferior temporal gyri was reduced following repetition-based learning, but not reactivations, a reduction that was negatively correlated with behavioral improvement. These results suggest that improving the efficiency of repetition-based learning with memory reactivations recruits distinct neural processes while leading to similar behavioral gains. These novel mechanisms demonstrate enhanced engagement of higher-order control and attentional resources following practice. Temporal-parietal resting functional connectivity changes also imply differential offline perceptual learning and memory representations. These findings may shed light on unique mechanisms underlying efficient learning approaches, providing valuable insights for their future applications.

Disclosures: T. Kondat: None. **H. Sharon:** None. **I. Tavor:** None. **N. Censor:** None.

Poster

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Program #/Poster #: PSTR346.13/DD18

Topic: D.06. Vision

Title: Class IIa HDACs support the encoding of familiarity and visual cortical oscillations

Authors: *M. ZIMMERMAN[1], A. MAXIMOV[3], A. A. CHUBYKIN[2];
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Abstract: The ability of the brain to translate sensory experiences into memory formation is a critical step to perceiving the world around us. This understanding of the novel and known stimuli through experience equips us to respond appropriately through daily life. With exposure to a specific stimulus comes about waves of dynamic experience-dependent changes in the brain that affect neuronal wiring, synaptic function, and the formation of memory. In response to sensory experience, early response genes (ERG) encoding transcription factors (TFs) induce changes through downstream target genes. Class IIa histone deacetylases (HDAC) are a family of chromatin-binding proteins that help regulate TFs access to DNA. Specific isoforms of this family, namely HDAC4 and HDAC5 genes, are necessary for normal cognition, synaptic plasticity, and the control of ERG pathways during memory formation and encoding. The role of these genes in subserving visual familiarity, however, remains to be known. By utilizing an HDAC4,5 double knock-out (DKO) strain of mice and acute head-fixed silicone probe recordings in the primary visual cortex, we were able to characterize the role of these isoforms in visual familiarity and memory. We discovered the emergence of low-frequency theta oscillations in the local field potentials that were specific to the familiar stimulus in the primary visual cortex (V1) of wild-type mice. The DKO mice, however, showed no defined peaks but rather sustained activation following the onset of the stimulus, likely due to the impaired ability of the network to undergo synaptic plasticity. Time-frequency analysis demonstrated a significant difference in low frequency (theta, alpha, and beta) band power post experience between the two groups. Further, the WT single unit activity showed the emergence of oscillations and definitive cycles following familiarity, while the DKO units were collectively more sustained with one single peak after visual stimulation. To confirm that these changes were not caused by impaired vision in DKO mice, we measured pupillary dynamics and discovered that both groups of mice demonstrated matching optokinetic responses during saccades. Collectively, our research findings highlight the disrupted neural activity observed in HDAC4,5 double knockout (DKO) mice in response to visual familiarity. Based on these compelling results, we consider this mouse strain to be a highly promising model for further investigations into impaired cognition, synaptic plasticity, and memory encoding.

Disclosures: M. Zimmerman: None. **A. Maximov:** None. **A.A. Chubykin:** None.

Poster

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Topic: H.06. Social Cognition

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BBRF Young Investigator Grant
Rockefeller Kavli Neural Systems Institute Pilot Grant
NIH NEI R21 Grant EY031486

Title: Examining populations of face-selective cells with calcium imaging in marmoset monkeys

Authors: *D. HILDEBRAND[1], S. OTERO CORONEL[1], J. DEMAS[1], S. GRØDEM[2], K. K. LENSJØ[2], G. H. VATNE[2], B. CHEN[1], F. TEJERA[1], M. FYHN[2], A. VAZIRI[1], W. FREIWALD[1]; [1]The Rockefeller Univ., New York, NY; [2]Univ. of Oslo, Oslo, Norway

Abstract: A major challenge to uncovering the computations and circuits that enable face perception is the disparity in the scales at which the face-processing system has been studied. We know a great deal about the broad properties of face areas from fMRI and individual 'face cell' tuning properties from single-cell electrophysiology. However, it remains difficult to investigate how populations of face cells work together to represent faces without simultaneous activity measurements from many individual face cells. To fill this gap, we developed an approach for recording calcium dynamics from inferotemporal cortical neuron populations in awake, head-restrained marmosets using two-photon mesoscopy. Using this approach, we are now recording from thousands of neurons within and surrounding the posterior dorsal (PD) face area. We are now analyzing these data to better understand face representations in PD.

Disclosures: D. Hildebrand: None. **S. Otero Coronel:** None. **J. Demas:** None. **S. Grødem:** None. **K.K. Lensjø:** None. **G.H. Vatne:** None. **B. Chen:** None. **F. Tejera:** None. **M. Fyhn:** None. **A. Vaziri:** None. **W. Freiwald:** None.

Poster

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Natural Science Foundation of China GrantNSFC 32271081

Title: Emergency and Development of Word Recognition Abilities in the Object Space Model

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Abstract: Reading systematically engages the lateral occipitotemporal sulcus, specifically the visual word form area (VWFA). While the recycling hypothesis posits that the VWFA emerges through repurposing a pre-existing region for recognizing written words, the original function of this region and how experience shapes its representation remains largely unexplored. The object space model proposed that this area may initially represent word-related features in non-word objects close to words in object space, subsequently expanding its representation through word training. Here we used functional magnetic resonance imaging (fMRI) and deep neural networks to test this hypothesis. Initially, we tested the word discrimination ability in the pretrained AlexNet, a widely used convolutional network trained on the ImageNet dataset. Surprisingly, the network exhibited significant word discrimination ability. Removing images containing words from the training dataset did not significantly affect this ability. Instead, we found that the pretrained network can effectively extract and utilize implicit, useful features from non-word images with DeepDream methods. Furthermore, training different networks with different sets of images revealed that images closer to the words in the object space contain more word-related features. Next, we employed fMRI techniques to measure the brain response of 7 subjects to 20 words and 80 non-word objects. The results revealed that objects close to words in the object space elicited stronger responses in the VWFA, bolstering the evidence favoring the object space model as an effective framework for explaining object representation in the human brain. Lastly, by systematically varying the correlation between word identity and task requirements, we found that task-irrelevant exposure hindered word representation and impeded word discrimination ability. Conversely, as the degree of association increased, both the representation area and word discrimination ability enhanced, indicating that neural networks develop the object space through supervised rather than exposure-based learning rules. This study provides compelling evidence that the VWFA initially functions to represent word-related features in non-word objects close to words within the object space, and reading training further refines the representations to fulfill real-life demands, aligning with supervised learning principles. Collectively, our research underscores the object space model as a comprehensive framework for understanding the emergence and evolution of category-specific brain areas, shedding light on future electrophysiological study.

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Poster

PSTR346. Visual Attention, Learning, and Categorization

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR346.16/DD21

Topic: D.06. Vision

Support: MIC under a grant entitled “R&D of ICT Priority Technology (JPMI00316)

Title: Insights from Artificial Neural Networks on Generalization of Color Information in Object Recognition

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Abstract: Once humans acquire knowledge of a novel object in a dimly illuminated environment, they often show capability to recognize that object under illumination. This phenomenon suggests that the inclusion of additional information, such as color, which was not utilized during the initial learning phase, does not impede subsequent recognition. This resilience would be attributed to the generalization ability of the trained function of the human brain. In the brain, color and other static features in visual information are processed along the ventral pathway of visual processing. Recent studies have proposed that Artificial Neural Networks (ANNs) effectively model this pathway. However, the most suitable type of ANN for computational modeling of the ventral pathway are under intensive debate. In this study, we aim to move this debate forward by focusing on how color information is processed by ANNs. We conducted transfer learning experiments using two types of pretrained ANNs: Convolutional Neural Networks (CNNs) and Vision Transformers (ViTs). We trained these ANNs to classify 100-class grayscale images. Then we compared the performance of these models on both color and grayscale images of the same set of objects to measure their tolerance to additive color information. Both types of models achieved accuracies exceeding 80% (with a chance level of 1%) for both the color and grayscale images. The CNNs showed greater accuracy for grayscale images than for color images. The result seems to be understandable by taking into account that the models was exclusively trained on grayscale images. The ViTs, however, exhibited superior performance on color images than grayscale images despite the absence of color information in the training set. Our results indicate that in the ViTs color information does not interfere with but enhances the classification function which was trained only by the grayscale images. We also conducted additional experiments of the transfer learning for the classification of binary images, and the ViTs more clearly achieved higher accuracy for color images than for binary images, which was not shown by the CNNs. In conclusion, our study revealed the generalization ability in color processing depended on the types of ANNs, and it underscored a similarity between human and ViTs. This finding would deepen our understanding of neural computations in the ventral visual pathway. As potential research, comparing performance on similar experiments between transfer learning of ANNs and unfamiliar-objects learning of humans could help advance our understanding of generalization of color information in object recognition.

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Poster

PSTR346. Visual Attention, Learning, and Categorization

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Program #/Poster #: PSTR346.17/DD22

Topic: D.06. Vision

Support: NIH R01 EY019041
DOD VBFF

Title: Representation of direction, color, and category in primate posterior parietal cortex during double-feature categorization

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Abstract: The ability to categorize objects into functionally-relevant groups (e.g. edible vs inedible) based on visual attributes such as shape and color is a remarkable feat of the primate visual system. Our group has extensively studied the neural representation of learned abstract visual categories in the posterior parietal cortex (PPC) of non-human primates. However, previous studies focused on categorizing visual stimuli based on a single feature (e.g. direction of motion). In many cases, multiple visual features of an object must be integrated in order to correctly assign it to a behavioral category. To understand the neural representations underlying more complex category structures, we trained one monkey on a double-feature visual categorization task in which stimuli are assigned into one of two learned categories based on both direction of motion and color. Six motion directions (36° apart) and six colors (ranging from red to yellow) were combined to generate a set of visual stimuli. The category boundary was defined such that color and motion features needed to be integrated in order to determine category membership. We recorded neural activity of 1179, 550, and 289 neurons from the lateral intraparietal (LIP), 7a, and medial superior temporal (MST) areas of PPC respectively using 24-channel Plexon V-probes (n = 30 sessions). Using two-way ANOVA ($p<0.01$) we assessed direction and color selectivity in each area. All three areas included neurons that were direction selective: 16.3% of units in area LIP, 3.6% of units in area 7a, and 13.1% of units in area MST. Color selectivity was less prevalent in 7a and MST (2% in 7a and 3.8% in MST) compared to LIP (11.6%). Using one-way ANOVA ($p<0.01$) we assessed category selectivity in each area. 2.6% of the neurons in area LIP, 1.1% of the neurons in area 7a, and 3.5% of the neurons in area MST were category selective. We additionally assessed the representation of direction, color, and category at the population level using a support vector machine decoder trained on pseudo-populations of neurons recorded during all sessions (80% of trials for training, 20% for testing). In LIP, we observed 60.0% (20% chance level), 45.6% (20% chance level), and 71.8% (50% chance level) direction, color, and category decoding performance, respectively. For 7a, decoding performance was 20.7%, 33.5%, and 58.7% for color, direction, and category. In MST, decoding performance was 26.4%, 51.1%, and 66.3% for color, direction, and category. Ongoing analysis focuses on estimating and comparing response latency and dimensionality of neural representation of color, direction, and category in these regions.

Disclosures: **R. Cooley:** None. **P. Moghimi:** None. **D.J. Freedman:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.01/DD23

Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Thalamic Arousal Mechanisms in Transient Visual Perception

Authors: *Q. XIN[1], C. J. CHU[2], S. MAJUMDER[3], S. AERTS[4], S. I. KRONEMER[8], D. S. JIN[3], T. YADAV[5], J. LIU[6], A. KHALAF[6], A. AVILA[9], B. GEOGHAN[9], R. KHOZEIN[9], T. TCHENG[10], M. MORRELL[11], M. J. CROWLEY[5], M. RICHARDSON[12], I. H. QURAISHI[7], H. BLUMENFELD[13];
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Abstract: Conscious perception of transient sensory stimuli is the foundation of many daily tasks and implicated in perceptual disorders. Understanding the neural mechanisms of this process is thus of broad interest. The thalamus is traditionally understood to participate in subcortical arousal systems that regulate temporally sustained states of consciousness and impact perception. However, it remains to be shown whether the thalamic arousal system also plays a more active role in modulating the perception of transient, moment-to-moment stimuli. To this end, we directly delivered neurostimulation to the thalamus in human subjects using implanted closed-loop thalamic neurostimulators in patients with epilepsy. By aligning the timing of the neurostimulation with visual perceptual events, we could test if neurostimulation augments the perception of visual images. Specifically, we recruited four patients with epilepsy chronically implanted with depth electrodes (RNS® System, NeuroPace, Inc.) targeting the thalamic intralaminar centromedian nucleus. During the behavior task, the participants were asked to report if they perceived a 50ms human face image presented with at-threshold opacity against a noise background. The participants were also asked to report the location of the image as validation. A 300ms burst of biphasic square waves of neurostimulation at 100Hz was delivered either (1) during the onset of the face image or (2) 2 seconds afterward. A third condition of no neurostimulation was included as a baseline. The charge density of the neurostimulation was individually titrated to each subject's maximum level avoiding side effects of paresthesias. Perceptual sensitivity was calculated as the percentage of trials where the participants reported that they perceived the image and subsequently identified the correct position. We found that

among the trials where the image was presented on the side of the screen contralateral to that of thalamic neurostimulation, perceptual sensitivity for the concurrent neurostimulation condition increased by 13.4% ±8% (n=4) compared to the no-neurostimulation condition. This effect was not observed in the delayed neurostimulation condition (0.4% ±14%). Moreover, the perceptual sensitivity in the concurrent stimulation condition appeared to be positively correlated with the charge density of the neurostimulation (spearman's R=0.60), albeit not reaching statistical significance, likely due to the smaller sample size. Our preliminary results suggest that the thalamic arousal system may actively regulate the perception of conscious events through its influence on arousal states at the moment of perception.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.02/DD24

Topic: H.02. Perception and Imagery

Title: Machine learning to detect the brain networks related to auditory conscious perception without subject report

Authors: *S. L. AERTS[1], A. MANGLA[2], K. L. CHRISTISON-LAGAY[3], S. I. KRONEMER[4], Q. XIN[5], T. YADAV[1], H. BLUMENFELD[3];
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Abstract: Neuroimaging methods such as functional magnetic resonance imaging (fMRI) can provide some insight into a patient's level of consciousness, yet the precise brain areas and timing of conscious awareness is still in debate. A major confound in consciousness research is the reliance on subject report in behavioral tasks, which biases previous work towards the search for brain areas responsible for perceptual report. We recently found that large scale cortical and subcortical networks are involved in perception even without report via a visual task. Our goal is to extend this work to auditory perception to determine if these findings generalize to across sensory modalities. To assess perception without the requirement for report, auditory stimuli will be *classified* as perceived or nonperceived by a machine learning model trained on reflexive pupil/eye movement. Subjects are presented at-threshold auditory stimuli and report their perception in the target ear only (Report Condition); during the same trial, an asynchronous sound is presented to the nontarget ear (No-Report Condition). Eye metrics are recorded throughout. In the Report condition, we found that subjects (n=25) performed well: perception

rate was 55% for threshold-level stimuli; false positive rate (blank trials) was 10%; sound identification accuracy for perceived target sounds was 88%, and accuracy for nonperceived targets was 34% (chance: 33%). Further, eye metrics differed between perceived and nonperceived auditory stimuli, despite the loud background noise of the MRI. We found significantly different post stimulus eye responses between perceived vs. nonperceived pupil diameter (200-2500ms), blink rate (1130-1880ms) and microsaccade rate (153-510ms) via permutation testing. Ongoing work will use eye-metric data from the Report condition to train a machine learning model to classify trials as perceived or not; this model will classify No-Report stimuli as perceived or nonperceived. The classifier will be used to examine functional MRI for reported perceived vs. classified perceived vs. nonperceived auditory stimuli to investigate auditory perception without report. If eye metrics can be used to classify auditory perception, it has the potential to be used widely to study conscious perception purified from the confound of overt report.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.03/DD25

Topic: H.02. Perception and Imagery

Title: Shared fMRI transient increases in subcortical arousal networks across sensory modalities

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Abstract: Subcortical arousal systems play an important role in controlling sustained changes in attention and conscious awareness. Recent studies showed evidence that these systems have a major influence on short-term dynamic modulation of attention. In this study, we investigate shared subcortical arousal networks across different perceptual modalities (vision, audition, taste, and touch) during transient changes in attention using block and event-related fMRI paradigms. To identify common subcortical networks across these perceptual modalities, we analyzed fMRI datasets collected while subjects (N=1581) are performing visual, auditory, tactile, and taste perception tasks. Eleven tasks (6 visual, 2 auditory, 1 tactile, and 2 taste tasks) selected from five public datasets were employed. The public datasets included Human Connectome Project dataset (visual and auditory tasks), UCLA Consortium for Neuropsychiatric Phenomics LA5c dataset (visual task), as well as datasets collected at Yale University (taste perception tasks), Glasgow University (auditory task), and the Jagiellonian University (tactile task). Percentage change in whole-brain BOLD signal was calculated and a cluster-based spatiotemporal permutation test was employed to identify the statistically significant changes in BOLD percentage change with

respect to baseline. A conjunction analysis was performed across perceptual modalities on the statistically thresholded brain maps to identify subcortical regions sharing common activity. Similarly, a disjunction analysis was performed on the statistically thresholded brain maps to identify brain regions unique for each sensory modality. The disjunction analysis showed that the primary sensory cortices are the main unique regions for each perceptual modality while the conjunction analysis revealed a shared network of subcortical arousal systems that show transient increases in midbrain tegmentum and thalamus across tasks and modalities, as well as less consistent increases in nucleus accumbens, nucleus basalis and striatum. Identifying these subcortical networks is critical for understanding mechanisms of normal attention and consciousness, and may help facilitate optimal subcortical targeting for therapeutic neuromodulation.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

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Program #/Poster #: PSTR347.04/DD26

Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: Uncovering spatiotemporal modulations in intracranial EEG during tactile conscious perception

Authors: *T. YADAV[1], T. BUI[2], S. MAJUMDER[2], K. L. CHRISTISON-LAGAY[1], D. S. JIN[2], J. DING[2], N. FREEDMAN[2], M. GUSSO[3], S. I. KRONEMER[3], S. L. AERTS[1], I. FREEDMAN[3], K. WU[3], I. H. QURAISHI[1], A. SIVARAJU[1], E. DAMISAH[1], H. BLUMENFELD[1];
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Abstract: A better understanding of the neural processes of normal consciousness can provide helpful insights about brain networks essential to conscious perception. We previously studied neural mechanism of normal conscious perception in the visual and auditory domain and found distinct intracranial EEG (icEEG) power changes in Perceived vs Not Perceived trials. We found early changes confined to sensory cortices for Not Perceived trials, while for Perceived trials icEEG activity also increased in cortical association networks. Our goal was to further investigate whether a similar pattern of activity would be observed using a tactile perceptual threshold task with icEEG recordings. The task involved delivery of a vibration (40ms) at the participant's perceptual threshold to their non-thumb fingers via vibrating tactor with stimulated finger determined in a randomized fashion, and no vibration delivered in 14% of the total trials. The perceptual threshold of each participant was adjusted from trial-to-trial using a minimized expected entropy staircase method such that the vibration was detected in only ~50% of the

trials. After each trial, participants were asked two questions: (i) whether a vibration was felt and (ii) which finger was stimulated ? Behavioral findings indicate that participants (n=10) perceived a vibration in 55.9% of vibration-present trials. The false positive rate was 8.7% for blank trials. When participants reported perception of the stimulus, they correctly indicated its location in 78.9% of trials; when they reported no perception, finger localization accuracy was 17.9% (chance level 25%). As a proxy for local neural processing, icEEG power in the broadband gamma frequency range (40-115 Hz) was compared for Not Perceived and Perceived trials. Early stimulus processing (<300ms) accompanied contralateral increases in gamma power in the somatosensory, frontal, and insular cortices in Perceived trials. During later processing, gamma power spread to the parietal association cortex along with bilateral increases in the frontal association cortices and temporal poles. These prominent changes outside the somatosensory cortex were not observed in Not Perceived trials. Statistical differences in gamma activity of Perceived vs Not Perceived trials will be assessed using a parcellation map analysis. Consistent with our previous results in visual and auditory paradigms, our findings so far suggest shared mechanisms of conscious perception involving a broad network of cortical regions across sensory modalities.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.05/DD27

Topic: H.02. Perception and Imagery

Support: Blattmachr Family
Loughridge Williams Foundation

Title: State-based and event-based mechanisms of awareness of action

Authors: ***D. JIN**[1], O. AGDALI[5], S. MAJUMDER[2], M. C. MCCUSKER[2], M. KHURANA[1], I. FU[1], S. I. KRONEMER[6], T. YADAV[1], A. KHALAF[3], K. L. CHRISTISON-LAGAY[7], S. L. AERTS[4], Q. XIN[8], J.-J. LI[1], S. MCGILL[2], M. J. CROWLEY[1], H. BLUMENFELD[7]; [2]Yale Univ., [3]Yale Univ. Sch. of Med., [4]Yale Univ. Interdepartmental Neurosci. Program, [1]Yale Univ., New Haven, CT; [5]695 Park Avenue, HN 311, BP-ENDURE, New York, NY; [6]NIH, NIMH, Bethesda, MD; [7]Yale Univ. Sch. of Med., Yale Univ. Sch. of Med., New Haven, CT; [8]Yale Neurol., Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT

Abstract: Loss of awareness of action (AoA), the ability to name complex motor actions just performed, is a common phenomenon in everyday experience. The spatiotemporal dynamics of

neural activity underlying this phenomenon are poorly understood. We hypothesize that actions performed with and without AoA can be characterized by differences in static (long-term, state-based) and dynamic (transiently changing, event-based) neural activity. Here, we study static mechanisms through experiment-long behavioral metrics, and differences in dynamic mechanisms through transient eye metric changes and event-related potentials in EEG. Our experiment is based on the classic board game Rush Hour, in which a target block is moved to a given location by moving surrounding blocks which impede its path. We induce loss of AoA through a distractor task which consists of watching a background movie playing behind the Rush Hour game, and after each run, subjects perform a three-minute free recall period in which they must recount details from the video. As subjects play the full game, periodically, the foreground puzzle disappears, and they may be shown a quiz asking about the move just performed in a four-option multiple choice question. After answering, subjects indicate their confidence in their choice on a sliding scale. During the task, 1000 Hz EEG and pupillometry are performed to acquire neural data. We considered correct/high-confidence answers to be aware, and incorrect/low-confidence to be unaware. High (above 75^{th} percentile) and low (below 25^{th} percentile) confidence were defined relatively within the subject's slider selections. Behavioral metrics indicated state-based mechanisms of AoA. Over the course of the experiment, long term decreases in subject quiz accuracy (-0.8% per run) and confidence (-0.7 percentile points/run) were noted. Furthermore, movement speeds prior to unaware actions (483 ms between moves) were faster than those of aware actions (534 ms), indicating a more rapid automatic movement pattern in unaware actions. In the EEG domain, the amplitude of pre-movement ERP positivities, and post-action N140 and P300 were noted to be decreased in amplitude in the unaware moves as compared to the aware moves. Post-move pupil dilation was delayed in unaware moves. Clear state-based and event-based differences characterize actions performed with and without awareness. These effects can be seen in differing long-term, behavioral metrics, as well as in short-term EEG and pupil metrics. Additional studies of AoA through functional neuroimaging will help further elucidate the neuroanatomical origins of these signals.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

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Topic: H.02. Perception and Imagery

Support: JST FOREST Program Grant JPM- JFR2041
JSPS KAKENHI Grant JP22K07327

Title: Brain networks involved in haptic three-dimensional information processing

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Abstract: Haptic 3D information has been found to be processed in a hierarchy characteristic in the human brain. In our previous fMRI study, we found that primary sensorimotor cortex (PSC) and bilateral posterior parietal cortex form task-related representations during roughness estimations (RE) and 3D curve estimations (CE). However, how these brain areas are connected and contribute to the haptic 3D feature estimation remains unclear. To address this question, we first analyzed fMRI data from 18 participants by using physio-physiological interaction (PPI) analysis to verify the task-based functional connectivity. We assessed the context-dependent contributions of 246 brain areas which were regarded as seed regions to other brain regions during RE and CE conditions. We found 6 seed regions including the bilateral precuneus, right superior frontal gyrus (SFG), left middle frontal gyrus (MFG), left secondary somatosensory cortex (S2), and right middle temporal gyrus (MTG), tended to increase the contrast of RE > CE on 10 target regions located at the right inferior frontal gyrus (IFG), bilateral inferior temporal gyrus (ITG), bilateral super parietal lobule (SPL), intraparietal sulcus area (IPS), and left inferior parietal lobule (IPL). In addition, we also found a seed region located on left IPL revealed significant activation in the bilateral SFG when considering CE > RE as a psychological factor. Thus, we totally found seven seed regions expressed significant effects in twelve target regions under the contrast effect of RE and CE estimation. Then, the fractional anisotropy (FA) of these regions obtained from the same participants' diffusion tensor imaging (DTI) was used to measure the structural connectivity. Furthermore, an independent resting-state fMRI functional connectivity of these regions were calculated by independent coefficient regression (ICR) analysis from another 137-subject dataset. Taken together, we found that the connectivity between seed regions and target regions was not apparent during the resting state, highlighting the specific pairing of seed regions and target regions within the task-related processing network of haptic 3D features. Additionally, the structural connectivity between seed regions and target regions also indicted the presence of the haptic feature network. Thus, our findings suggest that haptic 3D perception was processed following a hierarchy network, with the target regions exhibiting a tendency to process the haptic features under the seed regions modulation.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

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Program #/Poster #: PSTR347.07/EE1

Topic: H.02. Perception and Imagery

Title: Perceptually-matched images and afterimages share whole brain fMRI dynamics

Authors: *S. I. KRONEMER, M. HOLNESS, T. MORGAN, J. GONZALEZ-CASTILLO, B. AKIN, R. HUBER, V. E. GOBO, J. B. TEVES, D. A. HANDWERKER, P. A. BANDETTINI; NIMH, Bethesda, MD

Abstract: Conscious sensory perception originates from sensory inputs - exteroception (e.g., sight and audition) - and neurophysiology directly, without a concurrent real world event - interoception (e.g., imagery and hallucination). The precise neural mechanisms underlying exteroception and interoception are unknown. Examining interoception is challenging because phenomenal experiences are inaccessible and heterogeneous, and overt report introduces task demand confounds. We designed a novel paradigm that matches perceptual content between exteroception and interoception in order to compare their underlying neural mechanisms, while also studying the neural mechanisms of afterimages - an illusory image that appears following the disappearance of an inducing image. In an initial task phase, healthy, adult participants (N = 35) reported on the perceptual content of their afterimages of a face stimulus (e.g., sharpness, contrast, and duration) using a concurrent, on-screen stimulus that the participants manipulated in real time. These perceptual reports were subsequently used to construct a *"mock afterimage"*: an on-screen stimulus designed to perceptually match with the real afterimages participants experienced. In the subsequent task phase, participants were instructed to report the onset and offset of real and mock afterimages. Whole brain 7T fMRI (voxel size: 1.2mm^3; TR: 1000ms; Siemens, Inc.) and eye-tracking and pupillometry (EyeLink 1000 Plus; SR Research, Inc.) were recorded with the behavioral task. Preliminary behavioral results show that participants reliably experienced afterimages subsequent to the inducing stimulus. The perceptural strength of the afterimages (opacity and duration) was positively correlated with self-reported imagery vividness, which suggested a shared mechanism between afterimages and imagery. Preliminary whole brain, fMRI analyses show that the real and mock afterimages share activity in primary and higher-order visual cortex, fusiform gyrus, insula, and multiple task-positive frontal and parietal loci. Real afterimages have stronger responses in anterior cingulate cortex - a region that may act to monitor conflicts between sensory input and perception, like those of hallucination. These early findings support the use of afterimages as a model of interoception, and reveals that afterimage perception involves similar brain regions as in perceptually-matched visual perception. Future research aims to study the role of feedforward and feedback signaling in emerging afterimage perception by examining cortical-layer specific fMRI activity in primary visual cortex.

Disclosures: S.I. Kronemer: None. **M. Holness:** None. **T. Morgan:** None. **J. Gonzalez-Castillo:** None. **B. Akin:** None. **R. Huber:** None. **V.E. Gobo:** None. **J.B. Teves:** None. **D.A. Handwerker:** None. **P.A. Bandettini:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.08/EE2

Topic: H.02. Perception and Imagery

Title: Pupil size and phase as a real-time marker of perceptual sensitivity and whole brain activity

Authors: *V. GOBO[1], J. GONZALEZ-CASTILLO[2], J. TEVES[2], M. HOLNESS[2], P. BANDETTINI[2], S. I. KRONEMER[2];
[1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Rockville, MD; [2]NIMH, Bethesda, MD

Abstract: Conscious perception is influenced by normal and abnormal states of arousal. Arousal is closely linked to neuromodulatory activity, with foci in the thalamus and the brain stem. These areas are difficult to record in humans. Correspondingly, a proxy, accessible, and real-time marker for perception and arousal-linked neural activity would offer experimental and clinical benefits. An emerging candidate is pupil size. The pupil is both spontaneously dynamic and evoked by numerous cognitive, behavioral, and perceptual factors, independent of luminance. In this investigation, we aim to: (1) study if different phases of the spontaneous pupil predict changes in perceptual sensitivity, and (2) use fMRI to uncover the brain activity linked with spontaneous changes in pupil size. For our first aim, five healthy adult participants completed a visual perception task in parallel with pupil size recording, outside of fMRI (EyeLink 1000 Plus, SR Research, Inc.). Embedded in this behavioral task was an algorithm to detect in real-time four spontaneous pupil size phases: (1) local peaks, (2) local troughs, (3) rising, and (4) falling pupil size. When one of these pupil phase events was found, a face stimulus was shown at an opacity ranging from .01 to .25 in steps of .01. A random stimulus presentation condition, independent of pupil size was included as a control condition. Participants were instructed to respond with a button press whenever they perceived the stimulus. Preliminary results in our behavioral task demonstrate that local extrema and transitional phases in pupil size can be reliably achieved in real-time, and perceptual sensitivity is predicted by spontaneous pupil size phase. For our second aim, to map the whole brain activity associated with these pupil size phases, the Human Connectome Project resting state 7T fMRI dataset with concurrent pupillometry was analyzed. Preliminary analyses of resting state fMRI data shows wide-spread cortical and subcortical signals linked with pupil size and phase, particularly in visual sensory, arousal, and salience networks. In summary, these results introduce a new method for real-time monitoring of the pupil size and demonstrates that these phases are predictive of perceptual state and distributed brain activity dynamics. Together, these findings may be applicable in the development of treatment and diagnoses of individuals who experience abnormal conscious states.

Disclosures: V. Gobo: None. **J. Gonzalez-Castillo:** None. **J. Teves:** None. **M. Holness:** None. **P. Bandettini:** None. **S.I. Kronemer:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.09/EE3

Topic: H.02. Perception and Imagery

Support: NIH Grant R01EY023384

Title: Differences between seen and mental images across distinct acts of imagining

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Abstract: Mental images are lossy approximations to seen images. How do human imaginers distribute this loss of information across distinct acts of imagining? We consider the case of imagining a fixed, previously seen target image that is stored in long-term memory. It is possible that imaginers replace lost details of a seen target with a fixed guess that is constant across acts of imagining. They could also sample lost details randomly and independently with each act of imagining. We explored where the imagery strategies of five test subjects lay between these two extremes. Subjects memorized a target image and were instructed to then imagine it while viewing a display screen. Across a series of several hundred trials, polygon-shaped probes were displayed to the screen, and subjects pressed a button to indicate, for each probe, how many objects in their mental image were touched by the probe. They then completed a corresponding experiment in which the target image was seen, not imagined. We applied variational methods to these data to infer, for each subject and target, a distribution over seen images, and a distribution over mental images. We then measured the expected difference between seen and mental images sampled independently from the seen and imagery distributions for a fixed target (similarity). We also measured the expected difference between pairs of mental images sampled independently from the imagery distribution (self-consistency). We reasoned that if mental images are fixed across acts of imagining the same target, self-consistency would exceed similarity. We found this to be true for all subjects when the target image depicted a small number of objects. However, for complex targets with many objects the strategies of different subjects diverged. Some subjects' imagery distributions were more self-consistent than similar, while others' were more similar than self-consistent indicating that these latter subjects generated variable mental images that were more similar to the seen target, on average, than they were to each other. We speculate that these diverse strategies for distributing loss across acts of imagining may be related the diverse subjective experiences of imagery that individuals report.

Disclosures: K. Banerjee: None. **T. Naselaris:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.10/EE4

Topic: H.02. Perception and Imagery

Support: NIH Grant 1R15EY033512-01A1

Title: Probing the link between vision and language in material perception using machine learning and psychophysics

Authors: *C. LIAO[1], M. SAWAYAMA[3], B. XIAO[2];
[1]Neurosci., [2]Computer Sci., American Univ., Washington DC, DC; [3]Grad. Sch. of Information Sci. and Technol., The Univ. of Tokyo, Tokyo, Japan

Abstract: Humans visually recognize materials and estimate their properties to plan actions. Along with visual discrimination, verbal description is a crucial channel for storing and communicating the knowledge of materials. Given the diversity and complexity of materials, it is crucial to characterize material perception from both visual judgment and language to capture the high perceptual dimensions. How these two aspects connect to material perception remains unclear. We develop a framework to systemically create images of existing and novel materials by parameterizing the statistical structures of real-world photos using an unsupervised image generation model, StyleGAN. This allows us to explore the continuous space of material appearances and measure material perception. We showed that StyleGAN could synthesize realistic images of soaps, and its scale-dependent latent space can disentangle physical factors such as the object's shape, translucency, and color. Via transfer learning, we now fine-tune a StyleGAN, pre-trained on a large soap dataset (8085 images), on two smaller datasets of rocks (3180 images) and squishy toys (1900 images). To this end, we can generate images of three distinct materials with unique visual qualities. Furthermore, we can create novel material appearances without additional training by linearly interpolating between the learned models. For instance, by interpolating between soap and rock models, we can generate the ambiguous material, "soap-to-rock," which assembles the visual features of both materials. We examine how humans visually judge and verbally describe images of the original (soap, rock, squishy toy) and the interpolated (soap-to-rock, rock-to-toy, and soap-to-toy) materials. With 72 images as stimuli, observers (N=6) perform Verbal Description (VD) and Multiple Arrangement (MA) tasks. In VD, they describe the materials from five aspects (name, color, optical and mechanical properties, and texture). In MA, they spatially arrange the images based on the material similarity. By embedding the verbal descriptions with a pre-trained large language model, CLIP, we compare the text representation with the visual judgment within each observer. We find low-to-medium correlations ($0.12 \leq r \leq 0.44$, $p < 0.001$) between MA and VD with substantial individual variations. We also find that observers use colorfulness and softness as critical dimensions to assess material appearances across categories. In conclusion, while linguistic descriptions may only partially capture the nuances of perceived materials, vision and language jointly capture salient dimensions humans use to evaluate material properties.

Disclosures: C. Liao: None. **M. Sawayama:** None. **B. Xiao:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: H.02. Perception and Imagery

Support: JST PRESTO Grant Number JPMJPR185B
We appreciate the support of the WPI-IRCN Human fMRI Core, the University of Tokyo Institutes for Advanced Studies.

Title: Mind captioning: evolving descriptive text of seen and imagined content from human brain activity

Authors: *T. HORIKAWA;
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Abstract: Humans can recognize and recall complex visual content comprised of multiple perceptual elements, such as objects, places, and events, and their relationships. Representations of visual content should thus exist in the brain such that they describe the complex semantic information interrelated among those individual components. Previous brain decoding studies have successfully predicted individual visual components, and recent attempts achieved sentence-level predictions using image-captioning models and databases. However, their predictions are inherently restricted by external resources, limiting the capacity to accurately assess the full extent of semantic information in the brain and flexibly generate comprehensive descriptions of mental content. To overcome these limitations, we present a novel generative decoding approach that translates brain representations into text via features of large language models (LLMs), which excel at capturing complex semantic meanings conveyed by sentences while considering contextual word meanings. Our approach generates a descriptive text of mental content through iterative optimization, gradually aligning features of a candidate description with target brain-decoded features by replacing and interpolating words within the candidate. Crucially, we leverage an LLM pre-trained for masked language modeling to efficiently constrain the search space during optimization. To assess the effectiveness of our approach, we trained decoding models to predict language model (LM) features computed from captions annotated to video clips using fMRI activity measured while subjects viewed the clips, then confirmed reliable feature predictions for seen and imagined content using independent test datasets. By applying our text generation approach to the brain-decoded features, we achieved a gradual evolution of descriptions only by the guide of the brain, wherein the evolved descriptions captured key aspects of seen and imagined content with moderate accuracy. Importantly, since the LM features of generated descriptions showed increasing correlations with brain-decoded features, surpassing those of the best-matching descriptions retrieved from caption databases, our approach may generate descriptions that represent the semantic information encoded in the brain with higher precision. The present study refines our conception of the extent to which rich and organized semantic information of visual content is encoded in and can be decoded from the brain, offering a means to produce intelligible interpretations of intricate mental content by translating brain signals into linguistic descriptions.

Disclosures: T. Horikawa: None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.12/Web Only

Topic: H.02. Perception and Imagery

Support: NSERC Grant 14367

Title: Haptic Processing: A door into understanding sex differences in mental rotation ability

Authors: *D. AGUILAR, C. L. R. GONZALEZ;
Univ. of Lethbridge, Lethbridge, AB, Canada

Abstract: We have proposed that functional lateralization for sensorimotor control served as a platform for the later development of cognitive abilities and their hemispheric functional specialization (Gonzalez et al., 2018). For example, the left hemisphere specialization for visuomotor control is related to the left hemispheric specialization underlying language. Similarly, the right hemisphere specialization for haptic control is associated with this hemisphere's specialization for visuospatial cognition. In this study, we tested the later hypothesis; we examined hand differences in a haptic object recognition task and hypothesized a left-hand, right-hemisphere advantage. Additionally, we investigated a potential relationship between haptic processing and performance on the Shepard & Metzler, Mental Rotation Test (MRT). Two-hundred and nineteen participants (130 female) divided into five age groups (young children, older children, teenagers, young adults, and older adults) completed the MRT and a haptic task (without vision) using the right and left-hands. Results confirmed our hypothesis: In the haptic task, participants were faster and made fewer errors when using the left versus the right hand. Furthermore, we found a sex difference in that males performed better in both the haptic and the MRT tasks. Finally, a negative relationship was found between the haptic and the MRT tasks. These results demonstrate the right hemisphere dominance for haptic and spatial cognition and suggest that the well-known male advantage for mental rotation might be rooted in an advantage for sensorimotor (i.e., haptic) processing.

Disclosures: D. Aguilar: None. **C.L.R. Gonzalez:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

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Program #/Poster #: PSTR347.13/EE6

Topic: H.02. Perception and Imagery

Support: JST ERATO JPMJER1801
JST CREST JPMJCR18A5
JST Moonshot R&D JPMJMS2012

Title: Neural representation underlying absolute pitch perception and imagery: a magnetoencephalography study

Authors: *H. YANG[1], M. SHIMOMOTO[1], R. FUKUMA[1,2], A. OTSUKA[1], A. KAMBARA[1], H. KISHIMA[2], T. YANAGISAWA[1,2];
[1]Inst. for Advanced Co-Creation Studies, Osaka Univ., Suita, Japan; [2]Osaka Univ. Grad. Sch. of Med., Suita, Japan

Abstract: Absolute pitch (AP) is an ability that enables individuals to identify and reproduce specific pitches without an external reference. This study investigates the neural representation underlying AP and its impact on the brain's encoding and classification of pitch categories during both perception and imagery tasks, using magnetoencephalogram (MEG).
The study included 20 AP and 21 non-AP musicians. MEGs were recorded while participants listened to and imagined different tones. The recorded MEGs were preprocessed and analyzed as raw signals and estimated cortical currents. Decoding analysis for the perception task was performed using the cortical current averaged within a 100-ms time window, shifting from -500 to 1000 ms with a 50-ms time step.
The root mean square of the raw MEG signals during the perception task revealed stronger neural activity in the left hemisphere of the AP group compared to the non-AP group at 85ms, while no significant difference was observed in the imagery task. Cortical current maps showed prominent activity in the auditory cortex from 50 to 150 ms in both groups, with distinct cortical activity patterns in AP and non-AP groups. In the decoding analysis for the perception task, we noted a dynamic pattern of decoding accuracy across time. The decoding accuracy peaked at around 200 ms according to the tone's onset, achieving a maximum accuracy of 62.4%. Interestingly, the non-AP group exhibited a weaker neural response, yet demonstrated superior decoding performance (ts(39) < -2.19, ps < .03). These differences were more pronounced in the right hemisphere. In the imagery task, significant above-chance decoding accuracy was observed in the left frontal and temporal regions of interest (ROIs) for the AP group, and in the right temporal ROI for the non-AP group. Furthermore, the mixed analysis of variance showed a significant interaction between the group and hemisphere ($F(1,78) = 4.94$, $p = 0.029$).
Our findings suggest that individuals with AP exhibit a left-hemisphere preference in pitch-processing tasks. These results contribute to a better understanding of the neural processing of music in the brain, particularly in relation to AP perception.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.14/EE7

Topic: H.02. Perception and Imagery

Title: What moves us: The impact of auditory features on moment-to-moment music appreciation

Authors: T. KHANAM[1], H. ASRANI[1], S. SPIVACK[2], ***P. WALLISCH**[1];
[1]Ctr. for Data Sci., [2]New York Univ., New York, NY

Abstract: Prior research - including some of our own work - strongly suggests that appraisal judgments of music stabilize after just a few seconds. People can quickly tell whether they like or dislike a given song, even if they do not recognize it from previous exposure. However, most prior research focuses both on summary judgements - song ratings after the musical exposure, as well as group averages. Here, we take a closer look at the temporal microstructure of what musical features resonate with listeners, on a moment-by-moment basis and on an individual level. Specifically, we asked a large sample of research participants (n > 600) to judge a representative sample of songs by adjusting a slider in real time while listening to the music. We then quantify the time course of auditory features present in the songs, such as Mel-Frequency Cepstral Coefficients as well as spectral and chromatic aspects with Librosa. We then relate these behavioral measures and stimulus features with reverse correlation methods to get a sense for which auditory features trigger slider adjustments in a given participant.
We find that auditory features present in songs change appraisal ratings on a moment-to-moment basis, but are also modulated by both personality factors (as measured by the NEO-PI) as well as prior familiarity with the song. We conclude that the impact of auditory features is strong, yet idiosyncratic, which is often obscured when reporting only group averages. Thus, successful modeling of the interplay between the auditory features of songs and subjective experience of listeners necessitates to model the interactions between the sensory feature content, the personality of listeners as well as their prior experience explicitly.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.15/EE8

Topic: H.02. Perception and Imagery

Title: Probing Sensorimotor Expertise in Professional Driving with an Immersive Racing Simulator

Authors: Y. HONDA[1], K. KITAZOE[2], M. SAWADA[2], T. FUJIMOTO[3], G. TAMURA[4], *M. FUKUSHIMA[1];
[1]Deloitte Analytics, Deloitte Touche Tohmatsu LLC, Tokyo, Japan; [2]Sumitomo Bakelite Co., Ltd., Shizuoka, Japan; [3]Tom's Inc., Tokyo, Japan; [4]Sch. of Design, Tokyo Univ. of Technol., Tokyo, Japan

Abstract: Professional racing amplifies the complexity of driving as a sensorimotor task, demanding the continuous processing of multimodal sensory information and intricate motor control for swift vehicle navigation. In this study, we used EEG and eye-tracking in an

immersive racing simulator to explore the neural and behavioral basis of these skills. Our setup involved a 3DoF (pitch, roll, heave) racing simulator with a large hemispherical screen (3.4 x 2.3 x 2.1m), providing a realistic driving environment. This system offers a natural, unoccluded view to both central and peripheral vision during driving. We synchronously recorded eye movements, EEG, and driving control variables from two professional drivers on two different circuits. We also used a newly developed flexible electrode for EEG measurement, made of resin with a hydrogel-coated tip surface. This flexible hydrogel, containing water, moisturizes the scalp surface, enabling EEG acquisition with low contact resistance, irrespective of scalp dryness or head shape.We found that both drivers exhibited remarkable consistency in driving control (wheel angle, brake and acceleration timing, etc.) with notable differences in specific parts of the racing circuits. We also observed distinct differences in gaze movement distance between the two drivers. They showed larger gaze movement distances before and after curves, particularly after curves. During straight-line driving, gaze movement distance varied significantly, while during curve driving, it tended to be smaller. These observations suggest potential variations in concentration levels and risk perception during different driving scenarios. In addition, preliminary EEG data analysis suggests individual differences in the modulation of EEG alpha power, correlating with focused attention. This exploratory study provides initial insights into the sensorimotor expertise of professional drivers, with potential implications for training and performance enhancement in professional racing. We will continue to investigate further to expand our understanding of the complex sensorimotor processes involved in professional driving.

Disclosures: **Y. Honda:** A. Employment/Salary (full or part-time):; Deloitte Touche Tohmatsu LLC. **K. Kitazoe:** A. Employment/Salary (full or part-time):; Sumitomo Bakelite Co., Ltd. **M. Sawada:** A. Employment/Salary (full or part-time):; Sumitomo Bakelite Co., Ltd. **T. Fujimoto:** A. Employment/Salary (full or part-time):; Tom's Inc.. **G. Tamura:** None. **M. Fukushima:** A. Employment/Salary (full or part-time):; Deloitte Touche Tohmatsu LLC.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.16/EE9

Topic: H.02. Perception and Imagery

Support: NIMH Intramural Research Program (ZIAMH002783)

Title: Behavioral and neural factors underlying the perception of the audiovisual bounce effect

Authors: *I. GEPHART, T. MORGAN, J. GONZALEZ-CASTILLO, D. HANDWERKER, P. BANDETTINI;
Section on Functional Imaging Methods, NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD

Abstract: Visual and auditory information can have strong influences on one another as they are integrated in the brain. One example of this multimodal integration is the Audiovisual Bounce Effect (ABE), in which auditory information modulates visual perception. This perceptual effect occurs when two circles move diagonally from the top corners of a screen toward the center. The circles meet at the center and continue toward the bottom corners of the screen. If a sound is played as the circles meet, the likelihood increases that a viewer will perceive the circles as bouncing off each other rather than crossing paths (Sekuler et al. 1997, Manigli et al. 2012). Previous work has concentrated on how timing of the audio information (Sekuler et al. 1997) affects visual perception. In this work, we investigate how changes to visual stimuli impact the ABE. In other words, does visual context affect the integration of audiovisual information, and is this consistent across individuals? To understand which parameters (e.g., circle speed, size, collision angle, and audio offset) affect perception, twelve participants completed 1000 trials of the ABE in a behavioral experiment. We found that these previously unstudied visual factors are important to bounce perception. A logistic regression analysis with random effects showed that larger circles ($p < 0.01$), faster circles ($p < 0.01$), and smaller collision angles ($p = 0.04$) are significantly more likely to be perceived as bouncing off each other. Additionally, we observed an effect of trial history, suggesting that brain states may play an important role in the ABE. To further investigate this finding, we conducted a 7T fMRI experiment to localize the integration in the brain and explore if there are different brain states associated with bounce vs cross perceptions. We have collected a data set of 6 participants, each of whom completed 520 trials while their brain activity was recorded using 7-Tesla fMRI. Preliminary results from one subject show significantly increased activation in parietal areas, frontal eye fields, V4, STS, and higher order auditory cortex when participants perceive a bounce compared to when they perceive the circles passing through one another. These results corroborate previous work demonstrating the involvement of parietal areas in sensory integration (Bushara et al. 2002), but also expand the activated ROIs, motivating further investigation. To better understand the neural correlates of audiovisual integration, we plan to collect higher resolution fMRI data to examine the integration of signals in distinct cortical layers during multisensory perception in the brain.

Disclosures: I. Gephart: None. **T. Morgan:** None. **J. Gonzalez-Castillo:** None. **D. Handwerker:** None. **P. Bandettini:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.17/EE10

Topic: H.02. Perception and Imagery

Support: This study was conducted as part of Global Singularity Research Program for 2023 financially supported by KAIST.

Title: Brain imaging evidence for Architectural Affordances: A Multivoxel Pattern Analysis Approach Using fMRI Data

Authors: *W. SHIN[1], Y. SONG[2], J. JEONG[3];
[1]Program of Brain and Cognitive Engin., [2]Bio and Brain Engin., [3]Brain and Cognitive Sci., Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: As modern people spend most of their time indoors, understanding how we interact with architectural elements is crucial. In this regard, Gibson's affordance offers insights into how they can influence human behaviors, cognition and interactions by indicating the possible actions or interactions between agents and the environment. Thus, our research seeks to understand whether affordance theory extends to architectural elements (i.e., architecture affordance) and investigate its neurological basis to enhance our knowledge of human-environment interactions. Upon literature review, we identified three core properties of affordance: (1) automatic perception, (2) the incorporation of motor information, and (3) the effect of action possibilities between an agent and an object. Then, we hypothesized that the architectural affordances would result in the presence of brain areas that have all those properties when interacting with architectural elements. To confirm this, we conducted four experiments, each designed to evaluate distinct aspects of the core properties with the utilization of multivoxel pattern analysis (with SVM) on fMRI data. The first experiment (n=23, female=10, mean age=23.2, s.d.=3.0) aimed to examine the core property (1), testing whether brain activity during mere observation could distinguish between eight architectural elements. The second experiment (n=21, female=9, mean age=24.4, s.d.=2.0) examined the property (2), identifying whether the same brain region that has information about different imagined actions even when the same architectural element was associated. The third and fourth experiments examined the property (3), checking if the same brain region distinguishes the action possibilities associated with the function of the same architectural element (n=23, female=9, mean age=23.6, s.d.=2.2) and whether the same brain region distinguishes the different manipulating methods of the same element (n=23, female=10, mean age=24.1, s.d.=3.6). The findings provide evidence for the brain regions which met all the properties associated with architecture affordance, which include the occipital cortex, parietal cortex, motor-related brain areas (M1, PM, and SMA), and cerebellum. Unlike other areas known to be involved in object affordance, the involvement of the motor execution area enables faster actions, while the engagement of the occipital area contributes to processing contextual information related to changes in body coordinates. Our results highlight the significant role of visuomotor processes in architectural affordances, laying the groundwork for a neuroscientific approach to architectural design.

Disclosures: W. Shin: None. **Y. Song:** None. **J. Jeong:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.18/Web Only

Topic: H.02. Perception and Imagery

Title: Navigating the neural landscape of dreams through computational linguistics

Authors: *R. MALLETT[1], C. PICARD-DELAND[2], T. NIELSEN[2], M. CARR[2];
[1]Northwestern Univ., Evanston, IL; [2]Univ. of Montreal, Montreal, QC, Canada

Abstract: Investigating the neural underpinnings of dreams across various sleep stages provides critical insights into the complex processes underlying human cognition. While REM and NREM sleep and their mental fluctuations offer a valuable model to study the neural correlates of thought, limited sample sizes have hindered a comprehensive understanding of the distinct phenomenology between REM and NREM dreams. To overcome this, we compiled polysomnography data and dream reports from several studies over a decade, yielding a large sample size of 650 awakenings from 403 participants (277 NREM dreams and 373 REM dreams). We applied computational linguistics techniques, such as Linguistic Inquiry and Word Count, to differentiate between mental contents of REM and NREM dreams. Our analysis indicated more social content in REM sleep dreams, offering tentative support for the Social Simulation Theory of dream function and suggesting its unique application to REM dreams. Conversely, NREM sleep dreams featured less dense narratives (e.g., shorter clauses and increased punctuation), suggesting that narrative thought structure is obstructed during certain sleep stages. This research provides a comprehensive analysis of dream phenomenology across sleep stages, confirming previous observations and extending our understanding of the brain's ability to generate content in the absence of extensive external input during sleep.

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Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

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Program #/Poster #: PSTR347.19/EE11

Topic: G.04. Emotion

Title: Direct perception of affective valence from vision

Authors: *S. SADEGHI, E. D. DE ROSA, A. K. ANDERSON;
Cornell Univ., Ithaca, NY

Abstract: Since the beginnings of psychology affect has been posited as the basic subjective feeling component underlying all experience, arising directly from internal states of the body and closely related proximal senses. By contrast, in the distal senses such as vision, affect is thought to be indirect, largely mediated by higher level processes. Evidence from machine learning suggests the low-level visual features in the external environment predict affect, but their causal role, if any, remains unclear. Here we examined the direct perception of valence, the fundamental hedonic axis of affect, as a feature of the external world embedded in visual perception. A visual valence (VV) model of low-level image statistics trained to predict normative valence ratings in 8000 emotionally charged images ($p<0.0001$). When we tested the model on abstract paintings, valence ratings were explained even better than real photos,

confirming the causal status of VV independent of conceptual content. In a behavioral study (N=180 participants), when we displayed realistic photos inverted and each for only ~100 ms, subjects' valence ratings became even more aligned with VV compared to ratings in normative conditions ($p<0.001$). This stronger VV under limited access to conceptual valence demonstrated a decoupling of VV from conceptual analysis. Re-analysis of an fMRI data (N=20 participants) revealed correlates of VV in early and mid-level visual regions, whereas normative valence rating had stronger correlates in the default mode network. Overall, these investigations reveal that VV, derived from ecological visual statistics, is neurally and behaviorally distinguishable from the normative valence which relies heavily on conceptual analysis. Results suggest a direct perception of valence as an apparent objective property of the external world. An avenue of future work can shed light on the significance of directly perceived VV for emotion, perception, memory, decision-making, and development.

Disclosures: S. Sadeghi: None. **E.D. De Rosa:** None. **A.K. Anderson:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.20/EE12

Topic: H.06. Social Cognition

Support: National Natural Science Foundation of China (62293550, 62293551, 61977008)

Title: A hierarchical architecture for the cortical processing of naturlistic conversations

Authors: *R. AILI, S. ZHOU, C. LU;
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Abstract: Recent studies have well demonstrated that speech is hierarchically processed in the human brain, with small units being processed in the lower-order cerebral cortices and large units being processed in the higher-order cortices. Most of these studies, however, have been conducted during speech comprehension. Thus, it is unknown whether the same principle applies to interpersonal communication such as naturalistic conversation. To address this issue, herein we measured brain activity from two individuals simultaneously using functional near-infrared spectroscopy hpyerscanning while they were involved in a naturalistic conversation. After vectorizing the speech units (e.g., a sentence or a linguistic context) using the BERT model, Representational Similarity Analysis (RSA) was performed to investigate whether and how the similarity between speech units (i.e., between sentences or linguistic contexts) could predict the similarity of brain activity that was elicited by these speech stimuli. The results showed that, for both the speaker and the listener, small unit was associated with the bilateral middle temporal cortex (MTC), and medial prefrontal cortex (mPFC), while large unit was associated with the left Postcentral and mPFC. Additionally, while the left Angular Gyrus (AG) was involved in the

processing of small unit in the speaking brain, it was involved in the processing of large unit in the listening brain. Moreover, the MTC's and mPFC's activity were more similar between adjacent than distant sentences within but not across a linguistic context; while the PCC and mPFC were more similar between adjacent than distant linguistic contexts. Together, our results provided novel evidence for the hierarchical architecture of the human brain when processing speech unit with different linguistic sizes during a naturalistic conversation. Moreover, our results also indicated that there are both shared and different features between the hierarchical architectures of the speaking and the listening brains. These findings extend the hierarchical principle of speech processing from speech comprehension to naturalistic conversation.
Keywords: Natural language comprehension; Speech production; Brain hierarchy; fNIRS.

Disclosures: **R. Aili:** None. **S. Zhou:** None. **C. Lu:** None.

Poster

PSTR347. Human Perception and Imagery Within and Across Sensory Modalities

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR347.21/EE13

Topic: D.08. Multisensory Integration

Title: Graded brain fMRI response to somatic and visual acupuncture stimulation

Authors: D.-E. YOON[1], S. LEE[1,2], J. KIM[1], K. KIM[1], H.-J. PARK[1], V. NAPADOW[3], I.-S. LEE[1], *Y. CHAE[1];
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Abstract: Increased stimulation can enhance acupuncture clinical response; however, the impact of acupuncture stimulation as “dosage” has rarely been studied. Furthermore, acupuncture can include both somatic and visual components. We assessed both somatic and visual acupuncture dosage effects on sensory ratings and brain response. Twenty-four healthy participants received somatic (needle inserted, manually stimulated) and visual (needle video, no manual stimulation) acupuncture over the leg at three different dosage levels (control, low-dose, and high-dose) during brain fMRI. Participants reported the perceived deqi sensation for each acupuncture dose level. Brain fMRI data were analyzed by general linear model and multivariate pattern analysis. For both somatic and visual acupuncture, reported deqi sensation increased with increased dosage of acupuncture stimulation. Brain fMRI analysis demonstrated that higher dosage of somatic acupuncture produced greater fMRI response in sensorimotor processing areas, including anterior and posterior insula and secondary somatosensory cortex. For visual acupuncture, higher dosage of stimulation produced greater fMRI response in visual-processing areas, including V5/MT+ in occipital cortex. Psychophysical and psychophysiological responses to both somatic and visual acupuncture were graded in response to higher doses. Our findings

suggest that acupuncture response may be enhanced by the dosage of needling-specific and nonspecific components, represented by different neural mechanisms.

Disclosures: **D. Yoon:** None. **S. Lee:** None. **J. Kim:** None. **K. Kim:** None. **H. Park:** None. **V. Napadow:** None. **I. Lee:** None. **Y. Chae:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.01/EE14

Topic: E.04. Voluntary Movements

Support: University of Georgia Mary Frances Early College of Education and University of Georgia Office of Research

Title: Corticospinal excitability while preparing interception movements depends on the visual motion properties of the target

Authors: *J. R. MCCURDY, J. XU, D. A. BARANY;
Univ. of Georgia, Athens, GA

Abstract: To accurately time the interception of a moving target, visual motion information about the target must be dynamically integrated with an internal model of the movement plan. Previous evidence from non-human primates has shown that neural activity within the primary motor cortex (M1) is modulated by both time-varying aspects of the visual target's motion and motor planning. These findings suggest that visual motion information influences M1 output during movement preparation; however, it is unclear how visual and motor information is integrated in M1 to facilitate accurate performance. Here, we applied single-pulse transcranial magnetic stimulation (TMS) over M1 while human participants prepared to hit a moving target to investigate the influence of the target's visual motion properties on corticospinal excitability. Participants (N = 19, 9 M, 20 ± 3.6 years old) were instructed to abduct their right index finger to intercept a moving target when it arrived at a fixed interception zone. Each trial began with the brief presentation of a static target at one of two distinct distances (Close/Far). After a variable delay, the target approached the interception zone at one of two constant horizontal velocities (Slow/Fast). Motion duration was matched across two of the conditions (Fast-Far and Slow-Close), allowing us to isolate the effect of target kinematics, independent of preparation time. Motor-evoked potentials (MEPs) in response to TMS were elicited from the right first dorsal interosseus muscle at target onset (baseline) or at one of five different latencies (-500, -300, -250, -200, -150 ms) relative to the time the target arrived at the interception zone. We observed that interception movements were initiated sooner for faster moving targets ($F_{1,18}$ = 6.36, p = .021), and delayed when TMS was administered closer to target arrival ($F_{2.3,41.6}$ = 56.31, p < .001). Similar to the MEP time course observed in delayed-response tasks, MEP amplitudes were reduced relative to baseline when TMS was delivered at -300 ms (t_{18} = -7.49, p < .001), and

increased when TMS was applied -150 ms to target arrival (t_{18} = 7.99, p < .001). Notably, we observed that faster moving targets resulted in relatively greater facilitation at -150 ms compared to slower targets ($F_{2.2,38.9}$ = 3.30, p = .044), which may underlie earlier movement initiation. Altogether, our results suggest that visual motion properties—especially target velocity—of the moving target modulate corticospinal excitability during the preparation of interceptive movements.

Disclosures: **J.R. McCurdy:** None. **J. Xu:** None. **D.A. Barany:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.02/EE16

Topic: E.04. Voluntary Movements

Support: NIH CTSA UL1 award 2UL1TR002378

Title: Conditioned H-reflex in the flexor carpi radialis during sustained activation

Authors: *B. CRABTREE[1], M. R. BORICH[2], J. XU[1];
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Abstract: Human hand motor control is modulated by multiple neural descending pathways. Disruption of these pathways after neurological injuries, such as a stroke or a spinal cord injury, may seriously compromise hand function. Indexing the functional involvement of these pathways can thus be informative to the neural mechanisms and rehabilitation strategies after neurological injuries. Functional engagement of different pathways in motor control can be probed using transcranial magnetic stimulation (TMS) conditioned Hoffman's reflex (H-reflex) with various timing between TMS and peripheral nerve stimulation (PNS) (Nielson, 2016). It is believed that short-interval facilitation of H-reflex taps into the direct, monosynaptic projections within the corticospinal tract, and long-interval conditioning recruits more indirect, polysynaptic pathways. Most studies, however, were conducted in the lower limb muscles (Lopez et al. 2020). Conditioned H-reflex has been obtained from the upper limb, e.g., flexor carpi radialis (FCR) (Niemann et al. 2018), but effects with various interstimulus intervals (ISI) and different TMS intensities are yet to be fully explored. Here we addressed this gap by characterizing TMS conditioned H-reflex in FCR across a large range of ISIs and different TMS intensities. Eighteen healthy younger adults were tested condition H-reflex with sustained FCR activation at 10% maximum voluntary contract (MVC). H- and M-wave recruitment curves were obtained from each individual within the range of 1-35mA. For conditioned H-reflex assessment, PNS intensity was set at 50% Hmax on the ascending limb of the recruitment curve; two TMS intensities were used: 80% (N=9) and 90% AMT (N=9). Three types of trials, Conditioned (TMS+PNS at ISIs from -6 to 8ms with 1ms increment steps, and extra-long at 20, 30, 40ms), Unconditioned (PNS-only), and TMS-only trials, were randomized. H-reflex Facilitation at each

ISI was calculated as the mean peak-to-peak H amplitude in the Conditioned divided by that from the Unconditioned trials.
We were able to obtain reliable H/M recruitment curves from all 18 participants. Paired t-tests were used to compare Conditioned with Unconditioned H amplitudes. Our preliminary results showed that TMS conditioning at 90% AMT resulted in significant H-reflex facilitation (Facilitation Mean+/-SD 1.29+/-0.26; p = .002) but not at 80% AMT (Facilitation 0.94+/-0.13; p = .17). Facilitation at 90% AMT disappeared with long ISI (20-40ms) (1.00+/-0.28). These preliminary results indicate that TMS-conditioned H-reflex may be a promising method to probe functional engagement of the descending motor pathways for upper limb motor control.

Disclosures: B. Crabtree: None. **M.R. Borich:** None. **J. Xu:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.03/EE17

Topic: E.04. Voluntary Movements

Title: Eeg monitoring of the training effect in team sports (volleyball).

Authors: *S. TUKAEV[1,2,3], M. MAKARCHUK[1], S. FEDORCHUK[2], O. PRAVDA[2], A. POPOV[4], J. FERREIRA[5];
[1]Inst. of Biol. and Med., Natl. Taras Shevchenko Univ. of Kyiv, Kyiv, Ukraine; [2]Res. Inst., Natl. Univ. of Ukraine on Physical Educ. and Sport, Kyiv, Ukraine; [3]Fac. of Communication, Culture, and Society, Inst. of Publ. Hlth., Univ. della Svizzera italiana, Lugano, Switzerland; [4]Dept. of Electronic Engin., Igor Sikorsky Kyiv Polytechnic Inst., Kyiv, Ukraine; [5]Faculdade de Medicina, Univ. de Coimbra, Coimbra, Portugal

Abstract: In the highly competitive world of professional sports, the constant struggle is for the slightest improvement in their results. Improving the training process requires the identification of neurobiological markers, which make it possible to judge a steady increase in sports mastery and/or correct, an increase in the effectiveness of the training process. Changes in the brain's electrical activity during intensive muscle loading, and physical exertion may be such objective markers reflecting an increase in sportsmanship, indicating more effective motor, cognitive activity, and information processing. The aim of this study was to study changes in brain activity during relevant physical activity in dynamics (6 months of training). 12 volunteers, players of the women's volleyball team, and students of the National University of Ukraine on Physical Education and Sport (NUUPES) aged 18-20 years (Mage = 18.35 years) were recruited for this study. 6 elite female athletes who qualified for the reserve group of the National Handball Team of Ukraine (juniors) aged 18-19 years (Mage = 18.77 years) composed the control group. The study of the main group (players of the university women's volleyball team) was carried out in two stages: before the start of a new training process (September 2019) and at the end of its first stage, 6 months later (February 2020). The athletes of the reserve group of the National Handball

Team of Ukraine underwent a study during the training camp in the summer of 2019. EEG was registered using mobile SMARTING EEG (mBrainTrain, Serbia). during test training on a bicycle ergometer Concept II BikeErg (Concept2 Inc. GmbH, USA) at submaximal workloads. As a result of 6 months of training, the number of differences with professional athletes decreases but remains in the sensorimotor area. It has been shown that an increase in athletic performance is reflected in changes in the brain's electrical activity (alpha and beta EEG ranges). The increased spectral power density of the alpha rhythm in the motor cortex and frontal area in less experienced athletes indicates that they need more pronounced inhibitory control to suppress the processing of irrelevant information. As the skill improves, the efficiency of information processing in experienced athletes increases, which is reflected in the observed changes. The observed increase in beta activity during physical load is unambiguously associated with higher cortical activation, The obtained results may reflect an increase in professional performance.
Key words: sports mastery, peak load, mobile electroencephalography

Disclosures: **S. Tukaev:** None. **M. Makarchuk:** None. **S. Fedorchuk:** None. **O. Pravda:** None. **A. Popov:** None. **J. Ferreira:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

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Topic: E.04. Voluntary Movements

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Grant PRIN 2020 to L.F. and by the European Union H2020 - EnTimeMent (FETPROACT-824160) to Luciano Fadiga

Title: Scale-invariant changes in corticospinal excitability reflect multiplexed oscillations in the motor output

Authors: *M. EMANUELE[1], A. D'AUSILIO[2], G. KOCH[3], L. FADIGA[1], A. TOMASSINI[1]; [1]Fondazione Inst. Italiano di Tecnologia, Ferrara, Italy; [2]Univ. of Ferrara, Ferrara, Italy; [3]Fondazione S. Lucia I.R.C.C.S., Rome, Italy

Abstract: While roughly smooth at first glance, our movements possess a complex microstructure, characterized by subtle rhythmic discontinuities, such as submovements (2 Hz) and tremor (8 Hz). To date, it is not clear whether the motor commands transmitted from the primary motor cortex (M1) along the corticospinal pathway define the macroscopic as well as the

microscopic architecture of movements. Here we asked participants (N=14, 7 females, mean age=23.50, SD=2.03) to control the 2D position of a cursor by exerting bimanual isometric force with the index fingers against two force sensors and track a target moving along a circular path at a constant pace of 0.25 Hz. In addition to the task-instructed 0.25-Hz periodicity, the motor output exhibits spontaneous ~2- and 8-Hz fluctuations, most likely corresponding to submovements and tremor. During the task, we randomly delivered transcranial magnetic stimulation (TMS) over the left M1 and measured the modulation of motor-evoked potentials (MEPs, i.e., an index of corticospinal excitability) recorded from the left first dorsal interosseous (FDI) muscle in relation to multiscale motor output fluctuations. We show consistent modulation of corticospinal excitability over the three timescales, exhibiting multiplexed oscillatory dynamics that reflect the temporal structure of movement. Remarkably, despite the microscopic oscillations in motor output being two orders of magnitude smaller than the macroscopic ones, the amplitude of corticospinal excitability modulation remains identical at all scales. This suggests a substantial rescaling of the descending motor drive, possibly through a low-pass filtering operation occurring downstream of M1 (e.g., extrapyramidal descending tracts, spinal circuitries, muscle viscoelastic properties). While maintaining unchanged the amplitude of the 0.25-Hz motor drive, this rescaling mechanism may attenuate the higher frequency components at 2 and 8 Hz. By remapping the same corticospinal drive to different force ranges, the motor system may improve the depth of motor encoding at higher frequencies, leading to more accurate control across timescales.

Disclosures: **M. Emanuele:** None. **A. D'Ausilio:** None. **G. Koch:** None. **L. Fadiga:** None. **A. Tomassini:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

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Program #/Poster #: PSTR348.05/EE19

Topic: E.04. Voluntary Movements

Support: NSERC 2016-05336
CFREF-VISTA

Title: Visuomotor control networks change as a function of hormone levels in working-aged women

Authors: *N. SMEHA[1], D. J. GORBET[2], L. E. SERGIO[3];
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Abstract: Interactions with our environment require intact connections between frontal, parietal, and subcortical brain regions. While some of these interactions are direct, others require cognitive-motor integration (CMI), where the guiding visual information and motor action are

decoupled. Our group has shown sex differences in the networks controlling these visuomotor skills.[1] However, most of this research is based on data from pre-menopausal college-aged women. To address the knowledge gap around the impact of sex hormones on the neural control of movement, we examined females between the ages of 34 and 57 in their pre, peri, and postmenopausal stages. We hypothesized that the neural activity underlying skilled movement control would differ as a function of hormone concentrations. Participants underwent MRI scanning during which they performed standard and CMI eye-hand coordination tasks using an MRI-safe touchscreen. Participants were trained on the tasks until performance reached greater than 90% success. Estrogen, progesterone, and testosterone levels were also collected. A generalized psychophysiological interaction (gPPI) analysis was implemented to examine how functional connectivity between regions known to be involved in our CMI task was modulated by sex hormone concentrations. Our preliminary linear regression analysis showed that in the standard condition, lower levels of testosterone were associated with increased right prefrontal cortex-inferior parietal lobule functional connectivity (IPL; $p < 0.05$), and precuneus-right superior parietal lobule connectivity (SPL; $p < 0.05$). Further, an elevated estrogen:testosterone (E:T) ratio was associated with increased bilateral prefrontal cortex-IPL connectivity ($p < 0.05$), as well as precuneus-left SPL connectivity ($p < 0.05$). In the CMI condition, lower levels of progesterone were associated with increased left prefrontal cortex-IPL ($p < 0.05$), precuneus-bilateral SPL ($p < 0.05$), and right-left IPL connectivity ($p < 0.05$). Finally, a smaller E:T ratio was associated with increased functional connectivity between the right prefrontal cortex and SPL ($p < 0.05$). Thus, with equivalent performance, we observe hormone-related differences in the connectivity strength of the brain networks for skilled performance. These data show that the neural control of visually-guided movement is differentially affected depending on sex hormone levels. We suggest that the structure of the networks required for accurate movement performance changes with the fluctuations in progesterone, estrogen, and testosterone that occur later in life. 1. Gorbet D & Sergio (2007). EJN 25(4), 1228-1239.

Disclosures: **N. Smeha:** None. **D.J. Gorbet:** None. **L.E. Sergio:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

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Program #/Poster #: PSTR348.06/EE20

Topic: E.04. Voluntary Movements

Support: FNRS Grant

Title: Role of the primary motor cortex in effort-based decision-making

Authors: M. BOISGONTIER[1], P. VASSILIADIS[2], L. DRICOT[3], V. TOUZE[1], A. NOURREDINE[4], J. DUQUE[3], ***G. DEROSIERE**[5];
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Abstract: From insects to rodents to humans, animals must constantly decide whether to engage (or not) in physical efforts to reach rewarding goals - a process usually referred to as effort-based decision-making. The propensity to engage in effortful actions strongly varies from one individual to another and the neural basis of this inter-individual variability remains unclear. Nonetheless, models of decision-making suggest that differences in baseline, resting-state levels of activity in structures involved in executing actions - such as the primary motor cortex (M1) - may predict the propensity to engage in physical effort. Here, we asked whether inter-individual differences in the resting-state activity of different intracortical circuits in M1 predict the propensity to engage in effort. 50 healthy subjects realized an effort-based decision-making task in which they had to decide whether to execute contractions of different intensities to earn monetary rewards. Computational modelling of behavior in this task allowed us to quantify in each participant the subjective valuation of effort, the valuation of reward, intrinsic motivation and fatigue. We also administered a battery of neuropsychological tests allowing us to quantify inter-individual differences in apathy, depression, anhedonia and sensitivity to punishment and reward. We exploited three single-coil, paired-pulse transcranial magnetic stimulation (ppTMS) protocols to probe the level of activity of three M1 intracortical circuits in each subject at rest: $GABA_A$ inhibitory circuits, indexed by short-interval intracortical inhibition (SICI), $GABA_B$ inhibitory circuits, indexed by long-interval intracortical inhibition (LICI) and glutamatergic intracortical circuits, measured as intracortical facilitation (ICF). Our analyses are still ongoing and involve quantifying the predictability of decision behavior and neuropsychological scores (*e.g.*, apathy) using the ppTMS data.

Disclosures: M. Boisgontier: None. **P. Vassiliadis:** None. **L. Dricot:** None. **V. Touze:** None. **A. Nourredine:** None. **J. Duque:** None. **G. Derosiere:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS118055
University of Michigan Rackham Merit Fellowship
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Title: Dsgrid: a dual-site tms grid-search targeting method for personalized functional stimulation of parietal-frontal circuits involved in actions

Authors: J. DELUISI[1], E. GOLDENKOFF[2], *T. LEE[3], J. BRISSENDEN[4], S. F. TAYLOR[5], T. POLK[3], G. F. WITTENBERG[6], M. VESIA[2];

[1]Kinesiology, Psychology, [2]Kinesiology, [4]Psychology, [5]Dept. of Psychiatry, [3]Univ. of Michigan, Ann Arbor, Ann Arbor, MI; [6]Neurol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Transcranial magnetic stimulation (TMS) can be used to focally modulate a brain region to assess effects on other brain regions. TMS combined with functional MRI can give information about causal connectivity. Another approach to measuring connectivity uses two TMS coils, one over the primary motor cortex (M1) and one over another brain region. This dual-site TMS (dsTMS) approach assesses the modulatory effects of the other brain region on M1 by measuring the TMS-induced motor-evoked potential (MEP) as a physiological readout. This two-coil approach often derives TMS targets from scalp measurements based on the 10-20 electroencephalography (EEG) system or anatomical coordinates. However, this approach can lead to interindividual heterogeneity of the stimulation site that can affect the behavioral response to stimulation. Using a target site derived from person-specific functional connections could refine targeting methods by reducing off-target effects and better accounting for differences in individual functional neuroanatomy. We developed a more precise TMS targeting method for stimulating personalized functional network engagement using a dsTMS grid-searching protocol we term "dsGrid". This approach uses a hunting procedure to target personalized functional interactions in the cortical grasping network. Here, we demonstrate the mapping of functional connections in the motor system at the individual level with the dsGrid approach. First, we locate the left parietal target from the P3 EEG electrode positio. A circular search grid around P3, with positions separated by 1 cm, is created using a neuronavigation system. A conditioning stimulus (CS) over each PPC target in the search grid is applied before delivering a test stimulus (TS) to the left M1 while participants perform object-directed grasping movements with the right hand. The optimal scalp position for coil placement is where CS elicits the largest MEP from the right (response) hand muscle during the movement plan. The MEP at each point in the search grid was evaluated in 48 healthy individuals. We validated the dsGrid approach with 8 minutes of resting-state fMRI data. The targets acquired with the dsGrid method were compared to the location of maximum resting-state functional connectivity between M1 and PPC for each participant. Finally, we evaluated the modulatory effect of parietal theta burst stimulation on downstream motor plasticity prescribed by the dsGrid functional mapping. This dsGrid approach could improve the spatial targeting of parietal-frontal network stimulation and better account for individual differences in functional neuroanatomy in research and clinical settings.

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Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

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Topic: E.04. Voluntary Movements

Support: NRF-2021M3E5D2A01019542

Title: Human beta-burst activity represents interaction between stop and go processes in horse-race model for movement cancellation

Authors: *E. OH, S.-P. KIM;
Biomed. Engin., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: Movement inhibition, particularly the ability to cancel ongoing or preparing movements, plays a vital role in enabling humans to rapidly adjust their behavior to varied environmental demands. It has been found that movement cancellation is mediated by transient and burst-like oscillatory activity in the beta frequency (15-29 Hz) in humans. The horse-race model has been proposed to elucidate how motor inhibition occurs through a competition between go and stop processes. If the stop process finishes before the go process reaches an action threshold, movement stops; otherwise, it fails to stop. Two hypothetical models coexist to describe how these two processes compete: the independent model focuses on the outcome of the race between independent go and stop processes, whereas the interactive model hypothesizes interactions between stop and go processes at a neural level. In the interactive model, stop activation starts near stop-signal reaction time (SSRT) and inhibits the go-activation. Although behavioral support for the horse-race model has been well documented, neural substrates supporting the model remain relatively unknown. Therefore, this study aimed to find neural substrates of the horse-race model using beta-burst activity in the human electroencephalogram (EEG). EEG was recorded in 30 participants during the stop-signal task (SST) where 25% of a total of 800 trials was stop trials and others go trials (fully randomized). Stop-signal delay (SSD) was adjusted by a stair procedure based on individual stop performance in the previous stop trial. We calculated beta-burst rates and compared them in different conditions including correct go, failed stop, and successful stop. Consistent with previous studies, we verified that behavioral results satisfied the horse-race model showing shorter failed-stop reaction time than go-trial reaction time ($p < 0.001$). From beta-burst analysis, we found that frontocentral beta burst rates decreased in the go condition, while it increased in both the failed stop and successful stop conditions, showing significant a difference between the two stop conditions and the go condition in a time window near stop-signal reacting time (SSRT). However, no significant difference was found in the period from SSD to SSRT. Moreover, beta-burst rates exhibited an earlier increase under the successful stop than failed stop conditions. In summary, movement cancellation entailed increases in beta-burst rates near the SSRT than immediately after stop signal but delayed onset of beta-burst rates could lead to failed stop. Our results corroborate the interactive horse-race model by showing the effect of stop activation.

Disclosures: E. Oh: None. **S. Kim:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.09/EE23

Topic: E.04. Voluntary Movements

Title: Clinical Mapping Using Stereoelectroencephalography Based Passive Task Mapping

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Abstract: Clinical Mapping Using Stereoelectroencephalography-Based Passive Task Mapping

Introduction: Stereoelectroencephalography involves the placement of depth electrodes throughout the volume of the brains of patients with drug-resistant epilepsy to characterize their seizures. While intracranial electrodes have been used to map motor, visual, and language representation in the brain, this has been largely limited to the surface of the cortex in the setting of electrocorticography. As sEEG records volumetrically, we set out to describe, measure, and characterize the utility of sEEG in language and motor mapping.

Objective: We aim to describe, measure, and characterize the utility of sEEG in language and motor mapping, and compare it to existing fMRI and clinical stimulation-based mapping modalities.

Methods: Fourteen adult and pediatric patients performed motor and language tasks consisting of visually cued movement (hand, tongue, or foot) and language production (verb and noun). We segmented data into epochs of speech/movement and rest using parallel audio and EMG recordings for motor and language tasks respectively. As broadband power has been established as a correlate of neural population firing rate, its variation in each sEEG channel between active (movement or language production) and rest periods allowed for identification of movement/speech correlated cortex.

Results: sEEG-based passive mapping (SPM) allows for the identification of both cortical and subcortical activity across all channels of the sEEG array. As SPM detects local neural population firing naïve to the anatomy, it may identify redundant pathways that stimulation mapping may miss. Similar to fMRI, SPM may identify cortex that is active yet not necessary to produce language or speech. Together, we demonstrate that this holistic approach to functional interdependence using SPM, fMRI, and clinical stimulation mapping are complementary.

Conclusion: SPM may serve as a useful clinical tool to complement fMRI and clinical stimulation based mapping modalities.

Disclosures: M.A. Jensen: None. **A.L. Fine:** None. **E.C. Alden:** None. **D. Hermes:** None. **K.J. Miller:** None.

Poster

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Program #/Poster #: PSTR348.10/EE24

Topic: E.04. Voluntary Movements

Support: K12HD093427

Title: Personalized whole-brain activity patterns predict corticospinal tract activation in real-time

Authors: U. U. KHATRI[1], *S. J. HUSSAIN[2];
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Abstract: The corticospinal tract (CST) is the major descending pathway responsible for voluntary human hand movement. Poststroke CST integrity predicts motor recovery. Strengthening the residual CST could thus promote poststroke hand motor recovery. Recent work in healthy adults showed that TMS interventions enhance CST transmission and motor learning, when delivered during pre-defined sensorimotor rhythmic brain states reflecting strong CST tract activation (brain state-dependent TMS). Because each stroke survivor has a unique pattern of brain damage and motor impairment, poststroke brain state-dependent TMS interventions cannot be delivered during "one-size-fits-all" states. Rather, TMS must be delivered during personalized poststroke brain states reflecting strong residual CST tract activation. Here, we developed and tested a novel machine learning-based EEG-TMS system that identifies personalized strong and weak CST states in real-time in healthy adults. Healthy adults completed a single session involving 600 single TMS pulses to the right motor cortex (M1) at 120% of resting motor threshold (RMT) during 64-channel EEG and left first dorsal interosseous EMG recordings. This dataset was used to train personalized machine learning classifiers to identify strong and weak CST states, defined as those EEG activity patterns during which TMS evoked either large or small motor-evoked potentials (MEPs). We spatially filtered pre-stimulus whole-brain EEG data and calculated power spectral features from these data. We then trained several personalized linear discriminant analysis classifiers using cross-validated grid search and chose the best performing classifier for real-time analysis. We used this classifier to deliver single-pulse TMS (120% RMT) during strong and weak CST states in real-time. Preliminary results (N=11) show that personalized classifier performance measured via F1 scores was 68±5%. Our real-time algorithm accurately targeted personalized high CST states 93.4±4.5% and low CST states 93.1±6.5% of the time, and MEPs elicited during strong CST states were 31.1±26.4% and 33.3±43.7% larger than those elicited during weak states and random states (state-independent TMS), respectively. Our results show that personalized whole-brain EEG activity patterns predict CST activation in real-time. Findings represent the first step towards using personalized brain state-dependent TMS interventions to promote poststroke hand motor recovery.

Disclosures: U.U. Khatri: None. **S.J. Hussain:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NIH Grant NS112367

Title: Role of multisensory cortical and subcortical regions in visuo-proprioceptive recalibration in estimating hand position

Authors: *H. BLOCK, M. WALI, A. HSIAO, C. LO;
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Abstract: Accurate hand movement depends on proprioception (limb position sense) and vision. When these signals become mismatched, as when a visual cue of hand position is displaced from the proprioceptive cue, the brain shifts its visual and proprioceptive estimates to compensate (recalibration). The neural basis of visuo-proprioceptive recalibration is poorly understood but may involve multisensory brain regions linked to both vision and proprioception such as the cerebellum (CB), ventral premotor cortex (PMv), or anterior superior parietal lobule (aSPL). Here we ask whether disrupting activity in these areas with continuous theta burst stimulation (cTBS) affects how healthy young adults use visual and proprioceptive recalibration to compensate for a visuo-proprioceptive mismatch. Before and after cTBS of CB, PMv, aSPL, or sham stimulation in the right hemisphere (left for CB), participants estimated their left finger position with veridical visual and proprioceptive cues. This was followed by the mismatch block in which the visual cue was gradually shifted 70 mm away from the proprioceptive cue. The task was performed on a 2D virtual reality apparatus that prevented direct vision of the hands. Mirror placement made the visual display appear in the plane of the hands, which were positioned above and below a two-sided touchscreen. Participants were asked to use their right “indicator” index finger to indicate the perceived position of visual (V), proprioceptive (P), or combined (VP) cues about their left “target” index finger. Participants received no online or endpoint feedback or knowledge of results, to prevent motor adaptation. Change in performance on unimodal P and V trials are used to quantify proprioceptive and visual recalibration, respectively. To assess the effect of recalibration in left hand perception on left hand motor control, participants also made fast reaches to a visual target with their left hand before and after the mismatch block, also with no feedback. In data collected thus far (3-5 subjects per group), participants who received sham cTBS recalibrated vision 33 ± 8 mm (mean ± SE) and proprioception 12 ± 5 mm, consistent with previous work. The aSPL group recalibrated vision 34 ± 9 mm and proprioception 6 ± 2 mm. The CB group recalibrated vision 31 ± 6 and proprioception -4 ± 4 mm. The PMv group recalibrated vision 39 ± 5 mm and proprioception 13 ± 3 mm. These preliminary results may be consistent with activity in the cerebellum and aSPL having a role in proprioceptive recalibration.

Disclosures: H. Block: None. **M. Wali:** None. **A. Hsiao:** None. **C. Lo:** None.

Poster

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Topic: E.04. Voluntary Movements

Title: The influence of transcranial direct current stimulation on contralateral primary motor cortex excitability

Authors: E. W. WILKINS[1], E. KAWANA[1], E. LOPEZ MORA[1], D. HOUSTON[1], S. BOSS[1], R. J. YOUNG[1], Z. A. RILEY[2], ***B. POSTON**[1];
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Abstract: Transcranial direct current stimulation (tDCS) applied to the primary motor cortex (M1)has been shown in numerous studies to increase M1 excitability and improve performance in relatively simple motor tasks performed with the hand and arm. However, the influence of tDCS on contralateral M1 excitability during and after tDCS application has not been quantified. The purpose was to determine the influence of tDCS on contralateral M1 excitability. The study utilized a double-blind, randomized, sham-controlled, within-subjects, crossover experimental design. Fourteen young adults performed two identical sessions in counterbalanced order and separated by a one-week washout. Transcranial magnetic stimulation (TMS) testing was used to quantify cortical excitability in the contralateral M1 to which a single 20-minute application of anodal tDCS (current strength: 1 mA) was applied. TMS was given in 5 test blocks (Pre, D5, D10, D15, and Post). The Pre and Post TMS test blocks were performed immediately before and after tDCS, whereas the TMS test blocks performed during tDCS were completed at the 5, 10, and 15-minutes of stimulation time points. The primary outcome measure was the 1 mV motor evoked potential (MEP) amplitude (index of M1 excitability). MEP data was analyzed with a 2 *Condition* (tDCS, SHAM) x 5 *Test* (Pre, D5, D10, D15, Post) within-subjects ANOVA. The main effect for *Condition* ($P = 0.878$), main effect for *Test* ($P = 0.272$), and *Condition* x *Test* interaction ($P = 0.788$) were all non-significant. These results indicate that tDCS does not modulate contralateral M1 excitability. Since tDCS applied unilaterally to one M1 usually increases its excitability and improves motor performance of the contralateral hand it primarily controls, the current results imply that this stimulation montage likely has neither positive nor negative performance effects on the hand ipsilateral to the targeted M1.

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Poster

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Program #/Poster #: PSTR348.13/EE27

Topic: E.04. Voluntary Movements

Title: Sensorimotor control in the wild: Spatiotemporal reconstruction of human motor control

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Abstract: Motor control has been studied for decades, but more often than not the types of motor tasks that are studied in the lab are simple leading to a lack of generalizability for more complex tasks that are performed daily. These studies are often limited due to difficulty of efficiently measuring the full range of motions required for complex motor tasks. Here we introduce a modular open-source software written in Python. The toolbox allows the quantification of sensory, motor, cognitive, neural and behavioral measures of human decision making and performance during task execution. By integrating unsupervised real-time decoding of human body positional control using video cameras with haptic gloves, eye-tracking googles and a world-view camera worn by the participant, human behavior is digitized. The sensory (tactile, visual and auditory) stimuli are controlled in mixed reality, while the neural data is acquired via mobile EEG and fNIRS. The toolbox allows real-time control of external devices, thus could be used for close-loop control of brain and behavior using neuromodulation and bio-stimulation methods. Taking advantage of this platform, here we also introduce a new dart-game task where participants learn to target a stationary or mobile target presented on a dart board. We show that the adaptive sensorimotor control strategies in the task allow minimization of behavioral errors. By reducing the dimensionality in human motor behavior, via high-dimensional clustering of motion tracking, we identify the "motor primers" that predict success in this task. Identification of the motor control parameters to maximize success in sensorimotor tasks will not only help improve human performance but also will help shed light onto the neural mechanisms of sensorimotor learning.

Disclosures: J. Mostyn: None. **T. Celikel:** None.

Poster

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Topic: E.04. Voluntary Movements

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Title: Technical Feasibility of Rapid Deep Brain Stimulation Amplitude Changes Synchronized to the Gait Cycle in Parkinson's Disease Patients

Authors: *K. LOUIE[1], J. BALAKID[1], J. BATH[1], H. FEKRI AZGOMI[1], J. CHOI[2], P. STARR[1], D. WANG[1];
[1]Univ. California San Francisco, San Francisco, CA; [2]Univ. of Florida, Gainesville, FL

Abstract: Gait disturbances is a particularly debilitating motor impairment associated with Parkinson's disease (PD) and can be refractory to conventional therapies such as dopamine replacement therapy and deep brain stimulation. The initial success of adaptive stimulation paradigms in treating akinesia in PD has garnered much interest for its use to improve gait in PD. However, questions remains whether an adaptive algorithm can detect and change stimulation parameters fast enough to target specific gait phases in real-time. In this study we implement a fully embedded adaptive stimulation therapy targeted to the swing phase of the gait-cycle using the Medtronic RC+S bidirectional neural stimulator.An individual with PD recieved adaptive stimulation therapy with deep brain stimulation leads targeting the globus pallidus interna (GPi), and subdural cortical paddles overlying the primary motor (M1) and premotor (PM) cortices. Nine different stimulation settings were tested while the patient walked overground at a self-selected speed for 200 steps. Of the nine settings, eight settings used biomarkers associated with contralateral leg swing for adaptive stimulation control, and one setting was set to their clinically optimized continuous DBS stimulation setting. These nine stimulation parameters were pseudorandomized. Subcortical and cortical LFPs were recorded simultaneously at 500 Hz. Additionally, gait kinematics were measured using external sensors (Delsys and Xsens). Adaptive stimulation accuracy and gait metrics were calculated for each setting.We identified a patient-specific frequency band that can effectively drive an adaptive stimulation algorithm synchronized with the swing phase of the gait cycle. The most accurate gait biomarkers originated from the pallidum, and were able to detect and modify stimulation amplitude in at least 58% of swing phases. Compared to the patient's clinical gait parameters, we observed improvements in gait speed and reduced stride time in six out of eight adaptive DBS settings. However, there were no significant changes in stride length. Notably, two pallidal biomarkers that demonstrated the highest accuracy also resulted in decreased variance in gait speed.These results show the feasibility of implementing fast adaptive stimulation algorithms that work in the hundreds of millisecond time scale. Furthermore, these preliminary results suggest that gait in individuals with PD may be improved with adaptive stimulation therapy synchronized to their gait cycle.

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Poster

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Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2020-04255

Title: The influence of music listening on sensorimotor integration

Authors: *J. VANDER VAART, M. PERRIER, K. GRAHAM, R. STAINES, S. MEEHAN; Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: Optimal motor performance relies on the efficient integration of sensory information into the descending motor command. The integrative process depends on both cognitive and motor factors. Past work has demonstrated that differing attentional foci when performing a task can influence motor performance. It is possible that altering the sensory environment may also influence sensorimotor integration by changing the cognitive-motor balance. Therefore, the current study used music to alter the cognitive-motor balance and establish its effect on different somatosensory-motor circuits that converge in motor cortex to influence motor output. Short-latency afferent inhibition (SAI), a marker of cholinergic function, was used to determine the effect of music on different sensorimotor circuits converging in motor cortex. Participants completed 2 sessions. During the first session, participants practiced a continuous motor task, without SAI, involving contraction of the first dorsal interosseous muscle (FDI) to track a cursor across the screen. During the second session, SAI was assessed as participants listened to white noise, no noise, low tempo, and high tempo music while performing a baseline contraction task and a variable contraction tracking task. SAI was assessed by preceding transcranial magnetic stimulation (TMS) over the FDI motor cortex with electrical stimulation of the median nerve at the wrist. The interstimulus interval used was 22 ms. Controllable pulse parameter TMS stimulation induced either posterior-anterior (PA) current lasting 120 μs or anterior-posterior (AP) current lasting 30 μs to probe specific sensorimotor circuits that may be indistinguishable using the fixed stimulus duration of conventional TMS stimulators. Preliminary findings indicate that PA SAI was stronger when listening to low tempo and high tempo music compared to no noise and white noise, regardless of whether they were holding a constant baseline contraction or performing the variable contraction task. In contrast, AP SAI did not differ across audio type during baseline contraction however, during variable contraction, AP SAI was weaker in low tempo music compared to high tempo and no noise. These results are consistent with past work indicating the involvement of PA120 and AP30 circuits with movement planning and movement modulation respectively. The current results demonstrate that listening to music differentially influences specific sensorimotor circuits that make novel contributions to skilled motor performance.

Disclosures: J. Vander Vaart: None. **M. Perrier:** None. **K. Graham:** None. **R. Staines:** None. **S. Meehan:** None.

Poster

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Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2020-04255

Title: The effect of explicit instruction on sensorimotor integration during early skill learning: A short latency afferent inhibition study.

Authors: *M. PERRIER, J. E. VANDER VAART, K. R. GRAHAM, W. R. STAINES, S. K. MEEHAN;
Kinesiology and Hlth. Sci., Univ. of Waterloo, Waterloo, ON, Canada

Abstract: The ability to integrate afferent somatosensory information during motor learning is governed by two broad distinct processes, explicit and implicit. The explicit process involves intentionally acquiring factual knowledge, while the implicit process involves the capacity to acquire a new skill through physical practice without conscious awareness. During skill learning, the complex interaction between the two processes may modulate somatosensory-motor integration. Short latency afferent inhibition (SAI) that involves preceding a transcranial magnetic stimulus (TMS) with an anterior-posterior (AP) induced current lasting 30 μs with electrical stimulation of the median nerve (MNS) can be used to probe the functional influence of a specific subset of sensorimotor circuits. The sensorimotor circuits recruited by AP induced-current lasting 30 μs (AP30) are sensitive to attention and cerebellar modulation, two key components involved in continuous motor skill acquisition. The current study used AP30 SAI to examine the effect of explicit instruction and how it influences sensorimotor integration. Participants were randomly assigned to an explicit or implicit group. The experiment consisted of an initial training and a retention session separated by twenty-four to forty-eight hours. The first session involved SAI assessments prior to practice, during initial performance and practice of a continuous tracking task. The second session involved a delayed retention test and recognition test. The experiment proceeded similarly for both groups, except that the explicit group was informed of the repeated segment after the initial performance block on the first day. Explicit knowledge was confirmed using a recognition test prior to subsequent practice. The continuous tracking task involved adducting the index finger against a force sensor to control a cursor to track a three-epoch sinusoidal wave. The first and third epochs were randomly generated between trials and blocks, while the middle epoch was repeated. SAI was assessed by preceding the TMS stimulus with MNS by 22 ms. Preliminary results show that those in the explicit group exhibited greater SAI from initial practice in the first practice block immediately following the delivery of explicit instruction. SAI remained elevated for the duration of practice. In contrast, the implicit group experienced no change between initial practice and training. This suggests that explicit instruction alters sensory-motor circuits that are known to be sensitive to attention and cerebellar modulation. Such alteration may have unique consequences for skill acquisition and performance in different environments.

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Poster

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Topic: E.04. Voluntary Movements

Support: NSERC RGPIN-2020-04255

Title: The relative contributions of short-latency afferent inhibition and short-interval intracortical inhibition during skilled motor behaviour

Authors: *K. R. GRAHAM[1], J. VANDER VAART[1], M. PERRIER[1], R. STAINES[3], S. K. MEEHAN[2];
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Abstract: Short-latency afferent inhibition (SAI) and short-interval intracortical inhibition (SICI) originate from peripheral sensory and cortical conditioning of the motor cortex output. SAI is elicited by preceding a reference transcranial magnetic stimulus (TMS) pulse over motor cortex with electrical stimulation of the corresponding afferent nerve by ~20ms. SICI is elicited by preceding the reference TMS pulse by a subthreshold TMS pulse by ~2 ms. Limited research has explored the interactions between the convergent sensory-motor circuits indexed by SAI and the intracortical circuit indexed by SICI. The different sensory-motor and intracortical circuits may play critical roles in different phases of skilled movement. The present study examined the relative contributions of different sensorimotor circuits and intracortical circuits to motor planning and execution of skilled movement. Participants completed two sessions. In both sessions, the participants tracked a sinusoidal waveform of varying amplitude by pressing the side of their index finger against a force sensor. The movement started with a 4s baseline where participants maintained a constant 10% maximal voluntary contraction (MVC) in the target first dorsal interosseus muscle. After the baseline phase, the target waveform appeared preceded by an additional two second baseline (planning phase). The participants then tracked the target waveform. TMS stimuli were delivered during the baseline (3.5 seconds), early planning (500ms before movement onset), late planning (250ms before movement onset, at movement onset), and then during tracking (1 s increments during the movement when tracking force corresponded to 10% MVC muscle activity). To examine the relative contributions of different sensorimotor and intracortical circuits, SAI was assessed using posterior-anterior (PA) current lasting 120 μs and anterior-posterior (AP) current lasting 30 μs, two configurations known to recruit functionally distinct sensorimotor circuits. SICI was assessed using PA and AP current lasting 70 μs, a duration consistent with conventional TMS stimulators. Preliminary results showed that changes in SAI and SICI dominate over different phases of the movement. PA_{120} SAI appears to dominate over PA_{70} SICI and AP_{30} SAI during early planning, while PA_{70} SICI dominates later planning as movement onset approaches, and AP_{30} SAI dominates during movement execution. AP_{70} SICI showed no change from baseline over movement planning and execution. These results suggest that the different sensorimotor and intracortical circuits may have different functional roles related to separate phases of a skilled movement.

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Poster

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Program #/Poster #: PSTR348.18/FF4

Topic: E.04. Voluntary Movements

Title: Single-pulse TMS over rIFC evokes an electromyographic response

Authors: *M. FISHER[1], C. G. WADSLEY[2], K. BAKKEN[1], W. BYBLOW[3], I. GREENHOUSE[1];
[1]Human Physiol., Univ. of Oregon, Eugene, OR; [2]The Univ. of Auckland, Auckland, New Zealand; [3]Univ. of Auckland, Auckland, New Zealand

Abstract: Right inferior frontal cortex (rIFC) is a key region in the putative response inhibition neural network for interrupting motor activity. Interestingly, direct electrical stimulation of rIFC during intraoperative surgery reliably elicits negative motor responses in humans. Negative motor areas may play a role in the inhibition of coordination of fine movements. However, research establishing whether negative motor responses can be elicited outside of the surgical suite is lacking. The present study aimed to determine whether transcranial magnetic stimulation (TMS) can be used to elicit negative motor responses non-invasively. Right-handed participants (n = 14, 7 female, 25 ± 5 years) received neuro-navigated TMS based on MNI coordinates for the rIFC (expected negative motor site, X = 42, Y = 18, Z = -6) and occipital (OCC, X = 6, Y = -84, Z = -1) cortex as a control. Electromyography (EMG) was recorded during 10% maximal voluntary contraction in four muscles of the left arm and hand: first dorsal interosseous (FDI), abductor pollicis brevis (APB), extensor carpi radialis (ECR) and anterior deltoid (DELT). rIFC and OCC were stimulated at 130% of left FDI resting motor threshold. Each site was stimulated with the coil oriented to produce either anterior-posterior (AP) or posterior-anterior (PA) flowing-current relative to the underlying cortical surface. Evoked responses in the rectified EMG data were identified in the 50 - 150 ms window after TMS, and the difference in area under the curve relative to baseline (-105 to -5 ms pre-TMS) was calculated. A repeated-measures ANOVA included the factors site (rIFC, OCC), coil orientation (AP, PA), and muscle (FDI, APB, ECR, DELT). There was a significant main effect of site [$F(1,13) = 10.28$, $p < 0.01$] indicating single-pulse TMS of rIFC evoked an EMG response during tonic upper-limb contraction. This novel EMG marker may reflect the influence of rIFC over corticospinal excitability via cortico-cortical or cortico-subcortical pathways.

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Poster

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Topic: E.04. Voluntary Movements

Support: R01 NS123115

Title: Is motor system excitability during rest the same outside and inside a behavioral task?

Authors: *K. R. BAKKEN[1], C. HORTON[2], M. FISHER[2], C. WADSLEY[2], I. GREENHOUSE[2];
[1]Human Physiol., [2]Univ. of Oregon, Eugene, OR

Abstract: Resting corticospinal excitability may differ between out-of-task and in-task contexts. Here, we used single-pulse transcranial magnetic stimulation (TMS) and electromyography (EMG) to measure motor evoked potentials (MEPs) during out-of-task rest and in-task (intertrial interval) rest in healthy young adults (n=26, 16F, mean age of 21 ± 3 years). TMS was administered to right M1 at a range of intensities (90, 110, 130, 150, and 170% resting motor threshold) to derive MEP input-output recruitment curves in the left first dorsal interosseous muscle at rest in three contexts: 1) out-of-task, 2) finger-choice task, and 3) hand-choice task. Participants were instructed to relax across all contexts which was indicated by EMG quiescence. On each trial of the in-task contexts, participants prepared and executed motor responses before returning to rest during the intertrial interval. We hypothesized resting state excitability would be greater at intertrial intervals than out-of-task rest contexts, resulting in consistently higher average MEP amplitudes than those measured during the out-of-task resting state and a steeper slope of the recruitment curve. A lack of a difference would suggest out-of-task and in-task rest are similar states in healthy participants. Mean MEP amplitudes were compared across all three contexts and TMS intensities. There was no main effect of context [$F(2,25) = 0.518$, $p = 0.599$] nor an interaction between context and intensity [$F(8,25) = 0.561$, $p = 0.809$]. Bayesian analyses also provided strong evidence for no differences between contexts [$BFM = 9.398\times10^{-25}$]. No significant difference was found between maximum slopes across contexts [$F(2,50) = 1.19$, $p = 0.31$]. These data support the test-retest reliability of recruitment curve characteristics on an individual and group level and suggest rest is a uniform state of corticospinal excitability. In the future, these data may serve as a useful reference for comparisons with clinical populations.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Gain in the human motor system during action preparation

Authors: *C. G. WADSLEY, C. HORTON, I. GREENHOUSE;
Univ. of Oregon, Eugene, OR

Abstract: The transition from thinking of an action to initiating it appears effortless and reliable in healthy individuals. Action preparation is marked by suppression of excitability within the corticospinal (CS) pathway from primary motor cortex (M1). *Preparatory suppression* has been described with a dual-process model involving impulse control and competition resolution. However, recent findings have prompted a revised perspective, proposing that preparatory suppression actively sculpts CS output through *gain modulation*, a well-characterized neural computation in human sensory and animal motor systems. This study aimed to investigate the presence of CS gain modulation during action preparation. We hypothesized input-output (I/O) curves of CS excitability would exhibit a steeper slope during action preparation, revealing a multiplicative increase in motor system gain. Human participants (n = 37) performed two-choice delayed response tasks involving either left and right index finger or right index and right pinky finger responses. Single-pulse transcranial magnetic stimulation was used to derive motor evoked potential (MEP) I/O curves in the left first dorsal interosseous (FDI) muscle. I/O curves were determined during the intertrial interval (baseline) and when FDI was task-irrelevant, nonselected, or selected for the forthcoming response. Boltzmann sigmoidal functions were fitted to I/O curves to estimate peak slope, MEP_{max}, and the stimulation intensity required to elicit half of MEP_{max} (S50). Evidence was quantified using Bayes factor (BF) in favor of the alternative hypothesis. MEP amplitude results provided strong evidence of a Context × Intensity interaction (BF > 100). At low intensities, preparatory suppression was observed in nonselected and task-irrelevant contexts. However, at higher intensities, preparatory suppression was only observed in the task-irrelevant compared to baseline context, while there was no difference from baseline in nonselected and selected contexts (all BFs < 0.12). The Boltzmann sigmoidal function provided a good fit across all contexts (mean $R^2 = 0.98$). There was moderate evidence for a main effect of Context on the S50 parameter (BF = 41.27), expressed as a leftward shift in the I/O curve during selected compared to nonselected and irrelevant contexts, but not for peak slope (BF = 0.57) or MEP_{max} (BF = 0.21) parameters. These results indicate preparatory suppression is expressed differently across response contexts depending on stimulation intensity, and changes in CS gain during action preparation may be additive rather than multiplicative.

Disclosures: C.G. Wadsley: None. **C. Horton:** None. **I. Greenhouse:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.21/FF7

Topic: E.04. Voluntary Movements

Title: A Bilateral Comparison of Muscular Activation in the Tibialis Anterior: Implications of Training Status

Authors: *S. M. WASHINGTON[1], K. J. PENNARTZ[1], C. D. SMITH[1], D. R. HARRIS[1], A. C. AVALOS[1], L. K. RANKIN[1], B. E. ZAPATA[1], J. A. FUSSELL[1], J. M. WATSON[1], J. L. KELLER[2], A. L. HARRIS BOZER[1], M. J. LUERA[1];
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Abstract: Generally, training status influences muscle morphology and contractile properties of a muscle. The intensity of the contraction determines the resulting activation needed to sustain a contraction and the type of fiber used. As fiber distributions shift with training the size of a muscle and its ability to sustain various contraction intensities is reflected through the resulting activation. The purpose of this study was to bilaterally compare cross-sectional area (CSA) and activation of the TA in trained and untrained individuals. 4 lower-body resistance trained males (n=4; age=22 ±0.8, height=174.5 ±6 cm., weight=83 ±12.9 kg) and 4 untrained males (n=4; age=22.5 ±2.1, height=173.5 ±5.6 cm, weight=79.7 ±20.1 kg) completed this study. CSA was recorded using the GE LOGIQ portable ultrasound machine and analyzed in ImageJ for CSA measurements. Using an isometric dynamometer, the subjects performed 3 maximal voluntary contractions (MVCs), the highest of which was used to determine their randomized trapezoidal tracings at 25%, 50%, 75%, and 100%. A 64-channel high-density surface electromyography electrode array located on the TA was used to record activation. The peak RMS values were calculated via manual editing techniques across all percentages. For further analysis, the linear relationship between RMS values and MVC percentages were calculated. 2 separate 2-way mixed factorial analysis of variance (ANOVA) (group [trained v untrained] x leg [dominant v non-dominant]) were used to compare slopes and intercepts of peak RMS values across the trapezoidal tracings. An additional 2-way mixed ANOVA (group [trained v untrained] x leg [dominant v non-dominant]) was used to compare CSA of the TA. There were no significant group x leg interactions or main effects ($p>.05$) in the slopes and intercepts. Regarding CSA, there were no significant group x leg interactions or main effects ($p>.05$.) Despite lower-body resistance training, there is no difference in the activation or CSA of the TA in the dominant and non-dominant legs of trained and untrained individuals. This may result from the lack of emphasis on the TA muscle in resistance training programs.

Disclosures: S.M. Washington: None. **K.J. Pennartz:** None. **C.D. Smith:** None. **D.R. Harris:** None. **A.C. Avalos:** None. **L.K. Rankin:** None. **B.E. Zapata:** None. **J.A. Fussell:** None. **J.M. Watson:** None. **J.L. Keller:** None. **A.L. Harris Bozer:** None. **M.J. Luera:** None.

Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.22/FF8

Topic: E.04. Voluntary Movements

Title: Exploring Hemisphere Differences in the Human Pre-Motor Cortex during Bilateral Isometric Contractions

Authors: *J. WATSON[1], A. AVALOS[1], K. PENNARTZ[1], B. ZAPATA[1], C. SMITH[1], S. WASHINGTON[1], L. RANKIN[1], J. FUSSELL[1], D. HARRIS[1], J. KELLER[2], A. HARRIS BOZER[1], M. LUERA[1];
[1]Tarleton State Univ., Stephenville, TX; [2]Univ. of South Alabama, Mobile, AL

Abstract: The premotor cortex is involved in the planning and execution of voluntary muscle contractions through propagation of beta band oscillations, which can lead to an increase in control of skilled motor movements. Oscillations in the beta-band (13-30Hz) can be analyzed during motor behavior and are present in the premotor cortex during sustained isometric muscle contractions. Contralateral comparisons of the left and right hemisphere during bilateral isometric contractions can be produced by analyzing the beta-band frequencies in the premotor cortex. The purpose of this study is to compare mean power frequencies (MPF) of the beta-band in the premotor cortex (FC5-FC6) while participants performed varying contractions of the tibialis anterior (TA). For this study, 4 lower-body resistance untrained males (n=4; age=22.5 ±2.1, height=173.5 ±5.6 cm, weight=79.7 ±20.1 kg) performed three dorsiflexion maximal voluntary contractions (MVC) in which the highest force output was recorded. Subjects then performed randomized ramping trapezoidal tracings at 25%, 50%, 75% and 100% of the individuals MVC. The participants were placed in a custom-built seat with their hip flexed to 100 degrees and their knee flexed 50 degrees in an isometric dynamometer that recorded TA force production. A 64-channel EEG cap was used to record beta-band activation in the premotor cortex during each contraction. Signals were recorded (OT Bioelectronica), decomposed, and filtered by channel type in MATLAB (EEGlab). Cartool was used to compute fast fourier transforms. In OT Bioelectronica, the trapezoidal muscle contractions were recorded to corresponding 64-channel EEG signals. A factorial analyses of variance (ANOVA) (location [FC5, FC3, FC1] x intensity [25%, 50%, 75%, 100%]) x (location [FC6, FC4, FC2] x intensity [25%, 50%, 75%, 100%]) was used to compare MPF of the left and right hemispheres respectively. In regard to the right hemisphere, there were no significant location x intensity interactions or main effects ($p>0.05$). In regard to the left hemisphere, there were no significant location x intensity interactions ($p>0.05$): however, when collapsed by intensity there was a significant main effect for location ($p<0.05$). These data indicate that when the contralateral leg is dominant, there is a significant effect at higher intensity in the premotor cortex in the beta-band. These data elucidate the neurocortical mechanisms that underlie premotor cortex processing of motor activity in the TA.

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Poster

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Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.23/FF9

Topic: E.04. Voluntary Movements

Title: Does muscle quality and motor unit firing behaviors differ in trained vs untrained individuals?

Authors: *L. K. RANKIN[1], S. M. WASHINGTON[1], D. R. HARRIS[1], K. J. PENNARTZ[1], C. D. SMITH[1], B. E. ZAPATA[1], A. C. AVALOS[1], J. M. WATSON[1], J. A. FUSSELL[1], J. L. KELLER[2], A. L. HARRIS BOZER[1], M. J. LUERA[1];
[1]Tarleton State Univ., Stephenville, TX; [2]Univ. of South Alabama, Mobile, AL

Abstract: The tibialis anterior (TA) is primarily comprised of slow-twitch muscle fibers, which have low force producing capabilities. Consequently, achieving a desired muscle force requires a higher firing rate and eventual recruitment of high-threshold motor units. In trained individuals, muscle quality commonly determines skeletal muscle force production in various contraction types, and may determine its relative firing patterns. Differentiating motor unit recruitment between trained and untrained individuals has practical implications for optimizing performance and preventing injuries. The purpose of this study was to examine the differences in echo intensity (EI) and motor unit relationships during isometric dorsiflexion for untrained and trained individuals. 8 male subjects (untrained, n= 4; age= 22.5 ± 2.1, height= 173.5 ± 5.6 cm, weight= 79.7 ± 20.1 kg; trained, n= 4; age= 22 ± 0.8, height= 174.5 ± 6 cm., weight= 83 ± 12.9 kg) performed 4 randomized isometric dorsiflexion contractions on an ankle dynamometer. Trapezoidal tracings were performed at 25%, 50%, 75%, and 100% of their maximal voluntary contraction (MVC). Prior to testing for both groups, EI was obtained from the TA using ultrasonography. A 4-pin surface electromyography sensor was used to acquire signals from the TA. Signals were later decomposed into their motor unit action potential (MUAP) trains, validated, and assessed for behavioral properties including mean firing rate (MFR) and MUAP sizes. For further analysis, an independent samples t-test was run to compare EI between groups. 2 separate 2-way mixed factorial analysis of variance (ANOVAs) (group [untrained v trained] x intensity [25% v 50% v 75% v 100%]) were used to compare the slopes and y-intercepts of MFR v MUAP. Regarding comparisons in EI between groups, there was no significant differences ($p=0.107$). There was a main effect for intensity ($p<0.05$) and a significant group x intensity interaction in the MFR v MUAP slopes ($p=0.026$). There was no significant interaction or main effect of the intercepts ($p>0.05$). Concerning the main effect in intensity, values at lower intensities were found to be significantly different than values at higher intensities. Furthermore, trained individuals have adaptations of improved neural control and enhanced muscle fiber recruitment patterns that assist in activating a significantly lower number of motor units at lower intensity exercises. The absence of disparities in EI among the groups could be explained by the comparable presence of glycogen content in the muscle. Additionally, trained individuals' endurance in the TA muscle suggests they would be capable of enduring longer contraction types.

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Poster

PSTR348. Cortical Planning and Execution: Human Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR348.24/FF10

Topic: E.04. Voluntary Movements

Title: Beta Band Oscillations in the Human Motor Cortex During Bilateral Isometric Contractions in Untrained Individuals

Authors: *B. E. ZAPATA[1], A. C. AVALOS[1], K. J. PENNARTZ[1], J. M. WATSON[1], C. D. SMITH[1], S. M. WASHINGTON[1], L. K. RANKIN[1], J. A. FUSSELL[1], D. R. HARRIS[1], J. L. KELLER[2], A. L. HARRIS BOZER[1], M. J. LUERA[1];
[1]Tarleton State Univ., Stephenville, TX; [2]South Alabama Univ., Mobile, AL

Abstract: The human motor cortex assumes the vital role in movement coordination of limbs and their interaction in motor function. It regulates the facilitation of voluntary movements, and damage to this area can result in weakness of motor function to complete paralysis. Motor cortex signals are involved in movement of the body, and in this study, will be reported as voluntary contractions of the tibialis anterior (TA). The purpose of this study was to examine the relationship between average beta band (13-30 Hertz frequency) power in the human motor cortex while varying muscle contractions occur with Mean Power Frequency (MPF). Following a familiarization session, 4 lower-body resistance untrained males (n=4; age=22.5 ±2.1, height=173.5 ±5.6 cm, weight=79.7 ±20.1 kg) performed three isometric dorsiflexion maximal voluntary contractions (MVCs). The highest MVC was used to establish randomized percentage-based trapezoidal ramp contractions at 25%, 50%, 75%, and 100% MVC. All testing was performed in a custom-built seat using an isometric dynamometer where hip flexion was set to 100 degrees and knee flexion to 50 degrees. A 64-channel EEG (OT Bioelectronnica; reference on the cervical vertebra C7) was used to record neural activity during the protocol. Signals were recorded, decomposed, and filtered by channel type in Cartool (.02-50Hz), MatLab (EEGlab), and Notepad ++. Subsequent registered EEG signals were processed through Cartool and MatLab which derived RMS values. Two separate 2-way mixed factorial analyses of variance (ANOVA) (location [C5, C3 v C1] x intensity [25% v 50% v 75% v 100%]) (location [C6, C4 v C2 v] x intensity [25% v 50% v 75% v 100%]) were used to compare MPF values of the left and right hemispheres, respectively. There were no significant main effects or location x intensity interactions (.05). This suggests there may be contra-lateralization in isometric contractions in untrained individuals compared to traditional lateralization findings.

Disclosures: B.E. Zapata: None. **A.C. Avalos:** None. **K.J. Pennartz:** None. **J.M. Watson:** None. **C.D. Smith:** None. **S.M. Washington:** None. **L.K. Rankin:** None. **J.A. Fussell:** None. **D.R. Harris:** None. **J.L. Keller:** None. **A.L. Harris Bozer:** None. **M.J. Luera:** None.

Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.01/FF11

Topic: E.04. Voluntary Movements

Support: ERC-StG WIRELESS 678307
ERC-CoG EMACTIVE 101002704
NGEU, MUR and NRRP funds MNESYS PE0000006

Title: Memory-driven coding of graspable objects in monkey parieto-frontal areas

Authors: *A. MITOLA[1], M. MARANESI[1], L. BONINI[1], M. LANZILOTTO[2];
[1]Med. and surgery, Univ. of Parma, Parma, Italy; [2]Dept. of Psychology, Univ. of Turin, Turin, Italy

Abstract: Reciprocally connected parieto-frontal areas constitute an extended grasping network (EGN) transforming the physical features of graspable objects into grasping motor plans, continuously updated by visual feedback. However, the absence of visual feedback during grasping in the dark can be compensated by memory-driven mechanisms. Previous evidence suggests a role for the intraparietal area AIP in memory-guided grasping actions, but whether and how distinct cell classes in different areas of the EGN subserve these processes is still unknown. Here, we recorded the activity of 580 single neurons in three macaques from four areas of the EGN, namely, AIP and the premotor areas F5, F2, and F6, while monkeys performed a Go/No-Go grasping task in both light and dark conditions. During grasping in the dark, the animal had to remember the features of the initially presented object to grasp it correctly when the light was turned off. Three types of objects (ring, small and big cone), affording different grip types, were randomly presented in different trials. In addition, during a set of No-Go trials randomly interleaved with Go trials, the monkey had to fixate the visually presented object while remaining still to get the reward. Population and single neuron analyses showed that in AIP several neurons exhibit a temporally stable tuning for the same type of object along grasping-in-the-dark trials. In contrast, F6 neurons exhibit brisker object tuning in temporally specific phases of the trials. Areas F5 and F2 host a sizeable fraction of neurons with temporally stable tuning for objects, but only in the light. Finally, the stability of AIP tuning for objects observed during grasping-in-the-dark was significantly reduced during No-Go trials, supporting a more specific role of AIP in memory-driven coding of graspable objects. Preliminary cluster analysis of the recorded neurons based on their spike shape suggest that different class of cells (e.g. narrow and broad spiking neurons) play specific roles in sensory- and memory-driven coding of objects in different areas of the EGN. Our findings contribute to link the local and system levels in the description of the cortical mechanisms subserving sensory and memory driven guidance of reaching-grasping actions in primates.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.02/FF12

Topic: E.04. Voluntary Movements

Support: ERC stg grant 678307
NGU MUR NRRP MNESYS (PE0000006)
ERC Consolidator grant 2020 EMACTIVE (Grant ID: 101002704)

Title: Neuronal correlates of spontaneous walking in the monkey ventral premotor cortex

Authors: *R. SINI[1], D. ALBERTINI[2], F. LANZARINI[2,3], M. MARANESI[2], L. BONINI[2]; [1]Italian Natl. Res. Council, Parma, Italy; [2]Dept. of Med. and Surgery, Univ. of Parma, Parma, Italy; [3]Ernst Strüngmann Inst. (ESI) for Neurosci. in Cooperation with Max Planck Society, Frankfurt am Main, Germany

Abstract: Quadrupedal walking is one of the most frequent whole-body behaviors of monkeys in the wild. It is characterized by a rhythmic pattern of forelimbs and hindlimbs movement, mostly generated by central pattern generators in the spinal cord. The cortical contribution to walking is more relevant when facing unexpected obstacles or directional changes, and it is mostly limited to mesial brain regions. In contrast, the ventral premotor cortex (PMv) is deemed to play a prominent role in the encoding of goal-directed, distal behaviors. Here, we leveraged a newly developed setup consisting of a large plexiglass room for neuroethological recordings (NeuroEthoRoom - NER) where two macaque monkeys were filmed during spontaneous walking. Simultaneous untethered recordings of multisite neuronal activity from PMv allowed us to correlate single neuron activity with specific phases of the walking as well as many other ethologically relevant behaviors. A sizeable fraction of the recorded neurons fired with a precise phase lock with the walking cycle of the contralateral forelimb, most often toward the end of the forelimb swing phase. Some walking-related neurons could respond specifically during walking and not during other spontaneous behaviors. Furthermore, some neurons responded differently when the monkey was walking on elevated wooden branches (placed in the NER) relative to the floor, suggesting that they might code postural aspects and muscle synergies involved in balance maintenance. Electrical microstimulation of the cortical sites where neurons were recorded from provided causal evidence of their involvement in the control of complex proximal and distal forelimb and face/mouth actions, in line with existing literature on the premotor mechanisms for the encoding of ethologically relevant behaviors. These results show that PMv neurons, classically deemed to be involved in the control of goal-directed, distal motor actions, can play a role in the encoding of more automatic naturalistic behaviors, such as quadrupedal walking, raising the intriguing possibility that neural signals recorded from regions primarily devoted to the control of hand-mouth actions might nonetheless be exploited to decode walking and other whole-body natural behaviors.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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ERC-CoG EMACTIVE 101002704

Title: Neuronal representations of self and other's action in the monkey putamen

Authors: *C. ROTUNNO[1], M. RENI[1], C. G. FERRONI[2], L. BONINI[1], M. MARANESI[1];
[1]Med. and Surgery, Univ. of Parma, Parma, Italy; [2]Univ. San Raffaele, Milan, Italy

Abstract: When observing the actions of others, an extended Action Observation Network (AON) encompassing cortical areas devoted to action planning and execution becomes active. Anatomical data in monkeys demonstrated that areas of the AON send convergent projections to overlapping territories of the putamen nucleus in the basal ganglia, suggesting that it may not only contribute to the selection of one's own action, but also to the representation of the action of others. To address this issue, we recorded single unit activity from the putamen of two male rhesus macaques (*Macaca mulatta*) during a Mutual Action Task in which the animal and an experimenter, facing the same device in a shared space in between them, took turns based on contextual cues, grasped or observed the other agent grasping the same multi-affordance object (*Social condition*), and finally received a reward if both partners correctly performed the task. Neural activity was also examined in an additional condition in which the task was performed by the monkey or the experimenter, alone, with a plexiglass barrier preventing the observing partner from interacting with the object (*Barrier condition*). Recordings were performed by means of linear multielectrode probes and neural data loggers, allowing to investigate neural activity at different depths from head-unrestrained animals. Preliminary data collected so far in the two monkeys show that more than most of the recorded neurons whose activity was modulated during the reaching-grasping epoch of the Social condition responded only during action execution (Self type), a small fraction only during action observation (Other type), whereas the remaining discharged in both conditions (Self-Other type). Among Self-Other type neurons, some exhibited an opposite modulation (facilitation vs suppression of their discharge) during execution and observation trials. Finally, the introduction of the barrier in between one of the agents and the target object produced an overall decrease in neuronal modulation amplitude with respect to the Social condition. Our preliminary findings support the existence of putamen neurons related to both self and others' manual actions, supporting the hypothesized involvement of the basal ganglia in the AON and indicating the need to causally investigate its overall modulatory impact on the functioning of the cortical AON.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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Topic: E.04. Voluntary Movements

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Title: Yawn selective neurons in the monkey ventral premotor cortex

Authors: *E. FERRETTI[1], F. LANZARINI[1,2], M. MARANESI[1], L. BONINI[1];
[1]Med. and Surgery, Univ. of Parma, Parma, Italy; [2]Ernst Strüngmann Inst. (ESI) for Neurosci., Frankfurt am Main, Germany

Abstract: Yawning is an involuntary behavior frequently regarded as a “fixed action pattern”, not influenced by sensory feedback and highly stereotyped among species, whose functional role is still open to discussion. Subcortical structures (e.g. the paraventricular nucleus of the hypothalamus and the brainstem) are deemed to play a major role in orchestrating the respiratory-motor pattern underlying yawning. However, the current knowledge about this behavior mainly rely on behavioral evidence collected from ethological observations, data from clinical conditions affecting yawning, and the outcome of chemical/electrical stimulation of the above-mentioned subcortical areas in rats. Despite its highly stereotyped nature, yawning can be displayed in highly various manner depending on endogenous and social factors, and can be partially voluntarily controlled. In primates, yawning may have a role in communicating the current physical and mental state of individuals and/or to synchronize their behavior in a group. Based on the motor representation of face and mouth actions in the ventral Premotor Cortex (PMv) and the presence of neurons with sensorimotor and social functional properties in this region, we hypothesized that PMv may host yawning-related neurons. To address this issue, we used wireless neural data loggers to record single neuron activity from the ventral premotor cortex of two freely moving monkeys (*Macaca mulatta*), tested separately, monitoring their behavior with a camera system synchronized with the neural recording system. We recorded PMv neurons during a variety of behaviors, including yawning and other mouth/face and forelimb behaviors (e.g. sucking, chewing, feeding, threatening, grasping). We found neurons responding specifically during yawn and not during other mouth-related actions. Interestingly, the discharge of some of these yawn-selective units did not correlate with parameters of mouth aperture. Our data support the hypothesis that PMv neurons encode yawning, possibly contributing to exert downstream control over yawn displays, by either facilitating or inhibiting it, depending on social and communicative purposes.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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ERC - CoG EMACTIVE 101002704
NGEU, MUR, NRRP Funds MNESYS PE0000006

Title: Premotor coding of natural behaviors in freely moving monkeys

Authors: *D. ALBERTINI[1], F. LANZARINI[1,2], M. MARANESI[1], E. H. RONDONI[3], M. LANZILOTTO[4], A. MAZZONI[3], L. BONINI[1];
[1]Univ. Di Parma, Parma, Italy; [2]Ernst Strüngmann Inst., Frankfurt am Main, Germany; [3]S. Anna Sch. of Advanced Studies, Pisa, Italy; [4]Univ. di Torino, Turin, Italy

Abstract: Our knowledge on the neural control of actions in primates derives mainly from studies of monkeys performing trained motor tasks under restrained conditions (RC), where the monkey sits on a primate chair and performs highly trained (often stereotyped) motor tasks. Despite the general assumption that RC provides results that translate directly to freely moving conditions (FMC), there is currently little evidence on whether and how this applies to premotor coding of natural actions. To test this assumption, we recorded premotor neuron activity from two monkeys (males, *Macaca mulatta*) using four 32-channels matrices of microelectrodes and a wireless recording system. The recordings were performed first in RC while the monkey executed several hand and mouth actions and then, within the same recording session, in FMC, i.e., while the monkey was freely behaving in a 2x2x1.8 m transparent enclosure named NeuroEthoRoom equipped with different enrichments. Three different sessions, recorded several months apart, were analyzed for each monkey and single cells could be steadily recorded in both conditions within each session (n = 225, 122 in Mk1 and 103 in Mk2). In FMC, recorded neurons displayed a firing rate similar to what observed in RC, but peak firing rate and firing variability were both higher, likely reflecting the broader spectrum of behaviors observed in FMC. We found cells that exhibited distinct, sometimes specific, activations for whole-body behaviors that could not be observed under RC: for example, ~30% of neurons responded to walking, ~20% to climbing and ~10% to yawning. Second, the neural signals recorded in RC hardly generalize to FMC: a classifier trained on RC could predict the encoded behavior in RC in ~80% of trials (with 5 to 9 behavior classes) but achieved a near-chance performance in classifying the same classes in FMC. Interestingly, neural responses linked to mouth movements were significantly more preserved across conditions, whereas hand actions showed lower generalization, possibly due to a different involvement of the postural/proximal component. Taken together, these results demonstrate the potential and necessity of neuroethological approaches in elucidating the neural control of natural behavior.

Disclosures: D. Albertini: None. **F. Lanzarini:** None. **M. Maranesi:** None. **E.H. Rondoni:** None. **M. Lanzilotto:** None. **A. Mazzoni:** None. **L. Bonini:** None.

Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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ERC CoG "EMACTIVE" 101002704

Title: Neuronal encoding of reaching-grasping actions in the monkey putamen

Authors: *M. RENI[1], C. ROTUNNO[1], C. G. FERRONI[2], L. BONINI[1], M. MARANESI[1]; [1]Med. and Surgery, Univ. of Parma, Parma, Italy; [2]San Raffaele Scientific Inst., Milan, Italy

Abstract: The putamen nucleus is the main input station of the basal ganglia complex, and is considered to participate in the selection and initiation of actions by virtue of the rich afferent projections it receives from cortical areas of the so-called “cortical grasping network”. Although the cortical network is known to subserve the control of purposeful hand actions based on information about object properties, identity, contextual information, behavioral goals, and rules, whether and to what extent these information influence putamen neurons’ activity during reaching-grasping actions remain poorly understood. To address this issue, we recorded single unit activity from the putamen nucleus of two male rhesus macaques (*Macaca mulatta*) while they performed a reaching-grasping task instructed by previously learned contextual cues. Each monkey was trained to interact with a multi-affordance object (located in its peripersonal space) that had to be reached, grasped, and lifted within a precise time interval to obtain a reward. The object could be grasped either with a precision grip (PG) or with a whole hand prehension (WH) based on the visual cue presented during the instructive phase of the task. We recorded neuronal signals by means of linear multielectrode arrays allowing to investigate neural activity at different depths from head-unrestrained animals sitting on a primate chair during task performance. Preliminary data collected so far in two monkeys show that about half of the recorded neurons exhibited a modulation during the reaching-grasping period of the task performed by the animal, most often with an increase and sometimes with a suppression of their firing rate with respect to baseline activity. More than half of these units responded differently during the two examined grip types, in most cases with a preference for the precision grip, that is known to require finer motor control, consistently with previous literature describing an involvement of the basal ganglia in parametrizing specific properties of this kind of prehension. Our preliminary data support the hypothesis of a contribution of the putamen nucleus in the selection and execution of finer aspects of reaching-grasping actions primarily encoded in the cortical nodes of the lateral grasping network.

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Poster

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Title: Intracortical recordings in freely behaving macaques during sleep and wakefulness

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Abstract: The tuning properties and functional roles of individual neurons and brain networks in primates are usually characterized during wakefulness. Little is known about whether and how the functional properties of cortical neurons exhibit specific changes during different sleep stages such as REM and NREM sleep. The challenges of recording from freely moving primates and the lack of a validated method to distinguish wakefulness from sleep states using intracortical recordings (independently from classical EEG, EOG, and EMG) leave this issue unresolved. Here, we tackled this issue by using chronic intracortical multi-electrode arrays and neural data loggers to record neuronal signals at different spatial and temporal resolution including single-, multi-unit activity and local field potentials (LFP) from the ventral premotor cortex of two male rhesus macaques (*Macaca mulatta*). A synchronized infrared-sensitive video camera allowed us to track animals' behavior during wakefulness and sleep directly from their home cage. By analyzing the temporal dynamics of different frequency bands of the LFP, as well as multi- and single-unit activity, we identified repeatable patterns of variation associated with animals' behavior and their wake-sleep cycles that were consistent across sessions and animals. By leveraging a data-driven approach that makes no a priori assumptions about the nature and temporal dynamics of wake-sleep phases, we were able to identify neural signatures that alternate across behavioral states depending on behavioral assessment, allowing us to accurately predict animals' behavior and their nocturnal brain states. Our approach can pave the way to study the functional architecture of wake-sleep transitions at the cellular and network levels in freely moving primates, and allows us to elucidate the firing properties of functionally distinct classes of neurons characterized during wakefulness as a function of current brain state.

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Poster

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Title: Interactions between reward and task difficulty in M1 neural population activity

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Abstract: Even in the primary motor cortex (M1) - a region of the brain fundamentally responsible for voluntary movement - neural activity is not solely influenced by motor signals. Rather, non-motor signals, including attention and motivation, also influence neural activity. We have found that cued reward values offered to the subject before a movement are encoded monotonically in M1 spiking activity, where subpopulations of neurons showed either greater or lower firing rates with higher valued cues (Smoulder et al., 2023). In addition to reward, motivation can be influenced by a variety of factors, such as past experience, environmental cues, and the amount of effort required for a given action. To parse whether the observed motor cortical signals are driven by reward or motivation more broadly, we investigate how task difficulty impacts reward encoding in motor cortex. We trained a rhesus monkey to perform a challenging delayed center-out reaching task while simultaneously recording from populations of neurons in the primary motor cortex. We manipulated task difficulty by changing the size of the reach target that needed to be acquired, with ‘Tiny’ targets requiring more precise movements than ‘Huge’ targets. In addition to varying task difficulty, we also varied the reward that would be delivered if the reach was successful. These reward values were pre-cued, so the subject always knew both the reward and difficulty condition when planning and executing the reach. We observed that both increases in reward and increases in target size led to increases in task success rate. We then characterized how reward and difficulty modulated neural population activity. By treating the activity of each individual neural unit as an axis in a high-dimensional space, we can identify specific dimensions that capture reward-related variance. We used principal components analysis (PCA) to find that a single dimension, which we call the “reward axis”, captured the majority of the reward-related variance for both target size conditions. We further found that reward-related modulations were greater when reaching to Huge targets than when reaching to Tiny targets ($p < 10^{-12}$, Wilcoxon signed-rank test). Additionally, we identified a single dimension, which we call the "target size axis," that captures most of the target size-related variance for all reward conditions, and is nearly orthogonal to the reward axis. Together, our findings suggest that task difficulty modulates reward encoding in motor cortex in a manner consistent with changing reward expectation.

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Poster

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Title: A link between memory traces in motor cortex and savings

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Abstract: Relearning a motor skill is faster than learning from scratch. This phenomenon, called savings, is a hallmark of motor learning but its underlying neural mechanisms are not well understood. Here we report a neural population correlate of savings in the motor cortex. Savings is a behavioral quantity, and the causal relationship between behavior and neural activity is often unknown. To overcome this, we leverage a Brain-Computer Interface (BCI) learning paradigm where the mapping between neural activity and cursor velocity is defined by the experimenter. Using the BCI mapping, monkeys modulate their neural activity to control the computer cursor without generating overt movement. In this BCI paradigm, we define savings as improved online control of a newly-learned BCI mapping during a second learning block, relative to a first learning block.
We implanted one rhesus monkey with Utah arrays in the primary motor and dorsal premotor cortex, allowing the monkey to modulate the activity of tens of neural units to control the cursor while receiving visual feedback (online control). Each session started with 120 trials of intuitive control in which the monkey's natural population activity patterns allow the monkey to proficiently perform an eight target center-out task. Then, for the first learning block a novel BCI mapping termed a within-manifold perturbation (WMP) was instantiated. WMPs are mappings within the low-dimensional intrinsic manifold of neural covariance patterns and are learnable within a few hundred trials (Sadtler et al. 2014). Following learning, the intuitive mapping was reinstated for a washout block. Then the WMP was re-instantiated for a second learning block. We detected that the early online control of the WMP during the second learning block showed

significantly larger cursor movements towards the target than the early trials of the first learning block, i.e., we detected savings. What might be the underlying neural mechanisms of savings? We (Losey et al. 2023) recently detected a memory trace in the motor cortex. A memory trace is a change in neural activity, following learning, that improves offline control through the WMP despite the online control being through the intuitive mapping. If improved offline control of a newly-learned mapping is a result of the neural activity moving to a region in neural space that facilitates online control for that mapping, then we may ask: does the memory trace lead to savings? We found the size of the memory trace is significantly correlated with savings across hundreds of targets and tens of sessions. Overall, our results suggest that the memory trace that emerged after learning may be a neural substrate of savings.

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Poster

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Title: Motor cortex separately encodes reward magnitude and reward type

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Abstract: Incentives tend to drive improvements in performance, but only up to a point. When rewards get too large, performance can paradoxically suffer, a phenomenon known as “choking under pressure”. We have recently demonstrated that monkeys (Rhesus macaques) choke under pressure. Further, in the motor cortex (M1), we have found that reward magnitude causes a strong monotonic change in neural activity during reach planning, while having a non-monotonic interaction with directional planning. To further understand these effects, here we investigated how the type of reward interacts with reward magnitude encoding in M1. To investigate this, we trained one monkey to perform a delayed center-out reaching task for different magnitudes of pre-cued reward. Two reward types were used in different blocks of trials: juice, and water. We

found that the animal choked under pressure for both reward types, showing an inverted-U relationship between success rate and cued reward magnitude. However, behavior for a given reward magnitude was similar for both types of rewards, despite the animal showing a strong preference for juice (verified in a separate experiment). We recorded spiking activity from populations of neurons in both the primary motor cortex and examined activity during the reach planning period. Reward magnitude had a similar effect on neural population activity for both reward types: increasing reward caused a strong, monotonic change in neural population activity during the reach planning period. However, the neural encoding of water and juice were offset from one another, such that it was possible to define a "reward-type" axis separating juice reach preparation neural activity from water reach preparation. In summary, we find that reward magnitude encoding is insensitive to reward type, even though that information is present in the motor cortex. Our study implies that rich contextual signals are available in M1 during reach planning, even though they may have little effect on motor execution.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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Topic: E.04. Voluntary Movements

Support: NSTC 112-2321-B-001-007

Title: A holistic interactive diagram of neural dynamics in the sensorimotor cortices in a forelimbreaching task in mice

Authors: *Y.-W. WU[1], Y. CHEN[1], H.-T. YANG[1], P. ADHIKARI[1], Y.-S. LIU[1], Y.-Y. CHEN[1], S.-H. YANG[2];
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Abstract: Achieving precise motor control requires coordinated communication between multiple cortical regions, including the primary motor cortex (M1), the somatosensory cortex (S1), and premotor cortex (M2). While various mechanisms of corticocortical communication have been proposed, a comprehensive understanding of the interactive dynamics across sensorimotor cortices in a spatiotemporally detailed description is yet to be fully explored. In this study, we used a large-scale Microwire Electrode Array (MwEA) to simultaneously record deep-layer neurons across M1, M2, and S1 in mice performing a forelimb food-pellet reaching task, wherein the spiking activities of more than 1000 neurons are extracted. We applied a machine learning framework, Latent Factor Analysis via Dynamical Systems (LFADS), to extract population dynamics embedded in a low-dimensional manifold. Through canonical correlation

analysis, dynamics were categorized into three subtypes: a global representation across regions, pairwise interactions, and private signals that are confined within. Furthermore, we identified the possible encodings of dynamical compartments to the behavioral features. Additionally, we revealed the information flows from one region to another within the sensorimotor cortices by applying convergent cross-mapping. Altogether, we presented a holistic interactive diagram of the multiple cortices system from the perspective of populational latent dynamics.

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Poster

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Title: Different Neural Subspaces and Dynamics of Premotor Cortex Mirror Neurons: Challenging Assumptions of Similarity during Execution and Observation

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Abstract: Populations of mirror neurons (MNs) are assumed to discharge similarly when a subject executes a movement (execution) and when the subject observes a similar movement being made by another individual (observation). We challenge the assumption by investigating the neural activity of premotor cortex mirror neurons (PM-MNs) recorded from two macaque monkeys during the execution and observation of a delayed-response reach-grasp-manipulate (RGM) task involving four target objects. We aim to test the hypothesis that (1) different neural subspaces are recruited during execution versus observation, (2) neural dynamics differ under these two task conditions.To assess subspaces difference during execution versus observation, we projected the populational neural activity onto two sets of time-resolved subspaces derived separately from execution and observation data. Each set of time-resolved subspaces are identified at sequential time steps through the course of behavior trials. We then used the projected activity to decode the instructed target object, repeating for all subspaces of each set. We also used canonical correlation alignment to compare the latent dynamics between execution recordings from two different days and between execution and observation recordings from the same day.We found distinct time-varying decoding accuracy patterns when projecting the identical neural activity onto execution and observation subspaces for both monkeys. Specifically, neural activity of execution resulted in a prominent peak when projected onto their corresponding subspaces, whereas projection onto observation subspaces yielded consistent decoding accuracy. Similarly, projecting the observation activity onto their own subspaces

revealed peaks, while projection onto execution subspaces showed no peaks. Together, the presented peaks suggest PM-MNs adaptively adjust recruited subspaces to preserve the target information during both execution and observation but the way of adaptive adjustment is different between them. We also found that the latent dynamics extracted from execution and observation activity recorded on the same day were less similar than the execution latent dynamics extracted from execution activity recorded on two different days. These findings suggest that the neural computations underlying execution and observation are different, serving as evidence that challenges the similarity assumption.

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Poster

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Title: Neural activity in motor cortex evolves differently for continuously feedback-driven movements compared to discrete reaches

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Abstract: Carnival games contain a library of movements we can make, from throwing a ball, to playing whack-a-mole, to balancing a pole on your hand. These movements are driven by sensory feedback to different degrees—unlike throwing, pole-balancing requires continuous feedback-driven adjustments. Motor neuroscience has explored many feedforward tasks to great depth, but we understand far less about the motor control of highly feedback-driven tasks. This work attempts to illuminate this aspect of neural control, examining neural activity in motor cortex as monkeys performed a highly feedback-driven task similar to pole-balancing.
In our balancing task, called the Critical Stability Task (CST), monkeys make hand movements to control the position of a cursor on a screen. The task begins with the cursor in the center of the screen. Once the trial starts, the cursor's motion becomes unstable: the horizontal cursor velocity is proportional to the sum of hand and cursor positions, referenced to the center of the screen. Left alone, the cursor would fly to a side. The monkey counteracted this instability by moving

his hand opposite to the cursor. To be rewarded, the monkey must keep the cursor within 5 cm of the center for 6 s.
As a comparison, we interleaved CST trials with a more feedforward, whack-a-mole type task, where monkeys made sequential discrete reaches to randomly positioned targets (Random Target Task—RTT). Hand movements in the two tasks were statistically similar, and motor cortex population activity covaried similarly in both tasks, with activity exploring highly overlapping ~15 dimensional subspaces of the full population space. Likewise, we found that a single neural decoder could accurately predict hand velocity across both tasks (accounting for 95% of variance accounted for by single task decoders).
However, there was an asymmetry: the decoder that best predicted hand velocity in CST also predicted well for RTT, but the reverse was not true. This asymmetry suggests that contrary to our previous observations, the neural activity in the two tasks do not fully overlap. Upon further examination, we found that population activity could be partitioned into three separate neural spaces: one only explored by CST, one only explored by RTT, and one shared between both tasks, suggesting both shared and separate neural processing for the two tasks.
As a field, motor neuroscience has focused on the neural activity in relatively simple movements. The virtual balancing task we studied offers a window into the neural activity generating continuously feedback-driven behaviors, offering a stepping stone towards understanding the complex tasks we perform in our daily lives.

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Poster

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Topic: E.04. Voluntary Movements

Support: The Vision: Science to Applications (VISTA) program

Title: Multi-unit recordings in Ventrolateral Prefrontal Cortex during a head-unrestrained reach task

Authors: *J. LIN[1], V. NÁCHER[2], H. WANG[1], S. SUN[1], X. YAN[2], J. MARTINEZ-TRUJILLO[3], J. CRAWFORD[1];
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Abstract: Previously, we used single unit recording in monkeys to identify a region spanning the posterior tip of the principal sulcus that encodes a variety of gaze, head, and hand motion signals

during head-unrestrained reaches to visual targets. The main purpose of the current study is to understand how these signals are coordinated in time and neural space, i.e. across a memory delay and across different neurons. In order to do this, we implanted a 128-channel Plexon Array over the posterior ventrolateral prefrontal cortex (pVLPFC) in a female Rhesus monkey trained to perform a memory guided reaching task. The hand was initially placed at 1 of 3 varying locations of a waist level LED bar while gaze fixated centrally. A visual target was then briefly presented at 1 of 15 locations on a touch screen grid, followed by a visual mask. After a variable delay period, the fixation light extinguished, signaling a reach to the target. Animals were rewarded for touching within a spatial 'window' surrounding the target but allowed to move gaze and head as they wished. Neural and behavioral (eye, head, hand) signals were then recorded daily for three months while the animal performed this and other related tasks. In general, gaze shifts were followed by head and reasonably accurate hand motion, but gaze was less locked to the target and hand position after a memory delay (compared to a visually guided reach task). Preliminary analysis of array data in this condition has identified the presence of early response neurons that have an increased firing rate during target presentation and gaze onset, late neurons that have an increased firing rate near the end of the reach response and sustained neurons with increased firing rate that spanned both periods, similar to our previous single unit recording results. Further analysis will focus on what these neurons encode at the population level and how these codes evolve through time.

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Poster

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Topic: E.04. Voluntary Movements

Support: Natural Sciences and Engineering Research Council of Canada

Title: Sensorimotor control with biophysically-based thalamocortical spiking network model

Authors: K. J. HOESTEREY[1], ***E. M. MATISS**[1], M. L. LAPORTE[1], D. I. STANDAGE[2,1], G. BLOHM[1];
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Abstract: Large strides have been made in understanding the anatomy of the brain, however, how this anatomy contributes to complex computations - such as controlling movement - remains more elusive. Building cortical models that are representative of the brain's anatomy, including its neuronal diversity and connectivity, is one way of closing this gap. However, most systems neuroscience models only consider single homogenous groups of excitatory and

inhibitory neurons. Here we built a spiking neural network with cytoarchitecture and hierarchical connectivity inspired by the thalamocortical pathway to perform motor control in a ballistic reaching task. We simulated Izhikevich models of pyramidal, large basket, and spiny stellate neurons in the Brian2 simulator. Two network areas - loosely corresponding to sensory and motor areas - were assembled with laminar structure, resembling cortical layers 3, 4, and 5. Physiological data of mammalian microcircuitry informed how these neuron populations were interconnected within and between network areas. Where data was unavailable, neuron parameters were tuned within a physiologically justifiable range to prevent exponential growth or decay of activity. To condition the model on the input statistics, unsupervised pre-training was implemented via global spike-timing-dependent plasticity. Subsequently, a linear decoder consisting of a three-layer feed-forward neural network with a nonlinear transfer function was trained to interpret network activity and generate motor commands which move a point mass in 2D space. To map spiking activity to desired muscle activation patterns, we converted spike trains from individual layer 5 pyramidal neurons in the motor area into pseudo-rate codes by convolving them with a causal excitatory postsynaptic potential kernel. The decoder weights were adjusted with stochastic gradient descent to match optimal muscle force trajectories generated by a linear quadratic Gaussian regulator. This 2-step training paradigm was inspired by liquid state machines. Finally, the point mass position was fed back into the network as sensory input. Network performance was tested with centre-out reaches akin to typical experimental paradigms. Overall, the model was successful in generating motor commands that closely matched those of optimal trajectories in the ballistic reaching task while generating realistic spike patterns across all layers. Beyond its ability to perform sensorimotor control, our biophysically-based network model also paves the way for future research investigating how individual anatomical components contribute to cortical computations.

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Poster

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Topic: E.04. Voluntary Movements

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Allen Institute

Title: A feedforward circuit motif underlies apparently paradoxical neural coding

Authors: *K. DAIE[1], L. FONTOLAN[2], S. ROMANI[3], S. DRUCKMANN[4], K. SVOBODA[5]; [1]Allen Inst. for Neural Dynamics, Seattle, WA; [2]HHMI, [3]Janelia Res. Campus, Ashburn, VA; [4]Stanford Univ., Stanford, CA; [5]Allen Inst., Seattle, WA

Abstract: The spiking of single neurons is tuned to behavioral variables like movement, sensory stimuli, and decision variables, but the influence of this activity on behavior is often unclear. We developed statistical methods to estimate 'behavioral influence' (impact on behavioral choice per spike) of neural activity in recordings from anterior lateral motor cortex (ALM) of mice performing a delayed movement task. We found that early in the trial selectivity was only weakly correlated with its behavioral influence. The mismatch between behavioral influence and selectivity can be understood in terms of high-dimensional sequential activity: early dimensions carried little selectivity but exerted large behavioral influence, whereas late dimensions carried large selectivity but relatively little behavioral influence. These results were consistent with a feedforward network motif in which small amplitude signals along early dimensions are amplified by recurrent circuitry to produce late, low-dimensional attractor dynamics. Targeted, 2-photon photostimulation experiments revealed that activation of the early dimensions triggered the sequential activation of the later dimensions and caused predictable behavioral biases, consistent with the feedforward network motif. Our results 1) reveal a novel feedforward dynamical motif in the cortex and 2) highlight the behavioral relevance of small amplitude fluctuations in neural activity, which would be missed in most dimensionality reduction methods.

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Poster

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Title: Cortical encoding of stereotyped face and whisker movement in a multimodal behavior task in headfixed mice

Authors: *J. K. W. DE VRIES[1], N.-L. BAHR[2,1], J. FLANNERY[3,1], K. SEHARA[1], M. E. LARKUM[1], R. N. S. SACHDEV[1];
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Abstract: Cortical encoding of stereotyped face and whisker movement in a multimodalbehavior task in headfixed mice**J. de Vries[1], N.-L. Bahr[1,2], J. Flannery[1,3], K. Sehara[1], M. Larkum[1], R. Sachdev[1]*Institute of Biology, Humboldt-Universitat zu Berlin, Berlin, Germany[1]; Freie Universitat Berlin, Berlin,Germany[2]; Bernstein Center for Computational. Neurosci., Berlin, Germany[3]Cortical activity in sensory-motor circuits is related to motor planning, movement execution and sensoryperception. This activity can be triggered by thalamocortical feedforward and cortico-cortical feedbackinteractions. In this study, mice were trained to perform a go-cue triggered whisking to touch behaviourusing a single whisker, the C2 whisker to touch a sensor. Bilateral whisker movement, nose, and tonguemovement were all tracked by video recording. Silicone probes were advanced into motor and sensorycortices, and PoM thalamus. In a set of animals, opto-tagging was used to determine cortical depth and targetlayer 6b neurons. Our work shows that a fraction of all neurons recorded in sensory- and motor cortex andthalamus were active in the task and related to the initiation of whisking. A GLM model created to analysewhisking-to-touch triggered activity showed that 30% of motor cortical and thalamic units predicted whiskermovement, 15% predicted the correct cue response, and 8% were related to licking. The specific pattern ofactivity determined by levels of bursting, firing rate clustered together and had a distinct laminar profile.With additional optogenetics and extracellular recordings during behavior, we expect to develop a deeper un-derstanding of the flow of activity from L6b to L1 in M1 and S1 and to understand the role of PoM thalamusin driving activity in M1 and S1.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.18/GG2

Topic: E.04. Voluntary Movements

Support: NIH F31 DC020648

Title: Dopaminergic modulation of cortical motor circuits during gustatory sensorimotor transformation

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Abstract: The anterior-lateral motor cortex (ALM) is involved in the genesis of sensorimotor decisions related to licking. Although licking and taste are invariably linked, it remains unknown if ALM plays a role in the sensorimotor transformation of taste stimuli to lick decisions, and what neuromodulatory mechanisms are involved in this process. One potential mechanism for

taste-lick transformations is dopamine transmission through D1 receptors (D1Rs). Blockade of D1Rs within ALM affects both cue-evoked responses and motor planning of licking, suggesting a role for dopaminergic signaling through D1R-expressing (D1R+) neurons in modulating licking behavior. We examined local dopamine release dynamics and activity of D1R+ neurons in mice performing a taste-guided 2-alternative choice (2-AC) task. Mice were trained to perform five dry licks from a central spout before receiving one of two different taste cues to guide directional licking towards two reward spouts (lick left, "ipsilateral" vs lick right, "contralateral"). Fiber photometry recording of the newly developed dopamine fluorescence sensor, GRAB-DA2h, was used to monitor dopamine dynamics in ALM during behavior in the task. We observed phasic dopamine signals related to the preparation and execution of licking when mice licked for a taste cue, followed by a persistent ramping signal during the delay as mice prepared their directional lick response. We next examined if activity of ALM D1R+ neurons tracked these dopamine release dynamics and whether their responses are distinct from responses of non-D1R expressing neurons (D1R-). Two-photon calcium imaging was performed using transgenic D1R reporter mice to simultaneously record and identify populations of D1R+ and D1R- neurons in ALM. Individual D1R+ and D1R- neurons exhibit task-specific responses related to different behavioral epochs of the task. Examination of activity prior to dry licks revealed larger and earlier preparatory responses in D1R+ neurons compared to D1R-. Weak tuning to specific taste cues during the sampling was found in neurons, regardless of receptor expression. However, analysis of lick direction coding revealed a stronger bias for contralateral lick trials in D1R+ responses when compared to D1R- responses. Population selectivity for directional licking was found to be distinct between the two groups of neurons, with D1R+ population showing stronger selectivity during the delay epoch. Altogether, these findings suggest that ALM D1R+ neurons may be involved in initiating licking with a contralateral bias and provide novel understanding on how dopamine modulates motor cortical circuits during gustatory decision-making.

Disclosures: **J. Chen:** None. **A. Fontanini:** None.

Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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Title: Multiscale model of M1 circuits validated against in vivo data predicts cell-type-specific mechanisms, LFP sources, presynaptic inputs and neural manifolds across behaviors

Authors: J. V. MOREIRA[1], E. URDAPILLETA[2], B. A. SUTER[3], J. DACRE[4], S. A. NEYMOTIN[6], J. SCHIEMANN[5], I. C. DUGUID[5], G. M. SHEPHERD[7], W. W. LYTTON[2], ***S. DURA-BERNAL**[2];
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Abstract: We have developed a multiscale model of the mouse primary motor cortex (M1), simulating over 10,000 neurons and 30 million synapses, that predicts in vivo layer- and cell-type-specific responses during various behaviors. The model integrates quantitative experimental data on neuronal physiology, morphology, laminar density, cell type distribution, dendritic distribution of synapses, and local and long-range synaptic connectivity. To validate the model, we reproduced mouse M1 in vivo experimental results across different behavioral states and experimental conditions. Our M1 model replicated neuronal firing rates and oscillations dependent on cell class, layer, location, and behavioral state. The model captured the effects of experimental manipulations like noradrenaline receptor blockade and motor thalamus inactivation, offering multiscale mechanistic hypotheses for behavioral deficits observed. LFP oscillations emerged spontaneously (no oscillatory inputs) at physiological frequencies, including delta, beta, and gamma. LFP power in L5B shifted from lower (delta) to higher frequency bands (gamma) during movement, consistent with in vivo LFP data. Simulation analysis identified the specific populations responsible for generating the delta and gamma oscillations. We used the model to systematically explore the interaction between MTh and NA inputs and predict M1 output at the level of individual cell types at sublaminar resolution. Results supported the hypothesis that both high MTh and NA inputs are required for voluntary movement-related L5B activity, with the strongest response coming from lower PT5B during execution. A novel method to analyze the synaptic drive of presynaptic inputs to L5B populations revealed that PV5B mediated a switch from IT- to PT-predominant activity during movement. Dimensionality reduction of the simulated activity uncovered low-dimensional neural manifolds associated with different behaviors and manipulations. The model and analysis methods provide a quantitative theoretical framework to integrate and interpret M1 experimental data across scales, evaluate hypotheses, and generate experimentally testable predictions.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.20/GG4

Topic: E.04. Voluntary Movements

Support: NIH Grant 2T32MH067564
Whitehall Foundation
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Title: Reorganization of premotor and primary motor cortex dynamics across behaviors

Authors: *A. C. KRISTL, N. T. KOH, Z. MA, D. BASRAI, S. HSU, M. AGRIOS, S. P. SAVYA, A. SAIKI, A. MIRI;
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Abstract: The functional organization of mammalian motor cortex (MC) has been debated extensively since its discovery. One controversy surrounds the idea of premotor (PM) and primary motor (M1) areas that interact through a PM-M1 hierarchy. Because physiological methods have historically lacked the resolution required to parse direct interactions between PM and M1, evidence for such a functional hierarchy remains indirect and inconclusive. In addition, most studies of MC have involved animals performing over-trained single forelimb movements like reaching, leaving motor system function during much of the mammalian motor behavioral repertoire unexplored. To address the question of PM-M1 hierarchy across motor behavior type, we used a novel approach to assess direct interactions between mouse homologues of PM and M1, the rostral and caudal forelimb areas (RFA and CFA), and forelimb musculature during two contrasting behaviors: single forelimb reaching and naturalistic climbing in which mice used all limbs to climb across a constantly varying, unpredictable terrain. To compare the direct influence of RFA and CFA on muscles during each behavior, we optogenetically silenced either area during behavior and measured the fastest effect on forelimb muscle activity. During each behavior, one area had a significantly greater influence on muscle activity than the other - RFA during reaching, and CFA during climbing. Next, to determine if these varying influences on muscle activity are accompanied by varying RFA-CFA interactions, we compared the effect of inactivating either area on neural activity recorded with Neuropixels arrays in the other area. CFA inactivation caused a strong suppression of RFA activity during climbing but only a weak suppression during reaching. However, RFA inactivation strongly suppressed CFA activity during reaching. Finally, we probed for direct feedforward influence between RFA and CFA indicative of hierarchy by recording neural activity in both areas simultaneously and decomposing activity patterns using delayed latents across groups (DLAG). DLAG parses activity in each region into components either specific to each region, or shared across regions at a temporal lag. We found a stronger RFA influence on CFA than vice versa during reaching, but symmetrical influences during climbing. Our findings uncover hierarchical influence between RFA and CFA on muscles that reverses direction in a different behavioral context. We therefore

suggest that MC dynamics reorganize to produce different behaviors, in contrast with traditional ideas of a fixed PM-M1 hierarchy.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.21/GG5

Topic: E.04. Voluntary Movements

Title: Separability of cognitive and motor processes in the behaving mouse

Authors: *M. HASNAIN[1], J. BIRNBAUM[2], J. UGARTE NUNEZ[3], E. HARTMAN[3], C. CHANDRASEKARAN[4], M. ECONOMO[3];
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Abstract: Goal-directed behavior arises from an interplay between cognitive and motor processes in the mammalian brain. The neural dynamics underlying these processes are often engaged concurrently and in the same brain regions, making it difficult for experimenters to isolate the neural correlates of cognitive variables. This problem is made more acute by the presence of task-uninstructed movements which are encoded throughout the brain and are often correlated with ongoing cognitive processes. These findings motivate two questions: (1) are the neural dynamics associated with cognitive and motor processes separable- that is, are some cognitive processes fundamentally embodied? And (2) how does one identify cognitive neural dynamics in the presence of ongoing and often correlated movements? We simultaneously captured high-speed video and performed silicon probe recordings in the anterolateral motor cortex (ALM) while mice performed a two-context task involving multiple classes of cognitive processes: motor planning, urgency, and contextual encoding. Neural dynamics in the ALM showed signatures of each of these cognitive variables. We find that upcoming choice and task context are predictable from both neural activity and uninstructed movements. Additionally, we find that putative cognitive dynamics can be predicted on a single trial level from uninstructed movements. These results suggest that, if cognitive and motor dynamics are separable, estimates of cognitive signals may be corrupted by motor processes. To assess the separability of these cognitive and motor processes, we leveraged previous work demonstrating that separate neural processes can be multiplexed by occupying distinct subspaces. We extended this work to analyze neural and behavioral data on single trials, allowing us to isolate cognitive dynamics from the neural representation of movements which vary in timing and identity across trials. In doing so, we demonstrate how: (1) neural dynamics taken to represent motor planning, urgency, and contextual encoding are tightly linked to uninstructed movements, (2) cognitive and motor dynamics can be effectively separated into distinct subspaces, requiring only an estimate of when

an animal is moving, and (3) re-examining the neural correlates of cognitive processes with this method can yield new interpretations. This work calls attention to the importance of accounting for movements in studies of cognitive processes and suggests that previous descriptions of cognitive signals should be re-examined to account for the presence of uninstructed movements.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

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Program #/Poster #: PSTR349.22/GG6

Topic: E.04. Voluntary Movements

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Title: Oscillatory dynamics within the face sensory-motor network underpin facial expressions.

Authors: *Y. VAZQUEZ[1], G. R. IANNI[1], E. RASSI[2], S. V. SHEPHERD[1], S. SCHAFFELHOFFER[3], A. G. ROUSE[4], M. H. SCHIEBER[5], F. YAZDANI[1], F. ABOHARB[1], Y. PRUT[6], W. A. FREIWALD[1];
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Abstract: In primate societies, survival and success rely on interpreting and producing social signals, such as facial expressions. Despite their importance, the brain's generation of these social motor behaviors remains poorly understood. The primate brain contains multiple cortical facial motor representations in the frontal lobe and adjacent anterior cingulate cortex, suggesting a cortical facial motor network underpinning facial expressions. To investigate this hypothesis, we combined fMRI-targeting of facial cortical sensory and motor areas with parallel electrophysiological recordings in non-human primates performing facial expressions and ingestive movements.
Our findings reveal that sensory and motor cortical face representations within primary somatosensory (S1), motor (M1), ventral premotor (PMV), and cingulate (M3) motor cortices display unique spectral fingerprints in the alpha, beta, and gamma range, corresponding to different facial movement types. Spectral information in the alpha-beta and gamma range enables decoding of emotional facial expressions and voluntary ingestive movements, suggesting simultaneous activity patterns in lateral (M1) and medial (M3) motor areas, PMV, and S1.
We also examined rhythmic activity between these areas for functional interactions underlying facial expressions. We found significant oscillatory synchronization between sensory, frontal,

and medial motor areas in the alpha and beta range, with connectivity and information flow dependent on facial expression type. Our results indicate that cortical sensory-motor face regions form a network coordinating activity during facial expressions. This occurs primarily in the alpha and beta bands, where information is exchanged about timing and movement type, potentially allowing for coordinated signals to be sent downstream to the facial nucleus.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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Topic: E.04. Voluntary Movements

Support: MWK Lower Saxony ZN3422 "DeMoDiag"
Leibniz Collaborative Excellence K265/2019 "Neurophysiological mechanisms of primate interactions in dynamic sensorimotor settings"
German Research Foundation (DFG) Collaborative Research Consortium 1528 "Cognition of Interaction"

Title: Full-body actions beyond walking: the Playground Experiment for free solo and dyadic foraging combined with FairMOT-based action classification in rhesus macaques

Authors: *Z. AHMED[1,2], I. LACAL[1,3], M. NUSKE[4], R. VOGG[5], F. WOERGOETTER[3,4], A. ECKER[3,5,6], A. GAIL[1,3,7,8];
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Abstract: Systems neuroscience of goal-directed behavior aims to understand processes involved in perceiving, planning, and executing actions towards specific objectives. Various regions of frontoparietal cortex are known to contribute to goal-directed reach behavior, for example. Yet, the natural repertoire of skeletomotor behaviors in primates goes far beyond single-limb reaching or periodic movements like walking. More complex behaviors, such as foraging in complex or social environments, remain hardly explored. To overcome these limitations, we introduce a free-foraging paradigm called the Playground Experiment (PE), designed to induce a rich repertoire of full-body actions in rhesus macaques. We encouraged complex, ecologically relevant behaviors in our recently introduced highly modular Exploration

Room (ExR) setup by simultaneously offering monkeys 12 stations: two wall-mounted touchscreen-based kiosk systems (XBIs) providing fluid rewards upon touch, four flexible strings (artificial branches) providing access to hanging grapes when pulling them down against varying physical resistances, and six strategically positioned litter piles through which the monkeys had to search to retrieve treats. We propose a novel approach for tracking and classifying complex behavioral data by combining image-based 2D action classification with 3D keypoint tracking. Using our novel FairMOT-based action classification, we successfully identified transition behaviors during station switching and station-interaction behaviors in solo and dyadic PE using only 4-6 cameras to cover the ExR with close to 30 m^3. We can demonstrate that different station types evoked different full-body actions, which were consistent across sessions and across all five tested animals. The combination of PE and our classification approach enables the application of established epoch-averaged neural analysis methods. We provide examples of how step-velocity can serve as an indicator to differentiate relocating behaviors from goal-directed walking during unconstrained, continuous foraging and compare reaches during different patch-specific postures and their neural correlates in cortical sensorimotor areas to study at-patch behavior. Furthermore, we demonstrate the suitability of the ExR for dyadic experiments by directly comparing foraging strategies between social contexts. We conclude that the combination of the Playground Experiment (PE) with our FairMOT-based action classification approach enables the study of neural dynamics associated with a very rich repertoire of full-body actions in complex and social environments.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

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Topic: E.04. Voluntary Movements

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Leibniz Collaborative Excellence K265/2019 “Neurophysiological mechanisms of primate interactions in dynamic sensorimotor settings”
German Research Foundation (DFG) Collaborative Research Consortium 1528 “Cognition of Interaction”

Title: Neural correlates of sensorimotor decision-making during foraging in freely walking rhesus macaques

Authors: *I. LACAL[1,2], Z. AHMED[1,3], A. GAIL[1,2,4,5];
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Minneapolis, MN; [4]Fac. for Biol. and Psychology, Univ. of Göttingen, Göttingen, Germany; [5]Bernstein Ctr. for Computat. Neurosci., Göttingen, Germany

Abstract: Previous neurophysiological experiments have shown that action goals are represented in the primate sensorimotor cortex both during action selection and execution. These results were obtained in chair-seated animals with tasks enforcing action selection to precede execution. How well these findings apply to complex, ecologically more relevant contexts, where decisions are made during ongoing behavior, remains unknown. To bridge this gap, we investigate the neural basis of decision making during goal-directed, full-body movements with a go-before-you-know paradigm that requires monkeys to choose one of two alternative targets whilst walking towards them. Four rhesus macaques were trained to perform a walk-and-reach task in a 4.6 x 2.5 x 2.6 m enclosure (Exploration Room), equipped with two synchronized touchscreen-based kiosk systems (XBIs) serving as potential targets on a short side of the room. To start a trial, the monkey had to acquire a centered position at the opposite side of the room. Once the screens turned white, the animal was allowed to walk towards the offered targets within a pre-defined time window. During this movement time, a change in color of the two screens disclosed the reward associated to each target at varying stimulus-onset asynchronies (SOA). Full-body movements were recorded by 6-18 FLIR Blackfly cameras, safely mounted inside the experimental room in custom-developed, adjustable, animal-proof chasses (ExplorEye), for offline pose tracking in 3D. We recorded neural activity from primary motor (M1) and dorsal premotor cortex (PMd) from three monkeys with either a 128 or a 256 channels data logging system (Spike Gadgets). All monkeys learned the task and showed predominantly reward-based choices. The overt commitment to a target was synchronized with the walking cycle, suggesting an influence of the ongoing action on the time the choice was expressed. Firing rates increased significantly in M1 and PMd when the monkeys started walking compared to baseline (i.e., sitting at starting position). M1 firing rates strongly correlated with the animals' wrist velocity during walking. Conversely, the activity in PMd was predominately modulated by the direction of the full-body turn (left or right) when the monkeys committed to a target after the reward contingencies were disclosed. Our results suggest that reach-related regions of M1 and PMd are involved in selecting and executing full-body movements towards action goals that extend beyond immediate reach. Specifically, during approach behavior, M1 strongly reflects arm movements for locomotion and PMd sustains adjustments in walking direction required to reach a desired goal location.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

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Topic: E.04. Voluntary Movements

Support: NIH Grant 1R01NS104834-01

Title: The sensitivity of motor execution to sensory feedback is flexibly regulated

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Abstract: Mammals excel at carrying out flexible, context-dependent sensory-guided movements, a process that involves rapid cross-talk between sensory and motor areas. Here, we investigated the sensitivity of ongoing motor execution to sensory input in different sensorimotor contexts. Mice carried out a task in which on each trial they made a sequence of licks directed to a moving target (a lickport). Sessions contained two types of trial blocks, which alternated: (1) “standard blocks” that contained only “standard” trials involving a single, predictable sequence of target positions; and (2) “backtracking blocks” that contained a random mixture of standard trials and “backtracking” trials in which mice might have to abruptly switch between motor programs at a fixed position (“branch point”) in the sequence based on tactile feedback from the tongue. Electrophysiological recordings in the tongue premotor (anterolateral motor, or ALM) cortex revealed that population activity patterns could be separated along an axis that discriminated between block types. On single trials, the location of population activity along this axis correlated with the speed of motor switching on backtracking trials. Optogenetic stimulation of tongue/jaw somatosensory cortical (S1tj) inputs to ALM---precisely timed based on position in the sequence---produced larger deviations in neural activity during backtracking blocks and were more likely to induce licking movements resembling those of backtracking trials. Block-type-specific differences in the dynamical features of population activity trajectories could predict the perturbability of individual trials. Together, our data suggest that sensorimotor cortical networks can be configured to allow greater perturbability by sensory feedback to facilitate motor program switching.

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Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.26/GG9

Topic: E.04. Voluntary Movements

Support: NSF GRFP DGE-1746045

Title: Stability of coding for a dexterous reach-to-grasp task across motor cortical laminae

Authors: *E. A. DE LAITTRE[1], J. N. MACLEAN[2];
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Abstract: The stability over time of the relationship between neural activity and a given external variable remains a matter of dispute in many brain areas (Liberti, Schmid, et al, 2022; Gonzalez et al, 2019; Ziv et al, 2013; Marks and Goard, 2021; Driscoll et al, 2017). Whether neural representations are stable or changing ("drifting") has massive implications for *how* those representations could be read out by downstream neural populations and incorporated into neural computations driving perception, behavior, and memory.
Notably, studies of stability of neural coding for movements have generally focused on stereotyped, overtrained behaviors (Jensen et al, 2022; Liberti, Schmid, et al, 2022). The study of behaviors which exhibit higher trial-to-trial variance may enhance our understanding of the neural control of naturalistic and unconstrained behaviors. Such behaviors also allow us to move beyond trial-averaged analyses to single-trial analyses, such as instantaneous decoding, to quantify the stability of neural coding for a wider range of kinematic variables. Moreover, it is far from clear whether the laminar position of a motor cortical neuron corresponds to the stability of the single-trial mapping between its activity and kinematic variables over days.
We have collected an extensive neural and behavioral dataset spanning 24 consecutive days while mice learn a difficult skilled reach-to-grasp task which minimizes automaticity (n=5 mice, 24 days per mouse). We adapted the Whishaw single-pellet reaching task (Whishaw and Pellis, 1990; Chen et al, 2014; Guo et al, 2015) to be freely moving and self-initiated, and increased task difficulty by requiring greater precision in paw placement and spatiotemporally coordinated digit control. These steps evoked high variance in forelimb, paw, and digit movements and minimize automaticity even after the task is well-learned.
We study representational drift in motor cortical coding using fine-grained behavioral descriptions of paw and digit kinematics (Mathis et al, 2018; Nath, Mathis et al, 2019) and the calcium fluorescence activity of large populations of neurons (~300 per field of view) recorded across cortical layers and tracked across days in unrestrained mice. Using trial-averaged methods, our results corroborate previous findings of stability in motor cortex (Jensen et al, 2022): peri-stimulus time histograms (PSTHs) locked to reach onset are strongly correlated across days for the vast majority of neurons. Encoding models of single trials will enable insights into trial-to-trial and day-to-day stability/drift in the motor cortical representation of a skilled motor behavior.

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Poster

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Topic: E.04. Voluntary Movements

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Title: Deconstruction of reach-and-grasp behavior in mice

Authors: *E. NAGASE, S. M. SHUKLA, A. YAO, K. M. SANTOSO, E. STENZLER, D. LIPKIN, A. KAN, A. ARAC;
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Abstract: Reach-and-grasp movements are a set of complex skilled movements that involve the coordinated activity of multiple distinct muscle groups. To understand the underlying neural mechanisms, a detailed behavioral analysis of these movements is essential. Previous studies demonstrated the effects of internal and external task features on the performance. Here, we aimed to identify distinct movement patterns using marker-less 3D kinematic analysis of the reach-and-grasp movements in head-fixed mice as they reached for a single food pellet to grasp and bring it to their mouth. In principle, there are two aspects of the reach-and-grasp movement: 1) the change in position of the paw in 3D space and 2) the change in paw shape throughout the movement. We identified the 3D position and changes in paw shape using deep learning techniques. We found five phases of paw shape changes during successful movements. Combining the 3D positions and shape changes of the paw, we found that there were two distinct phases up until the grasp of the pellet. Kinematically, these phases were substantially different from each other. Moreover, in the phase after the grasp, movement toward the mouth was also distinctly different from the other two phases. Thus, we hypothesized that the neural computational principles governing these phases were also different. Additionally, given the difficulty of the task for the mice resulting in a post-training 50-60% success rate of reaches, in parallel with the other studies, we also investigated the kinematics of the failed reaches. The main question here was whether each failed reach was behaviorally the same or whether there were different types of failures. Our analysis identified three distinct failure types in well-trained animals. We propose that the neural mechanisms of these failure types must also be different. Our results identify features of reach-and-grasp behavior unique to mice, as well as features that are similar in some respects to the same behavior in primates. Furthermore, our results provide valuable information about how the neural mechanisms that control this behavior might be executed.

Disclosures: E. Nagase: None. **S.M. Shukla:** None. **A. Yao:** None. **K.M. Santoso:** None. **E. Stenzler:** None. **D. Lipkin:** None. **A. Kan:** None. **A. Arac:** None.

Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.28/GG11

Topic: E.04. Voluntary Movements

Support: NIH NS109315
Whitehall Foundation

LLHF
NVIDIA

Title: Shaping of M1 dynamics during reach-and-grasp behavior in mice

Authors: S. M. SHUKLA, E. NAGASE, A. YAO, K. M. SANTOSO, ***E. STENZLER**, D. LIPKIN, A. KAN, A. ARAC;
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Abstract: The complex behaviors of animals arise from the coordinated activity of multiple brain regions. Although each of these regions demonstrate an anatomical and functional modularity, they are interconnected, and it is these interactions that generate complex behaviors, such as skilled upper extremity movements. Previously, it was shown that the primary motor cortex (M1) is directly involved in the execution of reach-and-grasp movements in mice. We first confirmed this finding by optogenetically inhibiting excitatory neurons in M1, which resulted in freezing of the reach-and-grasp movement when the light was turned on at any point in time. Several studies showed that M1 neural activity in simple reaching behavior was governed by dynamical systems and had low tangling, indicating primarily autonomous dynamics. However, in more complex behaviors such as reach-and-grasp or grasp-only, M1 dynamics were thought to be more strongly input-driven and had higher tangling. Here, our goals were 1) to define the dynamical properties of M1 during reach-and-grasp behavior in mice and 2) to explore how the interactions between M1 and other cortical and subcortical regions shape the M1 dynamics. To achieve these, we performed neural recordings using six Neuropixels probes targeting 10 cortical and subcortical regions simultaneously in head-fixed mice as they performed a reach-and-grasp task. Our previous work identified kinematically distinct phases of this behavior. We first modeled the M1 neural activity using the dynamical systems approach. Our analysis showed that in each behavioral phase, a different dynamical system governed how the neural activity evolved and that these dynamics were approximately linear. To model both the within- and cross-region dynamics of multiple brain regions, we utilized data-driven recurrent neural network (RNN) modeling. We trained RNNs with the neural data so that each node in the RNN reproduced the same firing rates of the recorded neurons given the initial conditions. This analysis demonstrated distinct contributions of different cortical and subcortical regions to M1 dynamics, as well as the importance of autonomous M1 dynamics for each phase of the behavior. We then validated some of these results using closed-loop optogenetic interventions with real-time marker-less behavioral analysis. Overall, our results provide unique insight and solutions into how M1 dynamics are shaped during a complex motor behavior.

Disclosures: S.M. Shukla: None. **E. Nagase:** None. **A. Yao:** None. **K.M. Santoso:** None. **E. Stenzler:** None. **D. Lipkin:** None. **A. Kan:** None. **A. Arac:** None.

Poster

PSTR349. Cortical Planning and Execution: Neurophysiology Across Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR349.29/GG12

Topic: E.04. Voluntary Movements

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NIH RF1 NS132025
NIH R01 NS113110
NIH R01 NS131229
NIH R01 NS112312

Title: Reorganization and recall of preparatory neural activities for learned motor actions

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Abstract: How are learned motor actions stored in the brain so they can be re-activated? Distinct patterns of preparatory activity in motor cortex instruct specific future movements. It remains unclear whether the pattern of activity producing the same movement is retained across sensorimotor contexts and stably maintained over time. We used longitudinal two-photon calcium imaging to track the same neuronal populations in the anterior lateral motor cortex (ALM) of mice for over three months. This was done during a tactile delayed response task in which mice discriminated object locations using their whiskers and reported choice using directional licking after a delay epoch. In an autonomous home-cage system (Hao et al eLife 2021), mice repeatedly learn sensorimotor contingencies reversals in which the same actions were instructed by distinct sensory stimuli (i.e. distinct sensorimotor context). Contrary to the notion of representational drift, preparatory activity for the same movement was stably maintained for over one month in expert mice under the same sensorimotor context. We trained a linear decoder to read out future lick direction based on ALM delay activity. A linear decoder trained in one session could accurately predict lick direction when applied to other sessions regardless of the time interval between sessions. To examine the stability of preparatory activity across sensorimotor contexts, we imaged the same ALM populations before and after contingency reversal learning. We observed a profound reorganization of ALM dynamics that resulted in a new pattern of preparatory activity after learning. A decoder trained on ALM delay activity in the original context was not able to predict lick direction in the reversed context. Interestingly, the previous preparatory activity pattern was not entirely lost. Re-training mice in the previously learned sensorimotor context re-activated the previous preparatory activity pattern. These data show that preparatory activity for the same movement is highly stable across time but preparatory activity is specific to sensorimotor context. Our results suggest that learned motor actions are stored as context-specific sensorimotor mappings, where distinct sensorimotor contexts evoke unique preparatory activity patterns leading to the same movement.

Disclosures: **J. Kim:** None. **N. Li:** None.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR350.01/GG13

Topic: E.05. Brain-Machine Interface

Support: R01 Grant NS107451

Title: Identification of Novel Biomarkers of Signal Quality Identified by Spatial Transcriptomics Analysis of Electrode Implanted Tissue

Authors: *A. SAXENA[1], Q. A. WHITSITT[2], B. EVANS[2], B. PATEL[2], B. HUNT[2], E. K. PURCELL[1,2];
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Abstract: The recorded signal from a brain implant often becomes less and less faithful over time. Electrode failure is, in part, credited to the glial encapsulation and the loss of neuronal density that typically occur around the electrode. Spatial transcriptomics can be used to look at the genetic changes driven by the implantation of the electrode and then compared with the traditional metrics of glial reactivity, neuronal loss and electrophysiological recording quality. Rats were chronically implanted with Michigan-style microelectrode arrays, from which electrophysiological recordings were taken. Brain tissue sections surrounding each electrode were then mounted on Visium microscope slides (10X Genomics) for spatial transcriptomics processing. The tissue was immunolabeled for neurons and astrocytes which provided both a spatial reference for spatial transcriptomics and a quantitative measure of glial fibrillary acidic protein (GFAP) and neuronal nuclei (NeuN) immunolabeling surrounding each implant. Hundreds of significantly differentially expressed genes (DEGs) were detected when the gene expression within 300 μm of the implant and the same region of the naïve tissue were compared. The amplitude of multiunit activity (MUA) and local field potential (LFP) were calculated from the raw electrophysiological data as markers of signal quality. The spearman correlation coefficients of neuronal density (ND), GFAP intensity,MUA amplitude and LFP amplitude against the spatial density of every gene available within 100 μm from the electrode were calculated. The obtained correlation coefficients revealed an inverse relationship between GFAP intensity and neuronal density. Similarly, correlation coefficients of MUA and LFP are plotted revealing a directly proportional relationship between the coefficients.The top 20 genes for positive MUA and positive LFP were observed to have a similar functionality to the top 20 genes associated with positive ND and negative GFAP (neuroprotection, proliferation, differentiation, cytoskeletal organization, and inflammation). Negative MUA and negative LFP were observed to have similar functionality with the genes associated with negative ND and positive GFAP which were known and unknown biomarkers of astrocytes, ferroptosis, neurodegenerative diseases, and cell apoptotic processes. Our approach identified novel potential biomarkers for signal quality and stability using spatial transcriptomics.

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Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR350.02/GG14

Topic: E.05. Brain-Machine Interface

Support: SPG Grant RG101412

Title: In Vitro Electrochemical Fouling Performance of Freestanding Boron-Doped Diamond Microelectrode using Fast Scan Cyclic Voltammetry

Authors: *M. PERILLO[1,2], B. GUPTA[3], J. SIEGENTHALER[5], R. RAHMAN[2], G. BANNA[4], B. KEPROS[5], R. RECHENBERG[5], M. BECKER[5], W. LI[4,5,2], E. K. PURCELL[2,4];
[2]Biomed. Engin., [3]Neurosci., [4]Electrical Engin., [1]Michigan State Univ., East Lansing, MI;
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Abstract: Real-time detection of neurotransmitter release in vivo is an important way to study neurological and neuropsychiatric phenomena. Fast scan cyclic voltammetry (FSCV) is an electrochemical technique for in vivo neurotransmitter detection that is typically facilitated with an implanted carbon fiber microelectrode (CFME). Chronic, as opposed to acute FSCV studies, are an attractive way to study various pathologies, as longitudinal data are particularly useful in models of progressive neurodegenerative disease. The CFME can present limitations for chronic implantation, in part due to its susceptibility to electrochemical fouling. Boron-doped diamond(BDD)is an alternative, electrochemically active material that is less prone to fouling than carbon fiber due to its higher sp^3 hybridized carbon content. Our group has developed a freestanding, all-diamond BDD microelectrode (BDDME) with polycrystalline diamond (PCD) insulation with customizable geometry on a subcellular scale that has shown promise in reducing fouling caused by biological materials. In this study, we demonstrate the performance of the BDDME vs. the CFME with fouling caused by oxidation byproducts from the serotonin (5-HT) redox reaction, i.e., “electrochemical fouling”. Different BDD surface treatments, namely physical cleaving, and laser treatment - which will increase the sp^2 content at the electrode surface - were used to explore their differences in fouling performance. We recorded the oxidation current response after 25 repeated injections of 5-HT in a flow-injection-cell and compared the current-drop from the first to the last injection to the same metrics from 25 injections of dopamine (DA), a neurochemical that is known to produce less fouling, oxidation-byproducts and have a stable repeated response. This work demonstrates the performance of the BDDME when it is repeatedly exposed to DA or 5-HT, which is additive to the development of a chronic, diamond-based electrochemical sensor for long-term neurotransmitter measurements in vivo.

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Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR350.03/GG15

Topic: E.05. Brain-Machine Interface

Support: NSF CAREER 1943716

Title: Spatiotemporal gene expression with single-cell resolution around implanted silicon and polyimide cortical electrodes of supra- and sub-cellular dimensions

Authors: *C. THOMPSON[1], E. K. PURCELL[2];
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Abstract: Microelectrode array technologies which interface with the nervous system are powerful tools in research and medicine. Implanted electrode arrays often suffer from progressive instability of recordable neuronal signals which can severely limit their longevity. The tissue response of the brain is believed to play a significant role in the progressive failure of implanted electrode arrays. To ameliorate or circumvent the tissue response, numerous next-generation electrode which feature various biomaterials and novel designs have been developed with varying degrees of success. However, recordable neuronal signals can still decline in apparently healthy tissue which present with minor glial scarring and normal neuronal densities. Therefore, it is essential that we better understand the tissue response as a means to inform and guide the design of cortical implants with greater biocompatibility. Recent transcriptional studies have provided novel context to the tissue response around implanted electrodes in the brain, yet there are few studies which directly compare the gene expression around implanted electrodes of different biomaterials and device feature size. In this study, we have utilized multiplexed fluorescence in-situ hybridization to compare the tissue response of implanted silicon and polyimide microelectrodes of standard (100x10um) and sub-cellular dimensions (10x10um) at 1-week and 6-weeks post-implantation. We have examined a panel of 100 previously identified genes associated with the cortical tissue response. We found that these genes are spatiotemporally expressed at the cellular level within neurons, oligodendrocytes, astrocytes, and microglia at the device interface. The results of this study provide new insight into the cellular dynamics of the tissue response and how cortical tissue responds to electrodes of different biomaterials and dimensions at the transcriptional level.

Disclosures: C. Thompson: None. **E.K. Purcell:** None.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

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Program #/Poster #: PSTR350.04/GG16

Topic: E.05. Brain-Machine Interface

Support: NIH R03NS127192-01

Title: Spatial transcriptomics of brain-electrode interface across cortical layers in the rat primary motor cortex

Authors: *B. GUPTA, M. P. WELCH, C. G. DORAN, A. P. VELTRI, E. K. PURCELL; Michigan State Univ., East Lansing, MI

Abstract: Implantable devices have created new opportunities to investigate electrical, chemical and optical signaling in the brain to study and treat neurological disorders. After insertion, brain implants can experience reactive tissue responses that interfere with neural signal recording and overall device performance. Significant increases in the presence of inflammatory microglia and reactive astroglia have been observed to encapsulate and surround the electrode while neuronal loss occurs within recordable radius of the device. Recent RNA analysis and transcriptional changes suggest a more complex and spatially stratified response at and around the device site than what can be observed with traditional immunohistochemistry. Previously, we used spatial transcriptomics to assess and confirm the differential expression of genes associated with typical elements of the biological response in transverse tissue sections. In this study, we aim to obtain both radial and depth-associated changes in transcriptional profiles surrounding devices by applying a spatial transcriptomics assay (Visium, 10X Genomics) to coronal tissue sections. Here, we collect and analyze coronal slices of brain tissue from rats with silicon electrode array implants for different time points (1 day and 1 week). Using spatial transcriptomics, we capture gene expression changes within and across cortical layers and identify spatial patterns of gene expression for any individual gene, or combination of genes, surrounding devices. These data add to the new and growing body of literature illustrating transcriptional changes surrounding implanted electrodes in the brain.

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Poster

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Title: The Effect of an antioxidant coating on the recording performance of planar silicon intracortical microelectrode arrays

Authors: A. G. HERNANDEZ-REYNOSO[1], ***B. S. STURGILL**[2], O. K. KREBS[3], G. F. HOEFERLIN[3], T. T. D. THAI[1], D. MENENDEZ[3], J. DUNCAN[3], L. DRUSCHEL[3], R. RADHAKRISHNA[1], T. J. SMITH[1], J. ZHANG[3], G. MITTAL[3], M. S. DESAI[1], S. F. COGAN[1], J. J. PANCRAZIO[1], J. CAPADONA[3,4];
[1]Bioengineering, Univ. of Texas At Dallas, Richardson, TX; [2]Univ. of Texas at Dallas, Richardson, TX; [3]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH; [4]Advanced Platform Technol. Ctr., Louis Stokes Cleveland Veterans Affairs Med. Ctr., Cleveland, OH

Abstract: Intracortical microelectrode arrays (MEAs) record neural activity to study neural circuitry and develop brain-computer interfaces. However, chronic use is limited by recording performance degradation. This is, in part, a result of the neuroinflammatory response which results in and is exacerbated by oxidative stress. Our group has developed a method of immobilizing an antioxidant - Mn(III)tetrakis(4-benzoic acid)porphyrin (MnTBAP)- to MEA surfaces. Here, our goal is to investigate how this coating and the intermediate functionalizing step- (3-aminopropyl)triethoxysilane (APTES)- affect MEA recording performance *in-vivo*.Silicon MEAs were implanted in the motor cortex (M1) of male rats and recorded twice weekly for 16 weeks. In total, 9 MnTBAP, 8 uncoated, and 7 APTES-coated MEAs were implanted. The proportion of electrode sites showing activity – termed active electrode yield (AEY) – signal amplitude (V_{pp}), noise level, and signal-noise ratio (SNR) were used as metrics of recording performance. For the AEY, a two-proportion z-test was used to calculate statistical differences between groups. For V_{pp}, noise and SNR one-way Kruskal-Wallis tests followed by Wilcoxon signed-rank tests was performed. All statistical tests were done to achieve a significance level of $\alpha = 0.05$.AEYs of MnTBAP coated devices were greater than controls at most time points between week 7-13 with AEY ranging from 55-65% for MnTBAP MEAs and 45-52% for control MEAs, but they converged thereafter. Interestingly, APTES MEAs outperformed the other groups on the day of surgery (AEY: 80% APTES, 34% MnTBAP, 45% Control, $p< 0.05$). While MnTBAP and control groups improved after the first week, APTES AEY declined to be similar to both groups at week 2 and declined again sharply at week 12. Noise levels were highest for control MEAs (medians ranging from 7-10uV) and lowest for the APTES-coated MEAs (4-6uV) at nearly all time points ($p<0.05$). From weeks 7-9, V_{pp} of controls (medians ranging 75-82uV) exceeded V_{pp} of MnTBAP MEAs (65-67uV). APTES V_{pp} was lower than both groups after the day of surgery. Importantly, the SNR of MnTBAP MEAs (median ranging 9.7-10.9) was greater than the other groups at nearly all time points (medians ranging 8.3-10.2 for APTES, and 8.4-9.1 for controls). These results suggest MnTBAP coating can improve chronic recording performance of intracortical MEAs by preserving signal and improving signal quality. Additionally, the APTES coating may benefit acute recordings, but has little benefit thereafter. Future studies will evaluate histological response and markers of oxidative stress to assess the antioxidant effects of the MnTBAP coating on surrounding tissues.

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Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

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Topic: E.05. Brain-Machine Interface

Support: DARPA N66001-15-C-4017
NSF 1533649

Title: Investigation of Fibrous Tissue Infiltration and Encapsulation of Utah Slant Electrode Arrays in Human Peripheral Nerve

Authors: *M. B. CHRISTENSEN[1], T. N. TULLY[2], E. S. STONE[2], T. S. DAVIS[3], C. J. THOMSON[2], D. J. WARREN[2], J. A. GEORGE[4], G. A. CLARK[2], D. T. HUTCHINSON[5]; [1]Surgery, [2]Biomed. Engin., [3]Neurosurg., [4]Electrical and Computer Engin., [5]Orthopaedics, Univ. of Utah, Salt Lake City, UT

Abstract: Penetrating microelectrode arrays show great promise in neuroprosthetic applications. When implanted into peripheral nerves, microelectrode arrays offer a high bandwidth interface for controlling and receiving feedback from prosthetic limbs. However, long-term stability of intraneural microelectrode arrays remains elusive. We have previously shown evidence suggesting that the deposition of fibrous tissue between implanted 100-electrode Utah Slanted Electrode Arrays (USEAs) (Blackrock Neurotech) and nervous tissue causes device migration out of the nerve. To further investigate this issue, we evaluated USEAs and associated adherent tissue that were removed from ulnar and median nerves in human subjects after chronic implantation. USEAs (n=4 USEAs from 2 subjects) and associated tissue were immersion fixed in 4% paraformaldehyde in PBS. USEAs were placed in blocking solution overnight and incubated with primary antibodies against MAC387 for macrophages. USEAs were then washed, incubated with the appropriate secondary antibodies plus DAPI, and washed again. Where possible, adherent tissue was removed from the USEAs prior to staining and imaged using second harmonic generation (SHG) to visualize Type I collagen, after which the tissue was stained similarly to the USEAs. Upon retrieval, all 3/4 USEAs were found to have either infiltrating presumptive connective tissue within the array or be completely encapsulated in that tissue. After staining, MAC387+ cells were observed covering the surface of retrieved devices, similar to results from previous studies examining USEAs implantation in animals and humans. SHG imaging of adherent tissue showed largely disorganized collagen structures, suggestive of fibrous tissue formation rather than organized epineurial collagen. These results further support previous findings that fibrous tissue ingrowth and/or encapsulation of USEAs is a barrier to optimal long-term performance of these devices.

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aspects of USEA. **J.A. George:** F. Consulting Fees (e.g., advisory boards); BIOS. **G.A. Clark:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); IP for aspects of USEA. **D.T. Hutchinson:** None.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

Location: WCC Halls A-C

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Program #/Poster #: PSTR350.07/GG19

Topic: E.05. Brain-Machine Interface

Title: Novel electrostatic-assisted assembly method for high alignment accuracy of modular flexible neural probes for recording channel count scaling

Authors: *M.-L. HSIEH, E. KO, E. YOON;
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Abstract: Reduction of brain tissue damage and improvement of neural probe recording capabilities are two main goals for implantable neural probe development. To reduce the damage introduced to the brain by the use of these implanted devices, much research has been devoted to transition to use of rigid silicon probe shanks into the use of flexible materials as the structural material for the implanted probe shanks. The use of flexible materials can minimize the relative motion between the brain tissue and the implantable device, thereby minimizing damage induced and improving recording quality and longevity in chronic experiments. In addition, flexible neural probes with high spatial resolution can be achieved by scaling the channel count of the neural probes. This pursuit of higher channel count for improved neural probe recording quality can be achieved by monolithically fabricating probes with extremely small linewidth and pitch, with the downside of potentially sacrificing device yield. Another method is to build modular probes with less channel count and assembling these modular probes together into a single device by stacking multiple probes into one. This second method requires less demanding fabrication linewidths and thus has a higher device yield, but requires a more demanding assembly process, since without proper alignment of the stacked probes, the device may not be usable for in vivo experiments. In this research, we aim to develop a novel electrostatic-assisted assembly method for polyimide-based flexible neural probes to overcome misalignment difficulty during assembly. Through the use of specifically designed alignment patterns and applied bias, electrostatic attraction force enables for simple self-assembly of the flexible neural probe shanks with minimal misalignment. Preliminary experimentation reveal that using the electrostatic-assisted assembly method, the misalignment between self-aligned layers of neural probe shanks can be reduced from 6μm down to 1μm when a voltage bias of 50V is applied between the two stacked layers. This assembly method provides a repeatable means to create scalable, flexible neural probe with minimal misalignment. The device that this novel assembly method enables is expected to be able to provide high quality recording signal with high spatial resolution and minimal tissue damage for chronic neuroscience studies

Disclosures: **M. Hsieh:** None. **E. Ko:** None. **E. Yoon:** None.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

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Program #/Poster #: PSTR350.08/GG20

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01 NS110564-01

Title: Characterizing the effects of electrical microstimulation on materials and cells in vitro

Authors: ***N. WILLIAMS**[1], M. PWINT[2], B. WU[2], Q. CAO[2], I. BUSCAY[2], E. CASTAGNOLA[3], T. X. CUI[2];
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Abstract: Electrical microstimulation is being explored as a treatment for several debilitating neurological conditions. However, there remain gaps in our understanding of the effects of microstimulation on individual cells, especially when it comes to non-neuronal cell types. Prior research has also shown that long-term microstimulation can accelerate the degradation of electrode materials. Here, we take a multimodal approach, studying both cellular and material changes due to microstimulation. We developed a transparent microelectrode array (MEA) system for observing cells during stimulation as well as a platform for tracking changes in electrochemical properties and MEA integrity after prolonged microstimulation. Considering that microglia are the dominant cell type adjacent to chronically implanted electrodes, and the important interplay between neurons and microglia, characterizing the microglia response is necessary for understanding the effects of microstimulation. To address this, primary microglia were seeded onto transparent MEAs and we measured the time course and spatial extent of membrane permeability changes under different stimulation conditions and electrode shapes. We found that ring shaped electrode sites led to a farther-reaching effect on membrane permeability (33.3um) compared to standard disk-shaped sites (25.8um) of the same surface area, in line with modeling results and indicating that site shape is an important design parameter. We also observed effects of acute stimulation in the following hours and days, leading to increased cell death and apoptosis. To determine if the effect was due to a soluble component, stimulated media was added to primary neurons that had not been directly stimulated, leading to decreased viability. To determine the rate of metal loss under different stimulation loads, stimulated media was assayed for metal content, showing that increased stimulation current led to increases in solubilized metal. Assessing electrode performance via electrochemistry and material integrity via optical and electron microscopy, we observed that electrode failure is not homogeneous but that individual sites fail at different rates, and that these failure modes arise due to different mechanisms such as; delamination, loss of electrode material, loss of electrode coating, failure of metal traces, etc. These experiments are ongoing, the results of which will help to inform

stimulation parameters that are both safe and effective at the cellular level as well as best practices for robust MEA design and further methods for characterizing electrode failure.

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Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

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Topic: E.05. Brain-Machine Interface

Support: NSF-NCS Grant 1926804

Title: Parylene photonic microimager: A flexible waveguide array for functional fluorescent imaging

Authors: *M. H. MALEKOSHOARAIE, K. SARNA, V. JAIN, M. CHAMANZAR; Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: To investigate cell-type specificity and analyze neural circuits, neuroscientists employ techniques such as functional fluorescent imaging. To achieve high spatial resolution using these techniques, novel methods for precise targeted light delivery and collection need to be devised to enable localized and minimally-invasive in vivo imaging of neuronal populations. We have recently demonstrated a fully flexible and compact waveguide platform called Parylene photonics. This platform utilizes biocompatible polymers, Parylene C and PDMS and is fabricated using scalable planar micromachining techniques. In our previous work, we demonstrated the application of Parylene photonics for light delivery and optogenetic stimulation. In the current study, we demonstrate the capability of this platform for microimaging of the localized neural activity. We have designed an implantable endoscope, consisting of an array of 20 adjacent Parylene photonic waveguides for functional fluorescent imaging. We conducted calcium imaging on brain slices from transgenic Thy1XSynCre mice expressing Calcium indicators in the cortex and hippocampus. Brain slices with a thickness of 350µm were held in a chamber perfused with ACSF and Carbogen above the input of the microimager. Layer 5 of the somatosensory cortex was electrically stimulated using a Platinum/Iridium concentric microprobe. A blue (450nm) laser was used to provide the excitation light. The evoked activity is manifested as a change of emitted fluorescent light, which was then captured by the waveguides at the microimager output port using a fluorescent microscope with 10x magnification. The results show that the miniaturized microimager enables visualization of the localized spatiotemporal activity of fluorescent-tagged neurons. This implantable microimager can be used in a gamut of applications, ranging from clinical intraoperative monitoring and research-based structural and functional imaging with cell-type specificity.

Disclosures: **M.H. Malekoshoaraie:** None. **K. Sarna:** None. **V. Jain:** None. **M. Chamanzar:** None.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR350.10/HH2

Topic: E.05. Brain-Machine Interface

Support: NIH/NINDS (R01-NS101013)
NIH/NIBIB (P41-EB018783)
McDonnell Center for Systems Neuroscience

Title: Histological changes to cortex and bone in non-human primates with chronically implanted epidural electrocorticography arrays

Authors: ***S. S. SIVAKUMAR**[1], P. DEMAREST[1], M. O. OLUFAWO[2], P. BRUNNER[2], E. C. LEUTHARDT[2], D. W. MORAN[1];
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Abstract: Electrocorticography (ECoG) electrodes serve as a minimally invasive tool to record electrical brain activity at high spatiotemporal resolution. Though a number of techniques are available for obtaining high-fidelity electrophysiological signals, ECoG implants do not induce host-tissue responses to as large an extent as observed with intracortical recording methods. Consequently, chronic ECoG array implantation is well-tolerated in non-human primate studies of cortical activity, and arrays may be implanted for months to years without significant behavioral impact. However, the effects of chronically implanted ECoG electrodes on underlying cortical tissue and overlying bone are poorly understood.
In this work, five rhesus macaques (*Macaca mulatta*) were implanted bilaterally or unilaterally with epidural ECoG arrays over somatomotor cortex. Each animal was sacrificed 3 to 6 years after array implantation, and heads were immediately preserved in 4% formalin solution. Computerized tomography scans of each head provided the locations of ECoG implants in standard stereotactic coordinates. The extracted skull, dura, and brain from each head, including implanted ECoG arrays, were decalcified and cut into 30-μm histological sections. Tissue specimens from both implanted and unimplanted sites were stained and analyzed for changes to gross morphology and cellular composition.
To our knowledge, this is to date the most comprehensive histological analysis of brain, dura, and bone tissue in primates after chronic ECoG implantation. With the goal of curating a histological atlas for use in future chronic cortical recording applications, here we show several results: thickening of the dura and expansion of lamellar bone overlying electrodes, presence of macrophages and foreign body giant cells, and evidence of reactive astrogliosis near implant sites. Despite these changes to tissue morphology, robust electrophysiological recordings were

obtained from each primate for up to 2 years post-implant, suggesting that ECoG may be a viable modality for long-term clinical applications.

Disclosures: S.S. Sivakumar: None. **P. Demarest:** None. **M.O. Olufawo:** None. **P. Brunner:** None. **E.C. Leuthardt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions. **D.W. Moran:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Neurolutions.

Poster

PSTR350. BCI and Neural Prosthetics: Performance and Properties

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR350.11/HH3

Topic: E.05. Brain-Machine Interface

Title: Mechanical Properties of PDMS Cuff Electrodes Containing Meandered Platinum-Iridium Metal Contacts

Authors: *M. SCHUETTLER[1], V. OPPELT[2,3], J. HOFFMANN[2,3], T. STIEGLITZ[4];
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Abstract: In recent decades, bioelectronic medicine has become important for development of therapies or diagnosis of neurological diseases. Stimulation of nerve tissue is an application of targeted electrical signals that has already been successfully used to treat various neurological diseases or to alleviate symptoms. Extraneural electrodes used for this purpose, which increasingly consist of polydimethylsiloxane(PDMS) as the insulating material and Pt10Ir as the contact material, are commercially available (CorTec GmbH). Due to the choice of materials, there are limitations in design & manufacturing. Metal is rather stiff and the silicone is flexible. To bring these two materials into the round shape of a cuff electrode, the metal contact is cut in meandering shape. This work will focus on the use of incised or meandering contact surfaces & their effects on the mechanical stability of the entire electrode. Here, the focus was on realistic mechanical stress scenarios of the electrode. Thus, the meander contacts were tested for tensile strength and on how the electrode behaves when crushed. Furthermore, the silicone adhesion behaves on the metal and the influence on the natural inner diameter of the electrode during self-coiling was considered. The design without meander has a statistically significantly larger inner diameter than the meandered samples. Non-meandered samples curl up into an oval shape. The inner diameters of the meandered designs among themselves show no significant difference. Considering the contact length of the meandered designs it is significantly smaller (5 %) than that of the samples without meander, since those with meander overlap to some extent at the cuts at the contact edge. The cyclic compression test is performed with two samples per design configuration. Samples without meander reach a maximum compression between 57 % and 59 %

during the test. Meandered samples are compressed by a maximum of 46 % to 54 %. The Pt10Ir film of the samples without meander cuts is buckled in several places after the cyclic compression test. The contact designs with meander cuts show no clear signs of mechanical damage. During the tensile test, the samples without meander (n=2) reach a maximum elongation of 31 %. Meandered samples are stretched by a maximum of 43 % to 47 %. The individual meanders are pulled apart during the tensile test. After the test, the meanders resume their original shape. No contact designs show clear signs of mechanical damage. Overall, the investigations showed that no significant difference between different meander widths could be identified and no deterioration of the mechanical properties compared to non-meandered contacts was evident.

Disclosures: **M. Schuettler:** A. Employment/Salary (full or part-time):; CorTec GmbH. **V. Oppelt:** A. Employment/Salary (full or part-time):; CorTec GmbH. **J. Hoffmann:** A. Employment/Salary (full or part-time):; CorTec GmbH. **T. Stieglitz:** F. Consulting Fees (e.g., advisory boards); CorTec GmbH.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.01/HH4

Topic: E.06. Posture and Gait

Support: NIH Grant R01HD082216

Title: Neural mechanisms underlying learned use of the paretic leg induced by random constraint force to the non-paretic leg during walking in chronic stroke

Authors: *H. LIM[1,2], S. YAN[1,2], W. DEE[1], R. KEEFER[1], I. HAMEEDUDDIN[1,3], M. WU[1,2,3]; [1]Shirley Ryan AbilityLab, Chicago, IL; [2]Physical Med. and Rehabil., Northwestern Univ., Chicago, IL; [3]Biomed. Engin., Univ. of Illinois at Chicago, Chicago, IL

Abstract: Objective: The purpose of this study was to determine whether random vs constant constraint force applied to the non-paretic leg during walking demonstrates learned use of the paretic leg and changes functional connectivity within cortical networks in chronic stroke survivors. This study also aimed to examine whether baseline motor variability of spatiotemporal gait parameters in the paretic leg is related to changes in paretic gait parameters after random vs constant perturbed walking. Methods: Twelve individuals with chronic stroke were tested under two conditions: random vs constant constraint force applied to the non-paretic leg during treadmill walking. Each condition consisted of 1-min walking without force (baseline), 7-min walking with force (adaptation), and then additional 1-min walking without force (post-adaptation). In random condition, the force magnitude randomly changed across steps between 30% and 100% of the predetermined force while constant condition kept the force magnitude at 100% predetermined force. We used electroencephalography and electromyography to assess

functional connectivity of the cortico-cortical and cortico-muscular networks, and paretic leg muscle activity during walking. We also measured spatiotemporal gait variables during treadmill walking. Results: Paretic leg muscle activity of the ankle plantarflexors increased after random perturbed walking and the changes retained at late post-adaptation. After constant perturbed walking, muscle activity of the paretic ankle plantarflexors and hip flexors increased but the changes did not retain. Random condition enhanced cortico-muscular connectivity from the lesioned motor cortex to the paretic ankle plantarflexors and increased functional connectivity between motor cortices while no changes were found after constant condition. Greater motor variability of the paretic step length during baseline was associated with greater increase in paretic step length after random but not constant condition. Motor variability of the paretic step length increased after random but not constant condition. Conclusion: Our findings suggest that constraining the non-paretic leg with random force during gait training may serve as a novel approach to facilitate learned use of the paretic leg during walking after stroke. The observed neural mechanisms underlying learned use of the paretic leg during walking with random practice provide valuable insights for the development of targeted gait training strategies post stroke. In addition, motor variability of the paretic gait parameters has a potential to assess individual's motor learning capability during walking.

Disclosures: **H. Lim:** None. **S. Yan:** None. **W. Dee:** None. **R. Keefer:** None. **I. Hameeduddin:** None. **M. Wu:** None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

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Program #/Poster #: PSTR351.02/HH5

Topic: E.06. Posture and Gait

Support: KAKENHI 22H03456
KAKENHI 20H05476

Title: Kinematics detail analysis reveals quality changes of the recovered forelimb function by forced limb use after hemorrhagic stroke in rats

Authors: S. UENO[1], S. TOMINAGA[1], D. MUSTIKA[1], T. SHIMIZU[1], K. KOBAYASHI[2], C.-G. JUNG[1], N. TAJIRI[1], *H. HIDA[1];
[1]Nagoya City Univ. Grad Sch. Med. Sci., Nagoya, Japan; [2]Natl. Inst. for Physiological Sci., Natl. Inst. For Physiological Sci., Okazaki, Japan

Abstract: Intensive forced limb use (FLU) after intracerebral hemorrhage (ICH) improves paralyzed limb motor function in rats, and a switch from the cortico-spinal pathway to the cortico-rubral pathway was observed by the rehabilitative training of FLU (J. Neuroscience 36,455-67:2016). Although success rate of pellet retrieval test was improved, it is still unknown whether quality changes of skilled reaching are shown by the rehabilitative training of FLU after

ICH. To analyze detail kinematics of the forelimb function, we applied a marker less pose estimation tool (DeepLabCut) based on deep neural network to ICH model rats. After ICH, mean distance between fingertip 1 to 4 (finger opening) showed negative correlation to success rate. However, it became positive correlation in FLU group. In addition, reaching distance in non-FLU group was longer than FLU. Thus, the quality of finger movements significantly differs between FLU group and non-FLU group. We further performed detail kinematics analysis in ICH model rats that had selective blockade of cerebello-rubral tract with DREADD system: AAV-DJ-EF1a-DIO-hM4D(Gi)-mCherry was injected into the cerebellar lateral nucleus and FuG-E-MSCV-Cre was injected into the parvocellular red nucleus. Interestingly, the blockade of the cerebello-rubral tract with clozapine-N-oxide (CNO) significantly reduced the success rate, keeping the positive correlation to success rate. In addition, reaching distance in FLU group with CNO is longer showing dysmetria-like phenomenon. Data suggest that the cerebello-rubral tract is related to the recovery of skilled forelimb function by FLU and that quality changes of skilled reaching are also induced by rehabilitative training by FLU after stroke.

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Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.03/HH6

Topic: E.06. Posture and Gait

Support: NIH Grant RO1NS115487

Title: Improving sitting reactive balance in children with cerebral palsy using a session of robotic hippotherapy

Authors: *S. YAN[1], I. HAMEEDUDDIN[2], H. LIM[3], W. DEE[3], R. KEEFER[3], A.-M. ROJAS[1], W. ZEV RYMER[1], D. GAEBLER-SPIRA[3], M. WU[1];
[1]Shirley Ryan AbilityLab, Northwestern Univ., Chicago, IL; [2]Dept. of Biomed. Engin., Univ. of Illinois Chicago, Chicago, IL; [3]Shirley Ryan AbilityLab, Chicago, IL

Abstract: Sitting posture control is crucial for children with cerebral palsy (CP) because many of them spend a lot of time sitting to perform daily tasks. Standing reactive balance training has shown promise for improving standing balance in children with CP. This study aimed to determine whether applying repeated perturbation force to the pelvis during sitting would enhance sitting balance in children with CP. We hypothesized that a hippotherapy session would improve sitting balance in children with CP. A custom cable-driven robotic horse was used to apply controlled forward and backward perturbation force to the pelvis while subjects sat astride. Twelve children with spastic CP (age = 7.3 ± 2.8 years) completed two hippotherapy conditions: CONSTANT and VARIED, with the order randomized. In the CONSTANT condition, the peak

force magnitude remained constant ($F_{constant}$ = ~20% of body weight, BW) for 15 minutes. A sine wave force profile was used with the magnitude of the peak force set at $F_{constant}$ with the frequency at 1Hz. In the VARIED condition, the peak force magnitude varied from trial to trial (30-100% of $F_{constant}$). Reactive balance was assessed by suddenly pulling the horse forward or backward for 1 second and maintaining the force for another 4 seconds. Such unexpected perturbation force (25% of BW) was tested immediately before and after each condition. Kinematic data and muscle activities of the trunk, head, and hips were recorded. After the VARIED hippotherapy session, a significant decrease in peak head extension angle (P = 0.034) and shortened neck flexor muscle reaction time (P = 0.013) were observed during backward perturbation. Additionally, the trunk extensor muscle reaction time significantly decreased during forward perturbation (P = 0.009). After the CONSTANT hippotherapy session, muscle activity of the trunk extensor significantly increased under forward (P = 0.035) and backward (P = 0.02) perturbations and the muscle reaction time of the trunk extensor significantly decreased during backward perturbation (P = 0.012). Subjects exhibited greater pelvic, trunk, and head angular range of motion (ROM) during the 15-minute CONSTANT session compared to the VARIED session. However, the VARIED session showed greater variability in pelvic angular ROM and increased muscle activities of the hip flexor, trunk flexor, and neck flexor and extensor. In conclusion, varied perturbation forces in hippotherapy can induce error variability in postural control, potentially facilitating motor learning in sitting posture among children with CP. Our findings may contribute to the development of interventions for improving sitting balance in children with CP.

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Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

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Program #/Poster #: PSTR351.04/HH7

Topic: E.06. Posture and Gait

Support: University of New Mexico RAC Grant

Title: Independent stance phase after a self-mobilization exercise program

Authors: *D. SHIBATA[1], Y. YOSHIDA[2];
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Abstract: Processing of sensory signals is critical for stability control. A self-mobilization exercise program was developed to realign spinal curvature and can effectively improve the stability control while a static standing. However, it is unclear if this adapted stability by the self-

mobilization would also be observed in dynamic motor tasks such as gait. This study aims to examine spatiotemporal parameters of gait in healthy adults before and after the self-mobilization exercise program. Thirty-two healthy young adults were randomly assigned to Exercise (Ex: $n = 16$) or Control group (Ctrl: $n = 16$). Ex group performed the self-mobilization while lying supine on a cylinder-shaped tube (98-cm length, 15-cm diameter), consisting of three warm-up positions and seven small motions. The duration of the exercise program was 15 min. Ctrl group laid supine on a flat surface with their legs flexed for 15 min. Before and immediately after respective interventions, subjects walked on a 11.8m-path for 8 trials at comfortable speed. A 10-camera 3D motion analysis system (VICON Ltd.) was used to compute spatiotemporal parameters of gait including speed, % stance phase left, % stance phase right, % double leg support, % initial double leg support left, and % initial double leg support right. To control the effects of gait speed, repeated measures ANCOVAs with speed as the covariate were used to examine changes in the gait parameters before and after the intervention within groups. The relations between pretest and posttest measures within groups were assessed, when appropriate, with linear regression analysis. We found a significant *time* × *speed* interaction in % stance time of the left leg only for Ex group ($F = 50.16$, $p = .02$). Linear regression analysis revealed that, in Ex group, pretest measures of % stance phase left decreased as a function of gait speed ($F = 13.15$, $p = 0.003$, $r^2 = 0.48$) whereas posttest measures did not significantly change ($F = 1.65$, $p = 0.22$, $r^2 = 0.11$). These findings indicate that % stance phase of the left leg was not decreased with the gait speed after the exercise program. The stance phase independent of the gait speed can be interpreted as an adaptation to postural changes induced by the exercise program and is against a tendency of healthy adults in response to faster gait speed. It should be noted that the stance phase of the right leg and double support phase for both groups decreased as gait speed increased. Thus, such an adaptation may depend on the dominant leg. While the exercise program increased static stability in standing, there seems no effect on dynamic stability in gait. The stance phase of the left leg became independent of gait speed after the exercise program.

Disclosures: D. Shibata: None. **Y. Yoshida:** None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

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Program #/Poster #: PSTR351.05/HH8

Topic: E.06. Posture and Gait

Support: DoD RESTORE Award No. W81XWH-21-1-0138

Title: Osseointegration of the lower limb prosthetic improves gait symmetry - a pilot study

Authors: *K. HANNA[1], B. LINDSEY[2], S. YAKOVENKO[1];
[1]West Virginia Univ., Morgantown, WV; [2]Johns Hopkins Univ., Baltimore, MD

Abstract: Nearly half of all amputees in the Unites States will abandon their prescribed powered prosthetic device due to problems with comfort and function that result from the socket interface. Osseointegration (OI) has inherent advantages over the socket design including reduced sweating, better force distribution and alignment, and increased range of motion; yet, the changes in limb control and coordination are not well documented. We recruited two lower-limb amputees with planned OI to describe biomechanical and pain perception changes resulting from OI. Both participants reported clinically significant decrease in the scores of pain categories of the NIH patient-reported outcomes (PROMIS: Pain Behavior 39% and 6%, Interference 55% and 22.5%, and Intensity 40% and 13.4%). The center of pressure analysis during quiet stance demonstrated a shift from asymmetric loading to the non-OI limb pre-OI to the symmetric loading post-OI. The mediolateral deviation of COP from the midline in two subjects changed from 1.4±0.27 cm and 1.58±0.38 cm towards the symmetric position with only small offsets 0.07±0.62 cm and 0.51±0.26 cm, respectively. Gait asymmetry was measured as the asymmetry index of stance duration in left and right steps during symmetric walking on an instrumented split-belt treadmill. Both subjects showed trends toward symmetric walking with the OI prosthetic (AI changed from -0.05±0.04 to -0.01± 0.05 and from 0.05±0.02 to -0.01± 0.02, respectively). The direction of change favored the use of the OI limb. The analysis of muscle coordination supports the view that OI reduced pathological hip abduction necessary for toe clearance, evident from the burst of *gluteus medius* at the onset of swing in the residual limb. This burst disappeared from the pattern in post-OI sessions. Together, gait analysis results indicated the increase in gait symmetry and efficiency. These results may serve as a statistical reference for expected effect sizes in further studies of post-OI biomechanics and pain measures.

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Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.06/HH9

Topic: E.06. Posture and Gait

Title: Effect of an exergaming-based dance training paradigm among people with chronic stroke

Authors: *S. SUBRAMANIAM;
Univ. of Illinois Chicago, Aurora, IL

Abstract: ***Background:*** Innovative upcoming interventions such as Active Balance and Cardio (ABC) care, which integrates aerobic dance and exergaming targets physical and cardiovascular functional impairments among people with chronic stroke (PwCS). Albeit, PwCS exhibit limitations (e.g., health care, medication cost, and travel) in laboratory/outpatient rehabilitation programs, making them inaccessible and exclude them from long-term maintenance. Effective ABC care as a *home-based exercise program* could eliminate limitations and can also be used as an maintenance therapy in PwCS. ***Aim:*** To evaluate the effect of ABC care on physical and

cardiovascular outcomes of a hybrid laboratory and home-based format of ABC care. ***Design***: Pre-post interventional study. ***Setting:*** Laboratory (phase 1) and home-based (phase 2) settings. ***Methods:*** Community-dwelling PwCS (n=8) participated in the study and received ABC care intervention using "Just Dance 3" for Xbox 360 console. The first 6 weeks training was provided in the laboratory setting, and was delivered in a high-intensity tapering method with the first two weeks consisting of 5 sessions/week, next two weeks of 3 sessions/week and last two weeks of 2 sessions/week, with a total of 20 sessions. Followed with 4 more wks of ABC care in the participant's house (3 days/wk), with a total of 12 sessions. For physical function, voluntary balance control was assessed with post-training changes in self-initiated center of pressure response time (RT), the movement velocity (MV), the maximum excursion (MXE) was examined. Data obtained for cardiovascular function; heart rate variability (HRV) consists of HRV for ten minutes in supine resting position. ***Results***: Post-training the RT was significantly reduced (pre vs. post, $p<0.05$). Similarly, post-training, MV and MXE were significantly higher ($p<0.05$). Number of steps during dance intervention significantly increased from the 1st to the 20th session ($p<0.05$). Similarly, post-training, MV and MXE were significantly higher ($p<0.05$). Number of steps during dance intervention significantly increased from the 1st to the 20th session ($p<0.05$). After training, participants demonstrated a significant improvement in autonomic modulation, indicating an improvement in LF, HF and LF/HF ($p<0.05$). ***Conclusions***: A combination of laboratory, transitioned to home-based maintenance therapy approach appears effective, thus promising for post-stroke rehabilitation. A larger randomized controlled trial is recommended to further investigate efficacy.

Disclosures: S. Subramaniam: None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

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Program #/Poster #: PSTR351.07/HH10

Topic: E.06. Posture and Gait

Support: NIH Award Number 1 R03 HD099426-01A1

Title: Evaluating the accuracy of a low-cost virtual reality system for motion capture of locomotor biomechanics: a gateway to clinical accessibility

Authors: *E. HERRICK[1], S. YAKOVENKO[2];
[1]Chem. and Biomed. Engin., [2]Exercise Physiol., West Virginia Univ., Morgantown, WV

Abstract: Gait is a fundamental physiological function that can be affected by age, injury, or pathology. The analysis of gait kinematics and dynamics based on motion capture provides metrics, rehabilitation targets, and real-time gamified rehabilitation in virtual reality (VR). However, the use of this approach has been hindered by the high cost and expertise required for its execution. VR systems (e.g., HTC VIVE) rely critically on 'good-enough' performance in

position tracking and low-latency closed-loop performance. In this study, we examine the use of these technologies for locomotor applications. We developed a computational pipeline that integrates motion capture into a VR engine (Unreal Engine and SteamVR) using 6 degree-of-freedom (DOF) sensors that provide 3 positions and 3 rotations for sensors placed on segments within kinematic chains for testing. For testing, we used two sensors calibrated to a goniometer set to multiple configurations (30, 60, 90, 120, 150, 180º). The reconstruction of posture was tested within the capture volume over a treadmill (about 1.5x2.5x2.0 m), where the integrated device was used to "paint" the volume in different orientations, which is a typical volume calibration procedure with optical motion capture systems. The errors were not dependent on position or orientation within this volume and were similar across all configurations. Full-body instrumentation required 11 sensors and co-registration of at least three representative landmarks for each body segment. Sensors were placed bilaterally on the foot, tibia, humerus, radius-ulna segments, lower- and upper-trunk (sternum and iliac crest), and head. Preliminary data shows that our method can describe foot contacts captured separately with a split-belt instrumented treadmill (Bertec) within the precision required for biomechanical analysis of gait. These results suggest that low-cost VR systems have the potential to make motion capture more accessible in clinical and in-home spaces to enable data-driven decisions for patient care.

Disclosures: E. Herrick: None. **S. Yakovenko:** None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

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Program #/Poster #: PSTR351.08/HH11

Topic: E.06. Posture and Gait

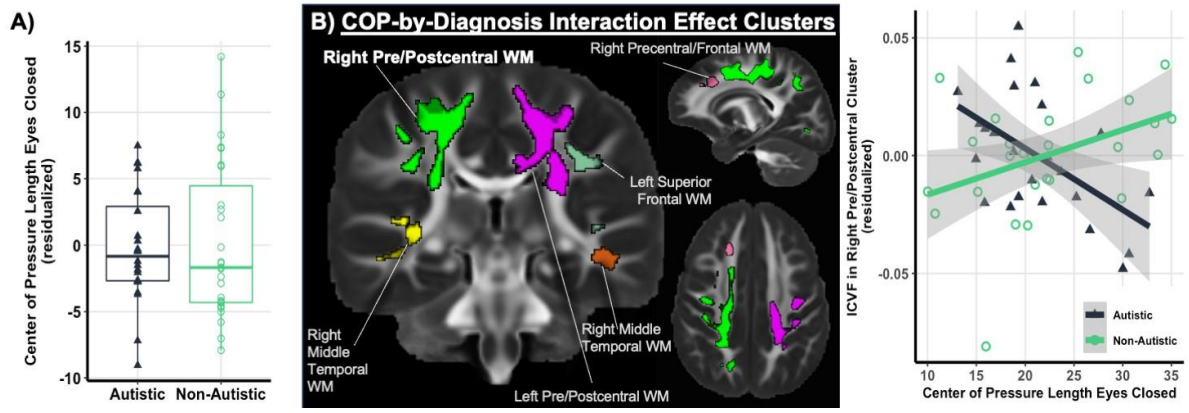
Support: Hartwell Foundation 's Individual Biomedical Award
R01 HD094715

Title: Neurite properties of sensorimotor white matter are unique related to postural stability in autistic and non-autistic children

Authors: *S. NAIK[1], O. J. SURGENT[1], J. M. GUERRERO-GONZALEZ[1,2], N. ADLURU[1,3], G. R. KIRK[1], S. R. KECSKEMETI[1], D. C. DEAN, III[1,2,4], A. L. ALEXANDER[1,2,5], B. G. TRAVERS[1,6];
[1]Waisman Ctr., [2]Dept. of Med. Physics, [3]Dept. of Radiology, [4]Dept. of Pediatrics, [5]Dept. of Psychiatry, [6]Occup. Therapy Program in the Dept. of Kinesiology, Univ. of Wisconsin-Madison, Madison, WI

Abstract: Postural stability challenges are highly prevalent in autistic populations (Fournier et al., 2010), and may be influenced by differences in visual-vestibular processing (Lim et al., 2017, 2018). Associations between postural control and neural structures involved in sensory integration have been found with autism-specific relationships in cortical and cerebellar

structures (Loram 2015). Yet, the role of white matter microstructure has not been thoroughly explored in the context of autistic postural control. Therefore, we investigated the relationship between white matter and postural control patterns in 52 autistic and non-autistic youth (6.0-10.9 years; 25 autistic). We calculated center of pressure length (COP) during 60 second, static, two-foot balance in two conditions: eyes open (EO), eyes closed (EC). We acquired multi-shell diffusion weighted imaging data with a 3T GE scanner, used TiDi-Fused processing (Guerrero-Gonzalez et al., 2022) to achieve 1mm isotropic apparent resolution, and calculated intracellular volume fraction (ICVF) at each voxel (Zhang et al., 2012). To explore neurite structure-postural control relationships, we used voxel-based analysis (VBA) with threshold free cluster enhancement and FDR correction. No group differences in COP were found in either condition [A]. In the EC condition, significant COP-by-group (autistic vs non-autistic) interaction effects were found to predict ICVF white matter clusters (43 clusters, 80,536 voxels) spanning sensorimotor pathways (superior longitudinal fasciculus, pre- and postcentral gyri [B]). These results indicate that while postural control in autistic and non-autistic youth is similar, neural contributions to performance may be distinct. This may especially be the case for sensorimotor white matter during the absence of visual input. Together, this work provides evidence to suggest unique neural mechanisms of sensorimotor integration in autistic and non-autistic youth and may inform our understanding of postural stability challenges in this population.



Disclosures: **S. Naik:** None. **O.J. Surgent:** None. **J.M. Guerrero-Gonzalez:** None. **N. Adluru:** None. **G.R. Kirk:** None. **S.R. Kecskemeti:** None. **D.C. Dean:** None. **A.L. Alexander:** None. **B.G. Travers:** None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.09/HH12

Topic: E.06. Posture and Gait

Title: Aerobic Exercise with a Robotic Hip Exoskeleton in Healthy Adults

Authors: *S.-H. LEE;
Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Decreased physical activity with increased sedentary behavior causes metabolic derangements and altered body composition. Regular physical activity and aerobic exercise can accelerate the human body's metabolic rate, help with weight management, strengthen bones and muscles, and enhance cardiopulmonary function. The purpose of this study was to investigate the effect of a wearable hip exoskeleton, the EX1, on muscle activation, heart rate, and cardiopulmonary energy consumption during aerobic exercise in healthy adults. Forty healthy adults (20 males, 39.51 ± 11.84 yr, range = 20-59 yr) performed aerobic exercise under three conditions: continuous aerobic exercise without the EX1 (C-NoEX1), continuous aerobic exercise with the EX1 (C-EX1), and interval aerobic exercise with the EX1 (I-EX1). Changes on muscle activation, heart rate, and cardiopulmonary energy consumption were measured during the three conditions to evaluate the effect of aerobic exercise with the EX1. Measured muscles included the rectus abdominis (RA), erector spinae (ES), rectus femoris (RF), biceps femoris (BF), tibialis anterior (TA), and gastrocnemius medialis (GCM). Muscle activation, heart rate, and cardiopulmonary energy consumption were significantly higher during aerobic exercise with the EX1 than without EX1. In particular, activation levels of all measured muscles were significantly increased in C-EX1 than C-NoEX1 ($P < 0.05$). Activation of RF, BF, TA, and GCM was significantly increased in I-EX1 than C-NoEX1 ($P < 0.05$). In addition, the time to reach a moderate exercise intensity level (64% of maximum heart rate) after starting aerobic exercise was greatly shortened in C-EX1 and I-EX1 (83% and 82%, respectively) than C-NoEX1, and EEm(Kcal/min) was significantly increased in C-EX1 and I-EX1 than C-NoEX1 ($P < 0.001$). Results of this study demonstrated that use of the EX1 during aerobic exercise increased muscle activities in trunk and lower extremities and cardiopulmonary energy consumption to the direction of enhancing physiologic effect of aerobic exercise based on the same exercise time without EX1. . These findings suggest that the wearable robotic hip exoskeleton, the EX1, can be used for increasing effectiveness of aerobic exercise in healthy adult.

Disclosures: S. Lee: None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.10/HH13

Topic: E.06. Posture and Gait

Support: JSPS KAKENHI 22K19742
JSPS KAKENHI 23H03299

Title: Exercise-specific reorganization in human spinal locomotor circuitry in sports athletes

Authors: *T. TAZOE[1], K. KAWAI[2], Y. NISHIMURA[1];
[1]Neural Prosthetics Project, Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Col. of Sports Sci., Nihon Univ., Tokyo, Japan

Abstract: Long term motor training reorganizes somatotopic arrangement in cortical motor areas. For instance, the hand representation in the cortical motor area is known to be larger in professional musicians than non-musicians, indicating that long-term intensive training induces dynamic topographical reorganization. In contrast to the cerebral cortex, it is unknown if similar reorganization can be observed in spinal motor circuits. Here, we demonstrated that the motor maps can be depicted for the human spinal motor circuits involving locomotor behaviors, and that the spinal locomotor maps were dramatically different among various sports athletes who were conducting daily motor training for specific types of motor behaviors. In a cross-sectional study, we recruited 32 college sports athletes; long-distance runners, road cyclists, weightlifters, and gymnasts (8 participants in each sport). The participants were in semi-prone posture on a bed with both legs suspended from the ceiling and received the burst of TVMS via a closed-loop paradigm while legs were fully relaxed. A 6 by 3 stimulation target grid was arranged over the back that covered 6 intervertebral spaces from Th11 to L5 and ~3 cm left to right from the midline. The bursts of TVMS induced the multiple patterns of cyclic bilateral leg movements depending on the participant group. In the long-distance runners and the road cyclists, a walking-like movement, cyclic leg movement alternating left and right was observed at broad stimulus sites while a hopping-like movement, rhythmic bilateral backward leg movement was induced at rest of stimulus sites. In contrast, the hopping movement was mainly induced at most stimulus sites and the walking-like movement was rarely induced at a few stimulus sites in the weightlifters. Interestingly, only gymnasts showed to induce a folding-like movement, rhythmic bilateral leg forwarding movements at the rostral parts in the stimulus grid in addition to the walking-like and hopping-like movements observed at the middle to caudal stimulus sites. These finding suggest that the human spinal motor circuits involving locomotor behaviours can be reorganized by long-term specific motor training.

Disclosures: T. Tazoe: None. **K. Kawai:** None. **Y. Nishimura:** None.

Poster

PSTR351. Posture and Gait: Aging, Injury and Exercise

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR351.11/HH14

Topic: E.06. Posture and Gait

Support: FAPESP 2018/04964-8

Title: Influence of stiff-knee and gait speed on locomotor coordination of individuals with stroke

Authors: *A. M. F. BARELA[1], O. BACCA[2], R. E. VAN EMMERIK[3], M. L. CELESTINO[4], J. A. BARELA[5];

[1]Univ. Cruzeiro do Sul, Sao Paulo, Brazil; [2]Univ. Industrial de Santander, Bucaramanga, Colombia; [3]Univ. of Massachsetts Amherst, Amherst, MA; [4]Univ. Federal de Pernambuco, Recife, Brazil; [5]Univ. Estadual Paulista, Rio Claro, Brazil

Abstract: Stiff-knee gait, slow walking speed, and locomotor coordination disturbances are common gait impairments observed in individuals with stroke. Considering that deficits in locomotor coordination can increase risk of falls, energy costs, and overuse effects in lower limb joints, we examined the influence of stiff-knee gait and walking speed on gait coordination between thigh-shank and shank-foot segments, and the coordination variability between gait cycles of these couplings. Twenty individuals with chronic stroke and 20 non-disabled individuals matched by age and sex were assessed. Individuals with stroke walked at self-selected comfortable speed and non-disabled individuals walked with an orthosis that limited right knee flexion up to 40 degrees at the average speed of individuals with stroke. We used a vector coding technique to analyze the frequency of four coordination modes (in-phase, anti-phase, proximal-segment-phase, and distal-segment-phase) during stance and swing periods of thigh-shank, and shank-foot couplings, and the variability between gait cycles of these couplings. We employed the conventional statistical procedures for the frequency of coordination modes and statistical parametric mapping for coordination variability. For the thigh-shank coupling, individuals with stroke presented lower frequency in the in-phase and higher frequency in the anti-phase, thigh-phase, and shank-phase coordination modes than non-disabled individuals. For the shank-foot coupling, individuals with stroke presented higher frequency in the anti-phase and shank-phase (stance and swing), and lower frequency in the foot-phase (swing) coordination modes than non-disabled individuals. The paretic limb presented lower frequency in the foot-phase and higher frequency in the in-phase (swing) coordination modes than the constrained limb in the non-disabled group. Regarding coordination variability, thigh-shank variability was higher for the paretic compared to the constrained limb during 3-17%, 35-47%, and 73-77% of the gait cycle, and the non-paretic limb variability was higher than the unconstrained limb during 56-61% of the cycle. Shank-foot variability in paretic limb was higher than the constrained limb during 97-100% of the cycle, and the non-paretic limb had higher variability during 14-18% and 65-68% of the gait cycle. The results revealed that a stiff-knee influences intralimb coordination mainly during the swing and variability mainly during stance, and individuals with stroke seems to compensate the diminished knee flexion by controlling the degrees of freedom differently than their non-disabled peers during locomotion.

Disclosures: A.M.F. Barela: None. **O. Bacca:** None. **R.E. van Emmerik:** None. **M.L. Celestino:** None. **J.A. Barela:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.01/HH16

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH F32HD092051
NIH P01HD075750
NIH R01HD098117

Title: Pregnancy and birth epigenetically shape the maternal oxytocin receptor gene

Authors: *A. M. PERKEYBILE[1], M. MCDONALD[2], W. M. KENKEL[3], C. CARTER[1], J. CONNELLY[1];
[1]Psychology, [2]Sch. of Nursing, Univ. of Virginia, Charlottesville, VA; [3]Psychological and Brain Sci., Univ. of Delaware, Newark, DE

Abstract: Obstetric practice routinely uses interventions such as induction of labor with synthetic oxytocin that alter endogenous oxytocin signaling during labor and delivery. Consequences of this intervention on the maternal brain are not well understood. Here we use the prairie vole to understand how pregnancy, natural birth, and labor induction with synthetic oxytocin impact epigenetic control of the oxytocin receptor gene, *Oxtr*. DNA methylation and gene expression of *Oxtr* were examined in virgin females, at term pregnancy, and within 90 minutes after an unmanipulated vaginal birth in new mothers. These same *Oxtr* markers were examined in a second cohort of term pregnant females after administration of exogenous oxytocin prior to labor onset, a model of labor induction in humans.
Results identify a switch in the regulatory state of *Oxtr* from late pregnancy to early postpartum in unmanipulated birth that appears to be facilitated in part by oxytocin. *Oxtr* methylation in the brain of virgin females is negatively associated with *Oxtr* gene expression. At term pregnancy there is no longer a significant relationship between these markers. Immediately following birth, this relationship shifts back to the negative association seen pre-pregnancy. This shift toward a negative association between *Oxtr* methylation and expression can be induced prenatally with administration of increasing doses of exogenous oxytocin. These findings indicate epigenetic control of *Oxtr* is dynamic across gestation and birth and is sensitive to oxytocin at term.
To build a translational model of birth to understand the epigenetic state of *OXTR* in the newly maternal brain of human mothers, we must identify easily accessible peripheral tissues that can serve as a proxy for central tissues. We examined the relationship between DNA methylation markers in the brain with markers in whole blood in our animal model. There is a strong positive correlation between brain and blood methylation in unmanipulated pregnancy and birth. After labor induction, this positive association is seen only at specific CpG sites. Results show DNA measures in blood may be useful in understanding central markers, but this may be CpG site-specific following oxytocin manipulation. We will also present data on *Oxtr* in the uterus, a tissue that may be especially important for understanding maternal outcomes given the role of oxytocin at birth in this tissue.
These results identify a need to better understand how commonly used birth interventions, such as labor induction or augmentation with synthetic oxytocin, impact *Oxtr* regulation in the maternal brain, potentially contributing to risk for adverse maternal mental health outcomes.

Disclosures: A.M. Perkeybile: None. **M. McDonald:** None. **W.M. Kenkel:** None. **C. Carter:** None. **J. Connelly:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.02/HH17

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Princeton University New Ideas in the Natural Sciences (Dean for Research Innovation Fund)

Title: Leveraging single nucleus RNA-sequencing to uncover mechanisms of paternal care in African Striped Mice (Rhabdomys pumilio)

Authors: *F. D. ROGERS[1,2], R. MALLARINO[2], C. J. PEÑA[1];
[1]Princeton Neurosci. Inst., [2]Dept. of Mol. Biol., Princeton Univ., Princeton, NJ

Abstract: The neural mechanisms that underly naturally occurring paternal care remain largely uncharacterized. Here, we leverage the natural behavior of African striped mice (Rhabdomys pumilio) in conjunction with single nucleus RNA-sequencing (snRNA-seq) to uncover putative mechanisms of paternal care. African striped mice are murine rodents in which both paternal and male alloparental care is naturally occurring and common. There is a significant body of literature implicating the medial preoptic area (MPOA) as a putative hub for maternal care, and by extension, paternal care. Using brain-wide Fos quantification, we find a significant positive correlation ($p < .005$) between immediate early gene activity (i.e., cFOS+ cells by IHC) in the MPOA and paternal phenotypes (vs. infanticidal and ambivalent phenotypes) in virgin male striped mice. To understand whether different cell types are activated or show distinct molecular profiles corresponding to behavioral phenotype, we used single-nucleus RNA-sequencing from the MPOA of 20 individuals across phenotypes: infanticidal (in Mus musculus and African striped mice), allopaternal, paternal (i.e., genetic fathers), maternal (i.e., genetic mothers), and behaviorally naïve controls. We sequenced more than 190,000 nuclei and are quantifying broader cell type composition and cell-type-specific immediate early gene expression across these six phenotypes at the level of the individual, group, and more generalized groups (e.g., broadly caring vs. infanticidal), and pairwise differences in cell-type-specific gene expression. These analyses will reveal potential mechanisms that support paternal care.

Disclosures: F.D. Rogers: None. **R. Mallarino:** None. **C.J. Peña:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.03/HH18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01 DA 052386

Title: The Effects of Transitioning from Morphine to Buprenorphine During Pregnancy on Maternal Care, Motivation, and Physiology in a Translational Rodent Model

Authors: *L. M. RICHARDSON, J. DURAN, A. M. MYERS, S. E. BOWEN, S. BRUMMELTE;
Psychology, Wayne State Univ., Detroit, MI

Abstract: The recent opioid epidemic has resulted in many opioid-dependent pregnant women receiving medications for opioid use disorder (MOUD). Buprenorphine (BUP) is a semi-synthetic opioid MOUD utilized to mitigate the negative effects of misused opioids on the mother and developing fetus. Clinically, BUP produces preferable outcomes for exposed infants as compared to methadone or illicit opioid exposure, however, BUP's effects on maternal neural networks that are critical for maternal caregiving behaviors are not well understood. BUP's mechanism of action (partial mu-agonist/kappa antagonist) varies from morphine's (full mu-agonist) and other opioids (i.e. methadone), which may result in a unique impact on the maternal brain during the transition to motherhood. The current study used a translational rodent model to investigate how transitioning from an opioid of abuse (i.e., morphine) to BUP during gestation affects maternal care, motivation, and maternal physiology compared to continued use of morphine, or BUP from preconception (drug administration began 7 days prior to mating) through early postpartum. Dams were assigned to one of 5 experimental groups: BUP continued ("BC", 1mg/kg, s.c.), morphine continued ("MC", 3 - 10 mg/kg, s.c.), morphine to BUP ("MB"; 3 - 5mg/kg morphine until Gestational Day (GD) 5, then 1.0 mg/kg BUP; s.c.), morphine to vehicle ("MV"; 3 - 5mg/kg morphine until GD 5, then 1.0 mL/kg saline; s.c.), or saline continued ("VEH", 1.0 mL/kg, s.c.). MB and MV groups switched to BUP or saline (respectively) on GD 5, to roughly mimic the time in humans when a woman would discover she is pregnant and may seek treatment (i.e. ~ 6-8 weeks of pregnancy). Dams' maternal behaviors were assessed through detailed observations of nest qualities and maternal care, as well as through several behavioral tests including a pup retrieval test (maternal motivation), a two-choice olfaction test (pup-preference) and restraint stress test (maternal physiology). All dams and their respective litters were sacrificed on postnatal day 2 (PN2). Our preliminary results indicate that opioid exposed dams had decreased nest qualities and spent less time on pup-directed care behaviors as compared to VEH or MV dams. These results suggest that transitioning from morphine to BUP during pregnancy does not result in differing effects compared to continued BUP exposure. Further, gestational BUP exposure warrants further investigation to elucidate potentially detrimental effects for both mother and fetus.

Disclosures: L.M. Richardson: None. **J. Duran:** None. **A.M. Myers:** None. **S.E. Bowen:** None. **S. Brummelte:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.04/HH19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIDA R01 DA049531
Tufts University Russo Family Award
Tufts Initiative on Substance Use and Addiction Award
Natalie V. Zucker Center Research Award

Title: Long-term effects of gestational exposure to medication for opioid use disorder: Neural correlates underlying offspring feeding dysregulation

Authors: *K. GILDAWIE[1], K. E. BUDGE[1], S. B. ISGATE[1], F. VASSOLER[1], E. YEN[2], E. BYRNES[1];
[1]Comparative Pathobiology, Tufts Univ., North Grafton, MA; [2]Pediatrics, Tufts Univ. Sch. of Med., Boston, MA

Abstract: The current, decades-long opioid crisis has resulted in escalating rates of opioid use disorder in women of reproductive age and increased prevalence of fetal drug exposure. While medication for opioid use disorder (MOUD), e.g., buprenorphine or methadone, improves maternal health outcomes, over 50% of infants born to mothers on MOUD experience hyperphagia, an early withdrawal sign that is associated with more severe withdrawal course. Recent human work found significant alterations in salivary expression of feeding related genes, including dopamine receptor type 2 (DRD2), neuropeptide Y2 receptor (NPY2R), and proopiomelanocortin (POMC) in neonates with opioid-induced hyperphagia. Using a translational model of MOUD, this work investigates potential neural mechanisms underlying the impact of gestational opioids on feeding dysregulation in neonatal and adult offspring. Adult Sprague Dawley females were implanted with osmotic minipumps filled with methadone (10 mg/kg/day) or buprenorphine (1 mg/kg/day) dissolved in dH_2O or saline control (2.5 µl/hour for 28 days) and mated. On postnatal day (PND)1, male and female pups were cross-fostered with drug-naïve dams. On PND2 and PND7, brain tissue was harvested and cryosectioned. RNAscope in situ hybridization was performed to visualize expression of feeding-related genes in the nucleus accumbens, a brain region involved in regulating feeding behavior. In adulthood, remaining subjects began palatable food self-administration to assess the motivational strength of a food reward in MOUD-exposed animals vs. controls, followed by analysis of gene expression via RNAscope. We found that maternal MOUD (both methadone and buprenorphine) results in reduced offspring body weight at PND1. At PND7, the number of puncta in NPY2R+ cells was lower in both male and female methadone-exposed pups when compared to either buprenorphine- or saline-exposed pups. We also observed long-term changes in feeding behavior, with adults gestationally exposed to methadone - but not buprenorphine - demonstrating increased motivated responding to a palatable food reward when compared to controls. These findings indicate that methadone-induced feeding dysregulation in adulthood is associated with decreased levels of NPY2R during neonatal development. These data provide insight into the potential neuromolecular underpinnings of MOUD-induced changes in neural modulation of feeding-related behaviors.

Disclosures: K. Gildawie: None. **K.E. Budge:** None. **S.B. Isgate:** None. **F. Vassoler:** None. **E. Yen:** None. **E. Byrnes:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.05/HH20

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH U19 Grant NS107616-01
NSF GRFP Grant

Title: A system for studying the neuroscience of mouse families: months-long recordings and interventional approaches to ensure pup survival

Authors: *L. PINEROS SCHUSTER[1,4,2], R. J. HENDERSON[2,4], V. IVAN[2,4], D. C. ANANTH[5], S. WINOKUR[2], H. A. ISSA[2,4], P. LEONE[4], R. FROEMKE[4,2], A. C. MAR[3]; [1]New York Univ., Brooklyn, NY; [3]Neurosci. and Physiol., [2]New York Univ., New York, NY; [4]NYU Langone, New York, NY; [5]North East Ohio Med. Univ., Ohio, OH

Abstract: Parental care helps ensure offspring health and wellness. Parenting in mammals is challenging as infants are largely helpless, needing near-constant supervision over prolonged periods of time. There is a high degree of rodent infant mortality in native habitats and in lab vivaria (Brajon *et al*. 2021), from hypothermia unless pups are kept warm by a caretaker or nest (Lynch and Possidente, 1978). Parents must balance caretaking with other activities for their own survival, e.g., foraging for food or water and regulating temperature, inevitably involving periods of absence from the nest.

To document the behaviors and decision-making involved in care of infant pups, we built a semi-automated, open-source system for continuous months-long 24/7 monitoring of mouse homecage life, with thermal imaging, multi-camera video recording from above/beside/below the nest, and environmental controls to standardize housing conditions across cages.

First, we monitored 38 wild-type, single-housed female mice and offspring, before, during, and after parturition, from initial mating over four consecutive litters. About half the dams had high litter survival rates ('high-pup-survival' dams, with >80% litter survival), but the other half had little to no pups survive ('low-pup-survival' dams, with 0% litter survival at litter 1 and <50% litter survival for litter 2).

The relative litter survival did not change or improve across litters in absence of intervention. Low-pup-survival dams kept neglecting pups and poor nest-building across litters, losing nearly all offspring within a day after birth. We could predict which mice would become low-pup-survival dams by inspecting nests built a few days before parturition.

Low-pup-survival dams minimally engaged in building/adjusting nests, and generally avoided pups. Conversely, high-pup-survival dams spent a substantial amount of time rebuilding and adjusting the nest over the first postnatal day and beyond. We co-housed a low-pup-survival dam with a high-pup-survival dam and her litter in between litters 2-3 of the low-pup-survival dam. When the low-pup-survival dam was bred again and singly-housed, litter survival rates thereafter were consistently much higher along with increased attention toward pups and nest (co-housed dams, litter 3 survival: 88.5±4.8%, N=9; isolate dams, litter 3 survival: 2.5±5.6%, N=5, $p<10^{-4}$). The high litter survival effect persisted for the low survival dams that were bred a fourth time.

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Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.06/HH21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: U19 Grant NS107616

Title: Mouse midwives: behavioral mechanisms that greatly improve maternal-infant survival during difficult parturition

Authors: L. F. SCHUSTER, ***V. IVAN**, R. HENDERSON, S. WINOKUR, A. MAR, R. C. FROEMKE;
Neurosci. Inst., New York Univ., New York, NY

Abstract: Oxytocin is an ancient peptide hormone with powerful actions in the brain and body, regulating mammalian reproduction and childcare (Gimpl & Fahrenholz 2001, Dulac et al. 2014, Froemke and Young 2021). Oxytocin is particularly important for uterine contractions during parturition for childbirth, and mice lacking oxytocin receptors do not have contractions (Takayanagi et al. 2005). Despite this lack of uterine function, oxytocin receptor knockout (OXTR KO) animals can be bred, although it has remained unclear what if any phenotypes or difficulties result from impaired OXTR signaling (Berendzen et al. 2022). In part this is due to challenges of observing and quantifying the details of mouse gestation and parturition, even in lab vivaria. With a recent report that human maternal mortality is on the rise (Hoyert 2023), it is critical to understand the physiology and behaviors related to parturition and successful childbirth.

We built low-cost open-source systems for months-long continuous 24/7 behavioral monitoring, with multiple cameras including under the nest. We have begun to describe the complexities of single-parenting and co-parenting, involving oxytocin and other neural systems to promote parental care and successful child rearing in mice (Marlin et al. 2015, Carcea et al. 2021, Schuster et al. 2022, Valtcheva et al. 2023). Under-nest imaging is possible as when pregnant mouse mothers (‘dams’) begin labor, they turn in circles and clear the cage floor, enabling unobstructed imaging of birthing. In most cases, singly-housed wild-type females undergo labor for hours, with a range of results in terms of pup mortality (Schuster et al. SFN 2023 Abstracts). We found that a co-housed non-pregnant female will investigate and interact with the dam during labor, including seeming to pull the pup out and clean it, or eating placenta- in line with previous reports of male rodent behavior (Jones & Edwards 1999, Lee & Brown 2002). However, as singly-housed females also usually successfully give birth, it is unclear if these interactions are advantageous.

We examined parturition in six singly-housed OXTR KO dams. We found that 5/6 dams died due to dystocia, and all pups died- in the surviving female, pups were transported to a wild-type lactating dam but did not latch and thus no nursing occurred. Remarkably, however, in five OXTR KO dams co-housed with a wild-type female which were bred a total of eight times, the engagement of the wild-type to remove stuck pups and otherwise 'assist' in labor led to survival of all dams across all pregnancies, and overall 93% of pups survived. Thus 'midwife'-type behavior in mice can greatly improve maternal-infant survival during difficult labor.

Disclosures: L.F. Schuster: None. **V. Ivan:** None. **R. Henderson:** None. **S. Winokur:** None. **A. Mar:** None. **R.C. Froemke:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.07/HH22

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: U19 BRAIN NS107616

Title: Neural responses to video-playback of parental behaviors

Authors: *K. QUIÑONES-LARACUENTE[1], A. CASLIN[2], S. WINOKUR[2], N. LÓPEZ CARABALLO[1], R. C. FROEMKE[3];
[1]NYU Sch. of Med., NY, NY; [2]New York Univ., New York, NY; [3]Otolaryngology, NYU Med., New York, NY

Abstract: Exposure to experienced parents can accelerate parenting in virgin mice (Koch and Ehret 1989, Marlin et al. 2015, Carcea et al. 2021). Our previous work has demonstrated that pup retrieval onset is accelerated in head-fixed virgin female mice passively observing videos of dams retrieving pups. We wanted to explore if mice would actively choose to watch parental videos. To test active engagement with parental behavior videos, we added an operant lever to allow the option to trigger the video in a 1:1 schedule interval. After training, mice pressed the lever for a 10 second parental care video once per minute (N=8 virgin female mice). In the same set of mice, swapping the video with a scrambled or abstract video reduced pressing to once every five minutes after four days, suggesting extinction of the instrumental conditioning. Another set of mice trained similarly to press a lever for the scrambled video pressed once every five minutes (N=6). These findings suggest that videos of parental behaviors favor the rewarding stimuli domain.
Visual input from the superior colliculus provides monosynaptic input to hypothalamic oxytocin neurons in the paraventricular nucleus (PVN), promoting onset of alloparenting in virgin female mice (Carcea et al. 2021). To determine the socio-visual responses of PVN neurons, we performed single-unit recordings from the PVN during passive and active video-playback of parental behaviors. Using tensor component analysis (TCA) (Williams et al. 2018), we examined

intra- and inter- trials activity in large-scale silicon probe recordings. In mice pressing a lever for parental videos, there are both excitatory and inhibitory responses to the video presentation, as well as two populations that separate after 10 video presentations (n=26). In contrast, when videos were passively shown, these subensembles did not form in either intra- or inter- trials (n=22), suggesting differences in the activity profile of PVN neurons in active versus passive social presentations.

Disclosures: K. Quiñones-Laracuente: None. **A. Caslin:** None. **S. Winokur:** None. **N. López Caraballo:** None. **R.C. Froemke:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.08/HH23

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH U19 Grant NS107616
NIH TL1 Grant 5TL1TR001447-08

Title: The neuroscience of maternal caregiving when sick

Authors: *S. B. WINOKUR, L. F. SCHUSTER, A. CASLIN, K. QUIÑONES-LARACUENTE, S. SHOKAT FADAEI, S. ORREY VALENCIA, R. C. FROEMKE; Neurosci. Inst., New York Univ., New York, NY

Abstract: Sickness causes substantial changes to physiology and behavior. Sickness behavior is a socially useful signal, increasing sensitivity to social threat and connection of sick animals (Eisenberger et al., 2017), and allowing other animals to respond to the health status of conspecifics (Devlin et al., 2021). Being sick as a parent introduces complex challenges to ensuring the survival of both parent and offspring. Mothers combating infection in the postpartum period show increased risk for anxiety, depression, and deficits with bonding and breastfeeding (Groer et al., 2015; Belfort et al., 2010). Though it is pivotal to treat maternal sickness for the well-being of mothers and their offspring, the mechanisms by which sickness influences the maternal brain and behavior is unclear. This project aims to address the central hypothesis that activity of oxytocin neurons within the paraventricular nucleus (PVN) is altered in response to acute lipopolysaccharide-induced sickness, as mediated by maternal caregiving experience. While the behaviors, circuitry, and molecular signals involved in parenting are complex (Dulac et al., 2014), work from our lab has shown the importance of PVN oxytocin neurons in enabling mouse maternal caregiving (Marlin et al., 2015; Valtcheva et al., 2021). The Froemke lab has developed a system for longitudinal continuous behavioral monitoring ideal for studies of the neural circuitry for maternal behavior (Schuster et al., 2022; Carcea et al., 2021). Using this system, we have found that sickness reduces the duration that first-time mothers spend on the nest caring for their young, which results in an elevated risk of pup mortality.

Interestingly, second-time mothers do not show these changes to caregiving when sick. Whole-brain cFos imaging indicates that PVN activity is decreased in sick second-time mothers relative to first-time mothers. We are also evaluating how sickness influences PVN activity by taking *in vivo* optogenetically tagged PVN oxytocin neuron recordings using silicon probe implants throughout the course of sickness during caregiving. Together, these results suggest that an inflammatory challenge differently influences maternal behavior depending on the degree of experience-related neuroplasticity the maternal brain has undergone.

Disclosures: S.B. Winokur: None. **L.F. Schuster:** None. **A. Caslin:** None. **K. Quiñones-Laracuente:** None. **S. Shokat Fadaei:** None. **S. Orrey Valencia:** None. **R.C. Froemke:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.09/HH24

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: U19 BRAIN NS107616

Title: Parental caregiving of sick offspring in mice

Authors: *A. CASLIN[1,2], L. SCHUSTER[2], K. QUIÑONES-LARACUENTE[2], G. KAUR[3], S. WINOKUR[1,2], S. SHOKAT FADAEI[2], R. C. FROEMKE[2];
[1]New York Univ., New York, NY; [2]New York Univ. Sch. of Med., New York, NY; [3]Neurosci., NYU Sch. of Med., New York, NY

Abstract: Maternal care is critical for offspring well-being, and must be directed and flexible depending on the perceived conditions and needs of offspring. However, it remains unclear which neural systems and mechanisms are important for mothers to sense and respond to the needs of their young. Sickness behaviors may serve as socially-useful signals that solicit comfort or caregiving, and the context of maternal care may reinforce prosocial behaviors. Here, we use long-term behavioral monitoring combined with optically-tagged in vivo electrophysiology, to test whether the maternal mouse oxytocin system contributes to caregiving. Using a multi-camera longitudinal behavioral monitoring system developed in the lab (Carcea et al. Nature 2021, Schuster et al. bioRxiv 2022), we characterized and compared parental behaviors of a mouse dam toward pups injected with lipopolysaccharides (LPS) vs. saline controls over the course of 48-hours. Dams were also tested in a three-chambered social preference assay. Using silicon probe implants in the paraventricular nucleus of the hypothalamus (PVN), a site of oxytocin synthesis, we further test how dam PVN neurons respond to sick vs. healthy pups. Our results show that dams display persistent increased physical contact (i.e., huddling behavior) toward LPS-injected offspring and non-offspring vs. saline controls 24h after injection with huddling returning to baseline levels 48h post injection. Dams also spend more time in the chamber with LPS-injected offspring than saline-injected offspring in the social preference test.

We do not see this effect with non-offspring. Our in vivo PVN recordings indicate that a subset of pup-responsive neurons are significantly increased by interactions with LPS-injected pups. These data suggest that dams exhibit increased caregiving behavior and approach toward sick vs. healthy pups which may be mediated by differential PVN activity.

Disclosures: **A. Caslin:** None. **L. Schuster:** None. **K. Quiñones-Laracuente:** None. **G. Kaur:** None. **S. Winokur:** None. **S. Shokat Fadaei:** None. **R.C. Froemke:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.10/HH25

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH R01HD104565
NIH R01HD109519

Title: Hypothalamic mechanisms controlling reproductive behaviors in females

Authors: *S. INOUE, L. GOZALI, R. YANG, H. JEONG, A. TANTRY, T. YANG, J. B. DING, N. M. SHAH;
Stanford Univ., Stanford, CA

Abstract: Behaviors are linked to the internal physiological states. In many species, including humans, females dramatically change their behaviors across the state of ovulation. Abnormal forms of such behavioral changes are linked to women specific symptoms such as premenstrual syndrome. Therefore it is important to understand neural mechanisms underlying behavioral changes in females. Females of many species including mice are sexually receptive exclusively during the estrus, ovulatory phase of the estrous cycle, while they are not receptive during other phases. Sex hormones such as estrogen and progesterone are required for both ovulation and female mating behavior. Although central and peripheral mechanisms of ovulation is well characterized, neural circuit mechanisms underlying the estrus-associated change of the behavior is poorly understood. We have previously shown that progesterone receptor (PR) expressing neurons in the ventromedial hypothalamus (VMH) are essential for female mating behavior via chronic ablation of PR+ VMH neurons. However, whether PR+ VMH neurons play a role in the cyclic change of female mating behavior is unclear. We examined whether PR+ VMH neurons play a role in the estrus-associated change of female mating behavior. We find that PR+ VMH neurons strengthen their functional connections during estrus with the anteroventral periventricular nucleus, depending on estrogen signaling in PR+ VMH neurons. We further find that these projections are essential for female mating behavior during estrus. These findings demonstrate that periodic remodeling in this behaviorally salient connections plays a critical role in associating female mating behavior with an internal physiological state. We further identified a transcriptomically-defined homogeneous population expressing Cholecystokinin A receptor

(Cckar) within the PR+ VMH population. The Cckar+ VMH neurons are the only neurons within the PR+ VMH neurons that regulate female mating behavior. I will present these and recent findings and future directions to discuss neural circuit mechanisms that control behavioral state changes in females.

Disclosures: S. Inoue: None. **L. Gozali:** None. **R. Yang:** None. **H. Jeong:** None. **A. Tantry:** None. **T. Yang:** None. **J.B. Ding:** None. **N.M. Shah:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.11/HH26

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Disrupted oxytocin receptor signaling in the posterior paraventricular thalamus increases anxiety-like and depression-like behaviors but has no effect on the onset of maternal behavior.

Authors: *A. HAUPT[1], S. K. WITCHEY[2], H. K. CALDWELL[2];
[2]Kent State Univ., [1]Kent State Univ., Kent, OH

Abstract: Oxytocin (Oxt) signaling via its receptor, the Oxtr, is important for the onset of maternal care. Previous work from our lab has found that genetic disruption of Oxtr expression in the nucleus accumbens (NAcc) shell results in a robust pup abandonment phenotype. However, it is unclear whether or not this observation is due to the disruption of the Oxtr exclusively in the NAcc shell or if knocking down Oxtr in other brain regions would result in a similar outcome. Thus, we chose to disrupt the expression of Oxtr in the posterior paraventricular thalamus (pPVT), due to its role in modulating stress response and avoidance behaviors, both of which can be involved in a dam's reaction to pups. We hypothesized that that the disruption of Oxtr in the pPVT would not impair the onset of maternal care or maternal behaviors. In order to test this hypothesis, adult female Oxtr flox/flox females were intracranially injected with either AAV2/2CMVCRE-wtIRESeGFP or AAV2/2CMVeGFP (control) in the pPVT. Females were then paired with C57BL/6J males until they were noticeably pregnant and then observed for the onset of maternal care. If maternal behavior was initiated, the latency to retrieve the first pup, latency to retrieve all the pups, time spent off the nest, time spent licking/sniffing, time spent self-grooming, and time spent rearing as well anxiety-like and depression-like behaviors were evaluated. Brains were collected and site checks were performed to verify the accuracy of the viral injections. While dams injected with AAV2/2CMVCRE-wtIRESeGFP displayed increased self-grooming behaviors during the maternal care test, as well as decreased time spent in the center of the open field test, and decreased time spent swimming in the forced swim test, there were no disruptions in the onset of maternal care and maternal care behaviors appeared normal. These results support the assertion that Oxtr expression specifically within the NAcc shell, but not the pPVT, is necessary for the onset of maternal behavior in female mice.

Disclosures: **A. Haupt:** None. **S.K. Witchey:** None. **H.K. Caldwell:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.12/HH27

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: UMass PBS Graduate Student Research Funds
NIH Grant DA055169

Title: Chemogenetic inhibition of medial preoptic area projections to the infralimbic cortex impairs maternal sensitivity in rats

Authors: *K. COPELAS, M. PEREIRA;
Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: Maternal behavior that is sensitive to the needs of the offspring in everyday interactions is essential for the healthy development and well-being in mammals. However, the brain mechanisms that allow the critical maternal ability to dynamically couple caregiving and affective interactions to resolve the needs of their offspring (referred to here as maternal sensitivity) are not well understood. Our prior work shows that the medial preoptic area (mPOA), a critical node of the circuitry regulating maternal behavior, is required for maternal sensitivity. The objective of this study was to determine the role of the mPOA neurons projecting to the infralimbic medial prefrontal cortex (mPOA-to-IL), a critical site involved in cognitive and executive functions necessary for optimal selection of behaviors, in maternal sensitivity. To this aim, we used an intersectional viral strategy to inhibit monosynaptic communication between mPOA and IL neurons during maternal interaction with offspring with varying needs. Primiparous female rats received Cre-dependent hM4Di Designer Receptors Exclusively Activated by Designer Drugs (DREADDs) into the mPOA, combined with retrograde AAV expressing Cre into the IL before mating. Maternal behavior testing occurred on postpartum days 7 and 8, 6-8 weeks after viral infusions. The study used a within-subject design to evaluate mothers interacting with offspring with varying needs following CNO inhibition of mPOA-to-IL projections. Our findings show that chemogenetic inactivation of IL-projecting mPOA neurons disrupts the ability of multiparous mothers to dynamically modify caregiving decisions and time commitment to resolve the needs of their offspring, highly contrasting the attuned behavior of control mothers. Together, this new work expands our understanding of the mPOA contribution to the expression of sensitive caregiving decisions.

Disclosures: **K. Copelas:** None. **M. Pereira:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.13/HH28

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: The Marsden Fund, Royal Society of New Zealand

Title: Fiber photometric analysis of nucleus accumbens serotonin release during the display of maternal behavior in female mice

Authors: M. R. PERKINSON[1], F. DENG[2], Y. LI[2], R. S. E. BROWN[1], ***J. S. LONSTEIN**[3]; [1]Dept. of Physiol., Univ. of Otago, Dunedin, New Zealand; [2]Sch. of Life Sci., Peking Univ., Beijing/Haidian/100871, China; [3]Neurosci. Program, Michigan State Univ., East Lansing, MI

Abstract: Maternal caregiving is a highly motivated behavior regulated by a complex network of brain sites and the integration of many neurochemical signals. Similar to many goal-directed behaviors, the nucleus accumbens (NAc) is required for high maternal motivation to interact with the young. Research on NAc control of maternal care focusses on its dopamine (DA) release and receptor activity, which disinhibits downstream targets to permit the display of caregiving behaviors. We recently found that the onset of caregiving is associated with a temporary peripartum increase in NAc serotonin 5-HT1A receptor expression and binding (Vitale, Ford, Ahearn & Lonstein, under review). Furthermore, we found that selective knockdown of these NAc 5-HT1A receptors disrupted postpartum mothering and affective behaviors (Vitale et al., under review). Because NAc 5-HT modulates local DA release, we here used *in vivo* fiber photometry to begin examining NAc 5-HT release dynamics when females interact with pups. Naïve female virgin C57BL/6J mice received stereotaxic infusion of the 5-HT sensor, AAV2/9-hsyn-5HT3.0 (BrainVTA, China) and implantation of a fiberoptic cannula into the NAc shell ($n=6$). Mice were pseudorandomly exposed to a: 1) palatable food pellet, 2) foster pup and 3) 3-D printed plastic pup for 3-10 min each. Changes in 5-HT-dependent (490 nm) fluorescence were determined with reference to scaled 5-HT-independent fluorescence signal (405 nm) as a proxy for 5-HT release. The first 20 min of recording were used to baseline the data as a z-score. The mean z-score was determined for one min before stimulus exposure and the one min immediately following first contact with the stimuli. Results reveal significant NAc 5-HT release in response to live pups (mean z-score=0.76±1.06 to 5.99±1.22, $p<0.01$; Student's paired t-test) with 5-HT release unexpectedly sustained for the duration of pup contact. This contrasts with our work (Perkinson & Brown, unpublished) showing that DA release in the virgin mouse NAc rises when females contact pups, objects, or food, but rapidly decreases soon after. Also differing from DA release, 5-HT release was exclusively released in response to pup contact and not to other rewarding stimuli. We are now measuring NAc 5-HT release in postpartum mice to determine reproduction state-driven changes. This study provides critical new information about how NAc 5-HT is related to the expression of maternal caregiving. Future studies investigating the temporal dynamics between 5-HT and DA release and receptor signaling in the maternal NAc will be essential to explicate the neurochemical control of caregiving and how it is disrupted in cases of impaired motherhood.

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Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.14/II1

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSERC PGSD3-557532-2021

Title: Plasticity in an amygdala-brainstem circuit facilitates flexible parental behavior

Authors: *C. J. BAIR MARSHALL[1], A. AGHA[2], R. C. FROEMKE[3];
[1]New York Univ., Astoria, NY; [2]NYU Langone, New York Univ., New York, NY; [3]NYU Med., NYU Med., New York, NY

Abstract: Like most mammalian infants, neonatal mice depend on parental care for all aspects of survival and wellbeing including nourishment, protection, thermoregulation, and socialization. However, despite their critical importance, parental behaviors in mice are highly flexible ranging from caregiving to avoidance and even infanticide depending on a range of external and internal factors (Dulac et al.,2014; Mei et al., 2023). In particular, pup-naïve female mice initially avoid pups whereas experienced females will approach, retrieve, and care for pups (Carcea et al., 2021). The transition from avoidance to care represents a dramatic naturalistic example of behavioral flexibility in mice, but the neural circuit mechanisms that underlie this behavioral plasticity are unknown. Because activity in the LC has recently been shown to be important for retrieval behavior in experienced females and CeA inputs to LC have been shown to be aversive in naïve mice (Dvorkin and Shea, 2022, McCall et al., 2015), we hypothesized that CeA-LC activity might contribute to the initial pup aversion responses characteristic of pup-naïve virgin female mice. To test this, we used fiber photometry and found that contact with pups increases activity in CeA-LC neurons in naive virgins but that these responses were blunted after cohousing experience with a maternal mouse and pups. Chemogenetic inhibition of CeA-LC neurons also resulted in increased pup approach and care behavior such as crouching. As we previously showed that oxytocin neuron activity is elevated during cohousing (Carcea et al., 2021), we then infused oxytocin antagonist into CeA which resulted in reduced learning of pup care behaviors compared to controls. Then using *in vitro* whole cell recordings, we found that oxytocin significantly reduced excitatory input strength in CeA, stimulation of CeA increased spontaneous firing in LC, which was blocked by corticotropin releasing factor antagonism. Taken together, our results suggest a model in which oxytocin released during cohousing reduces pup-related responses in CeA-LC neurons through synaptic depression of excitatory inputs, resulting in lowered CRH release in LC and reduced pup avoidance. Given the role of this projection in processes whose dysregulation can cause severe disruptions to maternal behaviors including anxiety and depression, understanding the mechanisms of plasticity for flexible

maternal behavior in this circuit could have significant clinical implications for the treatment of disordered maternal behaviors

Disclosures: C.J. Bair Marshall: None. **A. Agha:** None. **R.C. Froemke:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.15/II2

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Oxytocin signaling regulates maternally directed behavior during early life

Authors: *D. ZELMANOFF, M. KAUFMAN, J. DINE, A. LITVIN, J. WIETEK, O. YIZHAR; Dept. of Brain Sci., Weizmann Inst. of Sci., Rehovot, Israel

Abstract: Oxytocin (OT) has been shown to play an essential role in the regulation of various social behaviors, and serves as a potential biomarker and treatment in neurodevelopmental disorders, such as autism. While the role of OT signaling in maternal care is widely investigated, little is known about its involvement in social behavior during early life. In mice, the expression of the OT receptor (OTR) peaks during the second and third weeks of life, suggesting that OT signaling is important at this early stage, in which many neural circuits are undergoing maturation and refinement. We set out to explore the role of OT in the mother-infant interaction, a major generator of early-life sensory-social experience in humans and non-human mammals. Our results show that the activity of paraventricular OT neurons was strongly increased by a 3-hour period of maternal separation (MS), and returned to baseline after reunion with the dam and littermates. Behaviorally, we found that acute MS increased the emission of USVs and maternally directed behavior upon social reunion. These effects were attenuated by applying an OTR antagonist during MS, suggesting that OT release during MS is important for this behavior. To investigate the role of OT release with higher spatial and temporal precision, we established an optogenetic protocol for noninvasive transcranial photoinhibition in freely behaving pups using eOPN3, a potent and highly light-sensitive opsin. Using this approach, we found that optogenetic silencing of OT neurons during MS alters USV emission patterns during separation and disrupts the correlation between vocal behavior during separation and reunion. In summary, our findings reveal an important role of OT in experience-dependent social behavior in pups, opening exciting opportunities for mechanistic understanding of neural circuits in the early postnatal period.

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Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.16/II3

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Swiss National Funding (310030_212193)
NARSAD YI 27089

Title: A neural substrate for negative affect dictates female parental behavior

Authors: *S. LECCA[1], M. CONGIU[1], L. ROYON[1], L. RESTIVO[1], N. MAZARE[1], B. GIRARD[2], C. BELLONE[2], L. TELLEY[1], M. MAMELI[1];
[1]Fundamental Neurosci., Univ. of Lausanne, Lausanne, Switzerland; [2]Univ. of Geneva, Univ. of Geneva, Geneve, Switzerland

Abstract: Parental behaviours are essential for the survival of newborns. Such behaviours require an important motivational investment. The latter can be also driven by the will to limit negative affective states, which can emerge in the caregiver when coping with neonatal distress. However, whether neural circuits that process negative affect orchestrate components of parental behaviours remains unknown. The lateral habenula (LHb), a negative affect center, is excited by aversive stimuli and guides actions directed to avoid negative outcomes. Notably, lesioning the LHb impairs parental behaviours, suggesting that a yet unidentified function of the LHb may be to shape actions to end negative emotions individuals experience during interactions with a distress newborn. Here, to test this possibility, we employed a series of different approaches, including calcium imaging, optogenetic-based viral manipulations, trans-synaptic tagging and single cell transcriptomic of defined neuronal populations during parental behaviours in virgin female mice. Virgin females retrieve distress pups in the nest. Such action is efficient to reduce distress calls in pups. The latter alone are capable to induce a real time place aversion (RTPA) in virgin females. Accordingly, LHb neurons increase their activity upon pup distress calls. Moreover, LHb activity also increased during pup retrieval. LHb neuronal activity is required for both pup retrieval events and distress-calls-mediated aversion in virgin female mice, as bilateral LHb inhibition suppressed these behaviours. Intriguing, excitation upon pup retrieval was limited to a subset of LHb neurons, receiving input from the bed nucleus of the stria terminalis (^{BNST}LHb). Intersectional cell identification and transcriptional profiling of ^{BNST}LHb neurons associates this neuronal population to parental behaviours and outlines gene expression in female virgins that is similar to mothers but divergent from non-parental virgin males. From a functional standpoint, optogenetic activation and inactivation of the ^{BNST}LHb pathway maximizes and suppress, respectively, pup retrieval. Furthermore, the selective activation of ^{BNST}LHb cells led to a RTPA. Finally, tracking of single ^{BNST}LHb cell activity demonstrates specificity of this neuronal subset for encoding negative stimuli and pup retrieval. Altogether, our data suggest that ^{BNST}LHb neurons are sufficient to drive aversive behaviours and are unique to signal parental information. A neural circuit mechanism thus emerges that potentially bridges newborn distress signal with precise parental actions.

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Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.17/II4

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant IOS 2118607

Title: Effects of experience with pups on perineuronal net expression in the medial prefrontal cortex and medial amygdala of adult male and female California mice (Peromyscus californicus).

Authors: *M. C. ACOSTA[1], K. A. RAZAK[2], W. SALTZMAN[1];
[1]Evolution, Ecology, and Organismal Biol., [2]Psychology, Univ. of California, Riverside, Riverside, CA

Abstract: In biparental species, in which both parents care for their offspring, repeated exposure to infants can result in the induction of parental behavior in virgin males and females. Little is known, however, about the mechanisms underlying neural plasticity resulting from experience with infants. This study examined the impact of pup exposure on perineuronal net (PNN) expression in the medial prefrontal cortex (mPFC) and medial amygdala (MeA), brain regions implicated in the control of parental caregiving behavior, in male and female California mice (*Peromyscus californicus*), a biparental rodent. PNNs are thought to be one of the key structural mechanisms that act to restrict neuroplasticity. We tested the hypothesis that parents will show a greater abundance and labeling intensity of PNNs in response to pup stimuli compared to virgins exposed to pups for the first time. We stained sections with the PNN marker *Wisteria floribunda agglutinin* to examine PNN expression in adult virgin California mice exposed to pups 1, 2, or 3 times, as well as new parents. Control groups were similarly tested with a novel pup-sized object. Pup exposure altered PNN density, but not labeling intensity, in the mPFC and MeA of male mice. Virgin males exposed to pups twice exhibited reduced PNN density in the mPFC compared to virgin males that were exposed to pups only once. Moreover, pup-exposed fathers demonstrated a reduction in PNN density in the MeA compared to virgin males exposed to pups for the first time. In contrast, pup exposure did not alter PNN density or intensity in the mPFC or MeA of females. Additionally, PNN density in the mPFC was higher in males than in females. Our results suggest that dynamic changes in PNN expression in the mPFC and MeA occur with repeated exposure to pup stimuli in male California mice.

Disclosures: **M.C. Acosta:** None. **K.A. Razak:** None. **W. Saltzman:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.18/II5

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: the Environment Research and Technology Development Fund (S-17 1-3) of the Environmental Restoration and Conservation Agency of Japan

Title: Exposure to tris (1,3-dichloro-2-propyl) phosphate (TDCIPP) at periods of juvenile and adult suppresses sexual behavior, but not neonatal and adult in male rats

Authors: *S. IWATA[1], F. KATO[2], K. SATO[1], T. HATAKEYAMA[1,3], G. WATANABE[1,3], R. OHTA[4], R. YANAGISAWA[5], E. KOIKE[5], N. SUZUKI[6], M. KAWAGUCHI[1,2];
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Abstract: There is increasing concern about people's lifetime exposure to multiple high concentrations of Endocrine Disrupting Chemicals (EDCs) due to large-scale disasters. We found that two-hit exposure to TDCIPP during neonatal and adult stages results in greater adrenal toxicity compared to a one-hit exposure during the adult stage. However, since it is also known that TDCIPP has anti-androgenic effects, exposure to TDCIPP during the neonatal and juvenile stages are likely to also affect reproductive function due to the importance of sex hormones during these development periods. Therefore, in this study, we examined the effects on reproductive function of exposure to TDCIPP during either the neonatal or juvenile stage, followed by a second exposure during the adult stage. In Experiment 1, Wistar-Imamichi rats were exposed to TDCIPP (or oil control) during either the neonatal period (Postnatal Day: PD1-7), the adult period (PD101-107), or both. In Experiment 2, similar Wistar-Imamichi rats were exposed to TDCIPP (or oil control) during either the juvenile period (PD42-48), the adult period (PD142-148) or both. We exposed to TDCIPP 250 mg/kg/day for neonatal and juvenile period and TDCIPP 650 mg/kg/day for adult period. All exposures conducted by forced oral gavage. All test animals were allowed to experience sexual behavior prior to the adult exposure, and a sexual behavior test was conducted on the last day of exposure. Animals in Experiment 2 were further measured for organs weights such as liver, spleen, kidney, adrenal glands, testis, epididymis, and then sperm motility by using CASA system on 33 days after the last exposure. In Experiment 1, the group with only TDCIPP exposure to the adult period suppressed a part of sexual behaviors. However, the group with TDCIPP exposure to both the neonatal and adult periods did not show any significant differences compared to the control group. In Experiment 2, the group with TDCIPP exposure to both the juvenile and adult periods showed a complete loss of sexual behavior. In particular, there was a significant reduction in mounting compared to the control group. On the other hand, no effects were observed for organ weights or sperm motility. These results show that the timing of the first exposure to TDCIPP affected sexual behavior. Since mounting is considered to be indicator of sexual motivation, it is possible that the anti-

androgenic activity of TDCIPP manifested in the hypothalamus prominently, which is the central region for sexual behavior. These results indicate that the anti-androgenic activity of TDCIPP may have a time-specific effects on central functions such as sexual behavior.

Disclosures: **S. Iwata:** None. **F. Kato:** None. **K. Sato:** None. **T. Hatakeyama:** None. **G. Watanabe:** None. **R. Ohta:** None. **R. Yanagisawa:** None. **E. Koike:** None. **N. Suzuki:** None. **M. Kawaguchi:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.19/II6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIMH Intramural Research Program

Title: Behavioral and neuroimaging analysis of the marmoset maternal brain

Authors: *M. KRISHNAMURTHY[1,2], K. M. COLE[7], T. M. DRAZAN[2], P. A. TAYLOR[3], S. P. BRADLEY[4], K. F. BERMAN[5], P. J. SCHMIDT[6], Y. CHUDASAMA[2,4];
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Abstract: Pregnancy and the postpartum are accompanied by rapid fluctuations in endocrine signaling which can render substantial structural and functional changes to the maternal brain. Neuroimaging studies of the human brain have shown changes in gray matter volume and functional coherence during the postpartum period. These changes have implications for maternal behavior in the postpartum, but little is known about their timing and localization. Here we examined the potential relationship between brain functional coherence, hormonal profile, and maternal behavior across pregnancy and the postpartum in the common marmoset (*Callithrix jacchus*). We studied nulliparous females who became pregnant as well as non-pregnant controls at four timepoints: pre-pregnancy baseline, gestation 1 (50-70 days), gestation 2 (105-125 days), and postpartum (3-14 days). Structural (T1-weighted and DTI) and functional (awake resting state) MRI scans were collected on a 7.4T Bruker scanner, and imaging data were analyzed using AFNI (Analysis of Functional NeuroImages). Blood samples were collected for assay of estradiol, progesterone, cortisol, dehydroepiandrosterone, luteinizing hormone, and allopregnanolone levels. A custom designed T-shaped maze was used to assess infant-directed behavior by tracking the female marmosets' movement and gaze towards two infants, situated at either end of the T-maze. At present, five subjects (2 pregnant; 3 control) have completed datasets spanning the four timepoints and five subjects are undergoing data collection. Our first analysis of the current sample examines functional coherence within the default mode network

(DMN) since human imaging studies have reported increased DMN temporal coherence following pregnancy. These preliminary data reveal differences in temporal coherence between pregnant and control marmosets and indicate variations in the localization of these differences across pregnancy and the postpartum. In addition, the related behavioral data show decreased infant-directed behaviors among pregnant females during late gestation, followed by an increase during the postpartum period. These observations support previous findings of infant avoidance in nulliparous females prior to parturition and increased infant responsiveness during the postpartum. Ongoing analyses aim to explore changes to white matter microstructure and functional coherence in brain areas associated with social interactive behaviors.

Disclosures: M. Krishnamurthy: None. **K.M. Cole:** None. **T.M. Drazan:** None. **P.A. Taylor:** None. **S.P. Bradley:** None. **K.F. Berman:** None. **P.J. Schmidt:** None. **Y. Chudasama:** None.

Poster

PSTR352. Parental Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR352.20/II7

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NSF Grant 2032610

Title: Visualizing putative membrane androgen binding in goldfish brains

Authors: *V. C. ROSHKO[1], C. HELSENS[2], S. POTLURI[2], Y. DAVIS[2], R. THOMPSON[3]; [1]Emory Univ. Neurosci. and Animal Behavior, Atlanta, GA; [3]Neurosci. and Behavioral Biol., [2]Oxford Col. of Emory Univ., Oxford, GA

Abstract: Steroid hormones are most commonly known to induce long-term changes in behavior and brain function through genomic mediation. Recently, however, steroid hormones have been shown to induce rapid behavior changes, which would occur via non-genomic signaling. Neuronal membrane estrogen receptors have been well characterized, but, despite evidence supporting rapid behavior changes mediated by androgen hormones, neuronal membrane androgen receptors have not been as extensively described, especially within the brain. Here, we investigated whether we could identify membrane androgen binding, presumably to membrane androgen receptors, in goldfish brains. Ex-vivo male and female goldfish brains were coronally cut at the cerebellum and incubated with testosterone (T) conjugated to bovine serum albumen and fluorescein (T-BSA-FITC) or, as a control, BSA-FITC. Brains were then sectioned and visualized with fluorescence microscopy. Membrane binding appeared as rings surrounding cell nuclei in the ventral, lateral, and dorsal telencephalon, as well as lateral hypothalamus, and in the olfactory bulbs. We are currently working to verify the specificity of the signal by processing the brains incubated with the BSA-FITC, as well as by looking at brains co-incubated with T-BSA-FITC and excess unlabeled T. Our data thus provide preliminary support for androgen membrane

binding in goldfish brains, which could be a mechanism for rapid, androgen-induced behavior changes in this species.

Disclosures: **V.C. Roshko:** None. **C. Helsens:** None. **S. Potluri:** None. **Y. Davis:** None. **R. Thompson:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.01/II8

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Automating behavioural analysis in rodent sexual behaviour for neuroscience studies

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Abstract: Automating the analysis of behavior for neuroscience studies has been made possible by recent advances in machine learning. While this approach has been successful in studying sexual behavior in certain models like Caenorhabditis elegans and Drosophila, developing reliable machine learning models for rodent sexual behavior remains a challenge. In our study, we utilized video datasets from our research on steroid-independent male sexual behavior in B6D2F1 hybrid male mice. We employed DeepLabCut, a toolbox for pose estimation, to extract video frames and label 30 body parts for each mouse. These labeled frames were then used to train a model. For each video, nine behavioral tests were recorded simultaneously in the dark under red light, resulting in very low-resolution footage. This is not ideal for DeepLabCut, as distinguishing separate body parts reliably requires higher resolution. To overcome this limitation, we used high-resolution videos for model training and augmented the training data intentionally to be lower resolution. In this way, the model can be trained with accurate location of body parts, but on augmented videos with lower resolution. Additionally, we incorporated actual low-resolution videos labeled with only a few easily recognizable body parts as ground truth that will be used to optimize the model. By comparing the pixel errors between the machine-labeled poses and the ground truth, we can refine the model until it is more optimal. Once the videos are analyzed using the optimized DeepLabCut model, the poses will be inputted into Simple Behavioral Analysis (SimBA) and annotated for male sexual behavior to train behavioral classifiers. To optimize these classifiers, we will manually annotate the male sexual behaviors in the videos, which will serve as the ground truth for comparison when the classifiers are applied to the videos. The confusion matrix, which will compare the machine-annotated results with the ground truth, will be used as a benchmark to further improve the classifiers. This automation of sexual behavior analysis would expedite neuroscience studies to understand how the brain is involved in coordinating various aspects of sexual behavior.

Disclosures: **V. Chiang:** None. **J. Park:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.02/II9

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant RO1MH112593
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NIH Grant RO1NS123916
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U19 MG114830

Title: Love and War: Control of female social behaviors by hypothalamus

Authors: *M. LIU[1], A. NAIR[1], D.-W. KIM[2], H. ZENG[2], S. LINDERMAN[3], D. ANDERSON[1]; [1]Caltech, Pasadena, CA; [2]Allen Inst. for Brain Sci., Seattle, WA; [3]Stanford Univ., Menlo Park, CA.

Abstract: Female mice display contrasting social behaviors towards males based on their reproductive state, with virgins exhibiting sexual receptivity and lactating mothers displaying aggression. Furthermore, mating virgins show sexual acceptance during proestrus but reject advances during non-proestrus. The mechanisms underlying this qualitative switch in behavioral responses remain elusive. In this study, we employed single cell RNA sequencing and identified two distinct subtypes, alpha and beta, within the ventrolateral subdivision of ventromedial hypothalamus (VMHvl). We established that these subtypes causally control mating and aggression in females, respectively. Utilizing within-subject bulk calcium recordings, we found that beta cells exhibit heightened responsiveness to social cues during the transition from virginity to maternity, leading to the observed behavioral shift. Furthermore, longitudinal single-cell calcium imaging of alpha cells across the estrus cycle revealed the formation of approximate line attractor-like dynamics during proestrus. These dynamics were found to be modulated by the estrus state, disappearing during non-proestrus and reappearing upon re-entry into proestrus. Collectively, our findings indicate that distinct transcriptomic cell types within the VMHvl employ different strategies to regulate reproductive state-dependent social behaviors in female mice.

Disclosures: **M. Liu:** None. **A. Nair:** None. **D. Kim:** None. **H. Zeng:** None. **D. Anderson:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.03/II10

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: National Natural Science Foundation of China32022029

Title: The function of brain-heart axis during innate courtship behavior

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Abstract: The emergence of interoceptive representation and emotional states are long debated with diverse theories from a century ago. In animals, emotional states strongly influence bodily physiology, often referred to internal states, which support specific adaptive needs across taxa. During innate behaviors, animals exhibit optimized bodily physiology to fulfill essential requirements such as obtaining food, mating, and ensuring safety. Recent studies have probed the causal relationship between bodily feedback and central perception of emotional states. Yet, the emergence of emotional internal states in less developed animals is unprecedentedly deciphered. Here with genetically manipulated Drosophila melanogaster, by modified optical recording assay, we observed the dynamically regulated cardiac activity of male flies during courtship behavior, revealing the top-down innervation of bodily physiology from the nervous system during specific internal states. Additionally, we demonstrated that the perception-induced early death could be rescued by either blocking the elevation of cardiac activity or activating ejaculation-related neurons, highlighting the central role of cardiac activity in arbitrating the inverse demands between courtship and homeostasis.Furthermore, with a linear discriminative algorithm, we were able to distinguish the categories of cardiac activity in various internal mating states of flies, including virgin, mated, and unsuccessfully mated individuals. These findings provide evidence for the existence of the brain-heart axis coordinating internal brain states. Overall, our results suggest that even in the fruit fly, the brain-heart axis coordinates neuron activity, neuropeptide signaling, and cardiac activity to orchestrate transient challenges and long-term metabolic processes during innate courtship behavior. These findings, together with similar observations in rodents, support the notion that the evolutionary conservation of the sensation of being "flipped" is deeply rooted in the reproductive processes across species.

Disclosures: H. Li: None. **W. Zhang:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.04/II11

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: JSPS KAKENHI Grant Number 21J22555
JSPS KAKENHI Grant Number 19H05006
AMED under Grant Number JP21zf0127005

Title: Dopaminergic Signatures of male sexual behaviors in mice

Authors: *A. MIYASAKA[1,2], T. KANDA[1,4], N. NONAKA[2], Y. TERAKOSHI[1], Y. CHERASSE[1], Y. ISHIKAWA[1], Y. LI[5], H. TAKIZAWA[6], M. YANAGISAWA[1], J. SEITA[3], K. SAKURAI[1], T. SAKURAI[1], Q. LIU[1,7,8];
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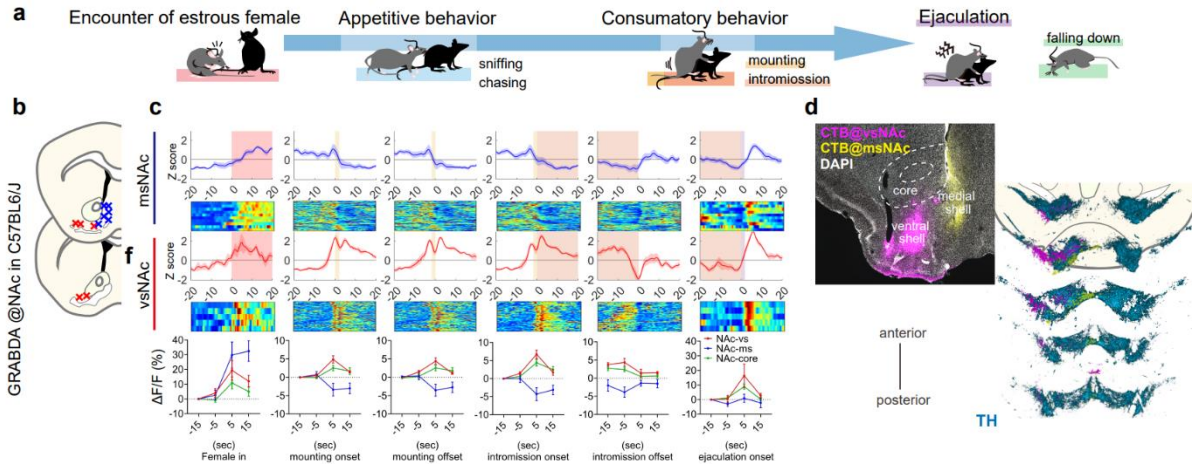
Abstract: Sexual behavior is crucial to reproductive success and is widely observed across various species. Male rodents display a stereotypical sequence of mating behaviors, which includes mounting, intromission, and ejaculation (Fig. a). Dopamine (DA) has been proposed as a key player in the regulation of mating behaviors. Nevertheless, the precise mechanisms and locations through which DA signaling governs male mating behaviors remain unclear. We hypothesized that DA signaling in the nucleus accumbens (NAc) modulates specific aspects of sexual behavior in male mice. Due to the anatomical heterogeneity of the NAc, it is imperative to accurately monitor DA release with high spatiotemporal resolution. In this study, we utilized fiber photometry to measure the DA dynamics with GRAB-DA sensor in NAc. Based on our GRAB-DA recording, we identified several subregions within the NAc (Fig. b). Specifically, the ventral shell region (vsNAc) exhibits increased levels of DA, while the medial shell region (msNAc) shows decreased DA levels during intromission phase (Fig. c). To determine the inputs to these NAc subregions, we employed a two-color cholera toxin B subunit (CTB)-mediated retrograde labeling. We found that the vsNAc receives input from the antero-lateral part of ventral tegmental area (alVTA), while the msNAc receives input from the postero-medial part of VTA (pmVTA) (Fig. d). To investigate the neuronal activity in alVTA and pmVTA, we recorded the intracellular calcium ion levels using GCaMP6s. We observed that the calcium levels recorded from alVTA exhibited an increase, whereas those from pmVTA showed a decrease during the intromission phase. Thus, the neuronal activity in the VTA subregions projecting to the specific subregions of the NAc corresponded to the DA release pattern. Through optogenetic manipulation of the dopaminergic terminals in the NAc, we discovered that the behavioral manifestation of sexual behavior in male mice was altered. These findings unequivocally showed that DA signaling within the NAc plays a pivotal role in the regulation of male sexual behavior in mice.



Disclosures: **A. Miyasaka:** None. **T. Kanda:** None. **N. Nonaka:** None. **Y. Terakoshi:** None. **Y. Cherasse:** None. **Y. Ishikawa:** None. **Y. Li:** None. **H. Takizawa:** None. **M. Yanagisawa:** None. **J. Seita:** None. **K. Sakurai:** None. **T. Sakurai:** None. **Q. Liu:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.05/II12

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: UVU Scholarly Activities Grant to KA
NSF S-STEM Grant #1833880

Title: Proopiomelanocortin deficiency and its effect on sexual behavior in mice

Authors: *K. ARGYLE[1], Z. THOMPSON[2];
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Abstract: Proopiomelanocortin (Pomc) is a gene expressed primarily in the arcuate nucleus (ARC) of the hypothalamus. The products of this gene include melanocyte stimulating hormone, adrenocorticotropic hormone (ACTH), and beta-endorphin. Alpha-MSH is involved in both sexual behavior and appetite regulation. ACTH is a peptide hormone that plays a role in glucocorticoid secretion from the adrenal cortex, and beta-endorphin is an opioid peptide that is closely linked to pain management and reward signaling. This makes Pomc a powerful influence on overall health, particularly in relation to body weight and fertility. Mutations in the Pomc gene result in significant deficiency of Pomc expression. In humans, this translates to extreme hyperphagia, early onset (and extreme) obesity, hypocortisolism, light skin, and red hair

pigmentation. It also seems to affect pubertal development. Several of these effects are also apparent in a mouse model of Pomc-deficiency. We are in the process of using this mouse model to help us determine the cause for the observed infertility experienced by Pomc-deficient mice, and potentially Pomc-deficient individuals as well. We plan to specifically evaluate the differences between wild-type (control) mice and affected POMC-deficient mice in precursor sexual behavior, libido, adherence to copulatory norms, and ultrasonic communication. We are capturing both video and audio recording of the sexual behavior interactions between Pomc-deficient male mice with wildtype female mice, as well as with Pomc-deficient female mice with wildtype male mice. We will compare these recordings to the interactions observed between male and female wildtype mice. These results will help us to understand whether Pomc-deficient exhibit normal sexual behavior, and how that may affect their reproductive success. This will also provide context for the relationship between Pomc expression and overall reproductive function that may exist in humans as well.

Disclosures: K. Argyle: None. **Z. Thompson:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.06/II13

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH grant R01 HD100007-01

Title: Efficacy of Bremelanotide (Vyleesi) and melanocortin 4 receptors in the nucleus accumbens to enhance sexual motivation in female Syrian hamsters

Authors: *J. M. BORLAND[1], A. L. KOHUT-JACKSON[1], A. C. PEYLA[1], M. A. L. HALL[1], P. G. MERMELSTEIN[1], R. L. MEISEL[2];
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Abstract: Characterized by diminished interest and initiation in sex, and a loss of pleasure during sex; diagnosis of sexual desire disorder among women are not only poorly understood from a psychological perspective, but also in terms of their underlying neurobiology. This led to approval of the drug Bremelanotide, trade name Vyleesi, to treat hyposexual desire disorder in women. However, very few clinical trials have been performed and almost nothing is known about its potential mechanism of action. Bremelanotide is a melanocrotin 4 receptor (MC4R) agonist; the melanocortin system is involved in the regulation of energy homeostasis and satiety. Thus, this study investigated the role of Bremelanotide on sexual reward in female hamsters and the role of MC4R in the striatum. Female hamsters experienced five, two or zero 10-min sexual interactions (paired with an adult male) in the conditioned place preference (CPP) arena. Female hamsters that experienced two 10-min interactions were also subdivided into groups that received saline, 50 ug/kg or 200 ug/kg Bremelanotide 30-min prior to sexual experiences. Two

or five days of sexual experience resulted in an increase in social preference. However, neither a low (50ug) nor a high (200ug) dose of Bremelanotide enhanced sexual preference. Following assessment of sexual motivation, brains were collected for assessment of mRNA expression of dopamine 1 and 2 receptor and MC4R in the striatum using Syrian hamster customized RNAscope probes. Collectively, these studies support the clinical ineffectiveness of Bremelanotide to enhance sexual motivation in women and for MC4R expression in the nucleus accumbens for sexual reward in females. NIH grant R01 HD100007-01.

Disclosures: J.M. Borland: None. **A.L. Kohut-Jackson:** None. **A.C. Peyla:** None. **M.A.L. Hall:** None. **P.G. Mermelstein:** None. **R.L. Meisel:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.07/II14

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Temporal copulatory patterns of female rat sexual behavior

Authors: *J. C. OYEM[1], R. HEIJKOOP[2], E. M. SNOEREN[2];
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Abstract: Temporal copulatory patterns of female rat sexual behaviorAuthors*John C Oyem, Roy Heijkoop, Eelke MS Snoeren Department of Psychology, UiT The Arctic University of Norway
DisclosuresJohn C Oyem: None. Roy Heijkoop: None. Eelke MS Snoeren: None.
AbstractFemale rat sexual behavior is a rewarding behavior typically studied in tests that focus on copulation behaviors. Often, analysis of female copulation parameters is limited to the number of paracopulatory behavior (hops and darts) as an index of female sexual motivation and lordosis responses as an indicator of the female's receptivity. Additional common behavioral outcomes are contact return latencies and percentage of exits, used to reflect the female's motivation and motivation to continue copulation. To an extent, this is based on the reductionist approach of analyzing one behavioral element. A deeper understanding of intricate behavior can be used to fully unravel the neurobiological mechanisms orchestrating behavior. In male rats, we developed a new behavioral assessment method that also includes mount bout patterns. This provided a deeper insight into the microstructural organization of temporal copulation patterns of behavior. Nonetheless, such an extended behavioral assessment method does not exist for female rats yet. Therefore, in this study, we aim to investigate in detail the temporal copulatory patterns of female rat sexual behavior. For this project, we will use low receptive (sub-primed) and normal sexually receptive (fully primed) females and test them in both paced and non-paced mating set-ups to characterize copulatory behavior in female rats. Moreover, we will explore how sexual experience affects these copulation patterns. A female copulatory bout will be

defined as a series of behavior starting and ending with either paracopulatory behavior or lordosis, with the so-called time-out as the interval between bouts. Analysis of these copulatory bouts, combined with the already existing parameters, will provide more insight into copulatory pacing in females as well as the micropatterns of their copulatory behavior. In sum, the goal of our experiment is to develop a behavioral assessment tool to get a deeper understanding of the temporal patterning of female sexual behavior while exploring the effects of different copulation conditions on these temporal copulation patterns in female rats.

Disclosures: **J.C. Oyem:** None. **R. Heijkoop:** None. **E.M. Snoeren:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.08/Web Only

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: CONACYT, CVU: 1006947

Title: The nitric oxide pathway participates in lordosis behavior induced by intrahypothalamic administration of apelin-13 in estradiol benzoate-primed rats

Authors: *A. LUNA HERNÁNDEZ[1], M. GARCIA-JUÁREZ, SR[2], O. GONZÁLEZ-FLORES[3];
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Abstract: Female sexual behavior is induced by several compounds in ovariectomized (ovx) estradiol benzoate (EB)-primed rats. The intrahypothalamic administration of apelin-13 facilitates lordosis behavior in this model; however, the cellular mechanism involved in that regulation is unknown. Thus, in this work, we explored the participation of the nitric oxide (NO) pathway in facilitating lordosis behavior induced by the intrahypothalamic administration of apelin-13 in ovx EB-primed rats. Fifty-six adult female Sprague-Dawley rats (250 g) were ovx, and one week later, they received bilateral implants directly into the ventromedial hypothalamus (VMH). All rats were primed with 5 µg of EB injected subcutaneously, and 39.5 hours later, they were bilaterally infused with 500 µg of the nitric oxide synthase inhibitor (L-NAME) or 22 µg of the soluble guanylyl cyclase inhibitor (ODQ) and with 0.12 µg of the protein kinase G inhibitor (KT5823) in the VMH, 30 minutes before the 0.75 µg apelin-13 infusion. The positive control group received 0.75 µg of apelin-13, and the negative control groups received an infusion of saline or 10% DMSO into the VMH. Lordosis behavior was tested at 30, 120, and 240 minutes after apelin-13 administration. The lordosis behavior induced by apelin-13 was significantly reduced by the previous infusion of L-NAME at 120 minutes ($p \leq 0.01$) and KT5823 at 30 ($p \leq 0.001$), 120 ($p \leq 0.001$), and 240 ($p \leq 0.001$) minutes. However, the VMH infusion of ODQ before apelin-13 infusion did not significantly reduce the lordosis behavior induced by apelin-13.

In conclusion, the NO pathway participates in facilitating lordosis behavior induced by apelin-13 in EB-primed rats.

Disclosures: A. Luna Hernández: None. **M. Garcia-Juárez:** None. **O. González-Flores:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.09/II15

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Neurobiological Mechanisms of Socially Influenced Sex Change in A. ocellaris

Authors: *G. J. GRAHAM[1], Z. V. JOHNSON[5], E. IBANEZ[2], C. G. PARKER[8], M. G. CONNOLLY[9], J. T. STREELMAN[6], E. PANCZYK[1], B. E. HEGARTY[7], M. MATHAN[1], K. N. LEATHERBURY[7], G. W. GRUENHAGEN[6], M. L. SHOTWELLL[3], J. S. RHODES[4]; [1]Psychology, [2]Mol. and Cell. Biol., [3]Vet. Med., Univ. of Illinois at Urbana-Champaign, Champaign, IL; [4]Psychology, Univ. of Illinois at Urbana-Champaign, Urbana, IL; [6]Biol. Sci., [7]Biol., [5]Georgia Inst. of Technol., Atlanta, GA; [8]Univ. of Maryland, Maryland City, MD; [9]Neurosci. Program, Univ. of Illinois Urbana-Champaign, Champaign, IL

Abstract: The false clown anemonefish *Amphiprion ocellaris* changes sex from male to female as a natural part of its life cycle. Sex change begins in the brain when a male fish senses it is in the alpha position in the dominance hierarchy. Ultimately, the brain, behavior, gonads, and sex hormones transform from male to female, however little is known about how this is accomplished. Previous work in our lab using single nucleus RNA- sequencing (snRNA-seq) identified substantial sex differences in cell composition and transcriptome of the telencephalon and preoptic area (POA), including more than double the number of specific glutamate and neuropeptide neuron types in females than males and hundreds of differentially expressed genes in other discrete cell populations. While this indicates the telencephalon and POA must reorganize at some point during sex change, the timeline relative to gonadal and hormonal transformation is unknown. Neurobiological changes that occur prior to gonadal feminization could facilitate gonadal transformation while changes that occur after could result from altered sex steroid hormones released from the new ovaries. Further, some of the sex differences in the brain we observed could underlie sex differences in behavior and others differential control of the gonads. The goal of this study was to determine the temporal relation between cellular and transcriptional changes in the brain, expression of sexually dimorphic behaviors, gonadal composition, and plasma sex hormone levels. We paired 12 males together and waited 6 months when sex change was completed in 25% of the sex-changing fish with the remainder in intermediate stages. We measured alloparental care (males are the primary caregivers of the eggs), took blood samples, and isolated the POA for multiome analysis (i.e., snRNA-seq and chromatin accessibility). We also included 6 male-female reproductive pairs as controls. We are

currently processing the multiome data. Preliminary results for the behavior, gonadal, and sex hormone measurements suggest behavioral sex change occurs after gonads and sex hormones switch to the female phenotype. We observed a large variation in the gonadal composition and plasma steroid hormone concentrations in fish at various stages of sex change. This individual variability will be useful for correlating stages of sex change with the cellular, transcriptional, and epigenetic changes in the brain identified from the multiome data. Results from this study will provide unprecedented insight into the neurobiological, transcriptomic, and epigenetic changes responsible for orchestrating sex change in *A. ocellaris.*

Disclosures: G.J. Graham: None. **Z.V. Johnson:** None. **E. Ibanez:** None. **C.G. Parker:** None. **M.G. Connolly:** None. **J.T. Streelman:** None. **E. Panczyk:** None. **B.E. Hegarty:** None. **M. Mathan:** None. **K.N. Leatherbury:** None. **G.W. Gruenhagen:** None. **M.L. Shotwelll:** None. **J.S. Rhodes:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.10/II16

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R35GM148349

Title: Neurogenetic Mechanisms Underlying Sexually Dimorphic Behavioral States in C. elegans

Authors: *G. REILLY[1], C. BAINBRIDGE[2], J. WANG[4], D. S. PORTMAN[3]; [2]Biomed. Genet., [3]Dept. of Biomed. Genet., [1]Univ. of Rochester, Rochester, NY; [4]Univ. of Rochester Sch. of Med. and Dent., Rochester, NY

Abstract: Biological sex is a fundamental dimension of internal state that can have deep influences on behavior. Understanding the mechanisms behind these influences can provide insight into how shared neural circuits are tuned to produce sex-specific behavioral variation. Biological sex can influence both short-term behaviors and longer, more persistent forms of behavior known as behavioral states. In *C. elegans*, persistent motor behavior, called locomotor states, is well-studied in hermaphrodites. On a patch of food, hermaphrodites will switch between two states of foraging and feeding, called roaming and dwelling respectively. However, while some work has examined motor states in males, these remain poorly characterized. Previous work from our lab has demonstrated that male locomotion is sex-specific- the sexual state of muscle tissue and the nervous system is essential for sex differences in speed and body posture. Therefore, biological sex may also similarly influence locomotor states. We trained a supervised machine learning Random Forest model to detect three locomotor states: roaming, dwelling, and tail chase. Furthermore, to measure the transition probability between states, we used a Markov model. While both males and hermaphrodites share the locomotor states of

roaming and dwelling, the characteristics of these differ by sex- the amount of time spent in each state and the transition probabilities between states display sexual dimorphisms. To understand how sex tunes these locomotor states, we manipulated the sex determination pathway to sex reverse the nervous system in both males and hermaphrodites. Interestingly, we found that pan-neuronally feminized males had similar locomotor state characteristics to hermaphrodites; the sex difference in the proportion of time, average state duration, and average speed/curvature of each locomotor state was eliminated in the feminized males. Furthermore, this difference persisted when a smaller subset of neurons, the amphid sensory neurons, were feminized, indicating that the sexual state of these neurons is sufficient for the sexual dimorphism seen in locomotor states. To uncover the mechanisms that biological sex leverages to achieve this sex-specific variance in locomotor states, we are testing mutants for neuromodulatory pathways. Preliminary data suggest that sex differences in the PDFR-1 signaling pathway contribute to sexual dimorphism in locomotor states. Together, our results provide a mechanistic framework for understanding how sex-specific neuronal tuning influences behavioral states.

Disclosures: **G. Reilly:** None. **C. Bainbridge:** None. **J. Wang:** None. **D.S. Portman:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.11/II17

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Investigating Mechanisms Underlying Estrous Cycle-Dependent Changes in Cue-Induced Cocaine Seeking Behavior

Authors: *E. E. WANNEN[1], C. M. CORBETT[2], J. A. LOWETH[3];
[1]Cell Biol. and Neurosci., Rowan-Virtua Grad. Sch. of Biomed. Sci., Stratford, NJ; [2]Grad. Sch. of Biomed. Sci., Rowan Univ. Sch. of Osteo. Med., Stratford, NJ; [3]Rowan Univ. Sch. of Osteo. Med., Cherry Hill, NJ

Abstract: Sex differences in patterns of cocaine use and relapse vulnerability have been widely reported in both clinical and preclinical studies, with ovarian hormones playing a critical role in mediating these sex differences. Our lab and others have shown that female rats in the estrus stage of the estrus cycle (Estrus Females), when ovulation occurs and estradiol and progesterone have just fallen from peak levels, show enhanced or incubated cue-induced cocaine seeking behavior following prolonged withdrawal or abstinence from extended-access cocaine self-administration. However, the role ovarian hormones play in mediating these effects remains unknown. Here we assessed whether blocking estrogen and progesterone receptors during proestrus, when estrogen and progesterone levels peak, would prevent down-stream signaling cascades needed to drive the enhanced seeking behavior normally observed in cocaine-exposed Estrus Females. Interestingly, acutely administering the selective estrogen receptor modulator tamoxifen (15 mg/kg) and the progesterone receptor antagonist mifepristone (10 mg/kg) during

proestrus did not prevent the enhanced cocaine seeking behavior observed the following day in Estrus Females compared to females in all other stages of the estrous cycle (Non-Estrus Females). Since tamoxifen can also act as an estrogen receptor agonist under certain conditions, it is possible that administering tamoxifen was enhancing, rather than inhibiting, estrogenic activity. Future studies will assess this possibility. Studies are also underway to investigate cellular and molecular mechanisms within the mesolimbic reward circuitry that could be contributing to changes in cue-induced cocaine seeking behavior and relapse vulnerability across the estrous cycle.

Disclosures: **E.E. Wannen:** None. **C.M. Corbett:** None. **J.A. Loweth:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.12/Web Only

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Effect of melatonin over anxious behaviour of preadolescents VPA-induce autism mice male: Pilot study

Authors: *C. PADILLA, A. GARZÓN-PARTIDA, C. CASILLAS-CHAVÉZ, N. HERRERA-LOZA, S. LUQUÍN, V. SÁNCHEZ-GONZÁLEZ, A. GÁLVEZ-CONTRERAS, R. GONZÁLEZ-CASTAÑEDA;
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Abstract: Introduction. Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by specific atypical behavioral patterns and different comorbidities. One of the most frequent comorbidities is anxiety, occurring in 11-84% of people with an ASD diagnosis. A notorious element of anxiety is the anticipation of uncertainty, dread, and fear. Different animal models of ASD have reported increased anxiety in experimental subjects compared to control groups. Melatonin (MEL) is a pleiotropic hormone that has demonstrated an anxiolytic effect in other disorders such as schizophrenia, however this effect has not been observed in murine models of ASD with prenatal exposure to VPA. Objetive: Evaluate anxious behavior in preadolescent mice with and without prenatal exposure to VPA after treatment with melatonin (MEL). Methods. Female CD1 mice were injected at day 12.5 intraperitoneal (i.p) with Valproate (VPA) at 500 mg/kg or vehicle solution of 0.9% alcohol in sterile injectable solution, to generate VPA and Control groups. On postnatal day (PD) 21, hatchlings were weaned, selecting only males. Starting on day 21 to 30 PD, four groups were created, each of them receiving i.p injections of 10 mg/kg of MEL, 5 mg/kg of luzindole + 10mg/kg of MEL or vehicle solution. On day 31PD all groups were tested in an open field test. Results were analyzed with an ANOVA test, with a p=0.05 significance. Results. VPA subjects treated with melatonin traveled longer distances within the open field (p=0.001, ANOVA) and there was an increase of entries to the central area (p=0.01, ANOVA) in comparison to untreated VPA and treated MEL+luzindole.

Untreated VPA and MEL+luzindole spent longer periods of time in the corners, compared to Control and melatonin treated groups (p=0.03, ANOVA). Conclusions. Our preliminary results suggest that mice prenatally exposed to VPA and treated with melatonin show an anxiolytic effect.

Disclosures: **C. Padilla:** None. **A. Garzón-Partida:** None. **C. Casillas-Chavéz:** None. **N. Herrera-Loza:** None. **S. Luquín:** None. **V. Sánchez-González:** None. **A. Gálvez-Contreras:** None. **R. González-Castañeda:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.13/II18

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01HD099084

Title: Neurokinin 3 Receptor neurons in the male medial amygdala integrate sociability, stress, and reproduction

Authors: *D. J. PAULIS[1], E. TORRES JIMENEZ[2], M. SILVA[2], V. M. NAVARRO[3]; [1]Neurobio., Harvard Med. Sch., Boston, MA; [2]Brigham and Women's Hosp., Boston, MA; [3]Med., Brigham and Women's Hosp. / Harvard Med. Sch., Boston, MA

Abstract: For successful reproduction, both activation of the hypothalamic-pituitary-gonadal (HPG) axis and socio-sexual behaviors need to occur. The neuropeptide Neurokinin B (NKB) acts at the neurokinin 3 receptor (NK3R), and NKB/NK3R signaling is known to play a role in regulating reproduction, stress, and social behaviors. Previous work revealed that activation of NK3R neurons in the medial amygdala (NK3RMeA) in female mice results in an increase in luteinizing hormone (LH) secretion. To understand if the same is valid in males, we aimed to uncover the role of NK3RMeA neurons in gonadotropin release and socio-sexual behaviors in male mice. To target NK3RMeA neurons, Tacr3-cre mice (N = 7) were injected bilaterally via stereotaxic injection with a cre-dependent AAV9-hM3Dq (excitatory DREADD). We discovered that chemogenetic activation with CNO (1 mg/kg) of NK3RMeA neurons significantly attenuates mean serum LH levels ($P < 0.05$). Next, we aimed to characterize the role of NK3RMeA neurons in socio-sexual behaviors. In the social partner preference test, the test mouse was given time to explore a 3-chambered arena prior to the introduction of a female mouse in estrous and a socially dominant male mouse, which were placed on opposite ends of the arena. We found that CNO-treated animals spend significantly more time in the center of the arena ($P < 0.05$), avoiding interaction with either sex. Notably, male mice showed a decrease in total distance traveled and velocity following CNO treatment during the test. We then tested male mice for sexual behaviors following sexual experience training sessions. Activation of NK3RMeA neurons with CNO significantly decreased male mounting behavior compared to

saline-injected behavior ($P < 0.05$). Given the impairment in socio-sexual behaviors, we tested whether the activation of NK3RMeA neurons would affect anxiety- and stress-related behaviors. Using the open field test, we found there were no significant changes in movement or preference for the periphery vs center of the arena. We also evaluated stress-induced repetitive behavior using a marble burying test and found that CNO-treated animals were more efficient at burying the marbles and showed persistent re-arranging, re-burying behavior throughout the test. Together, our data suggest that NK3RMeA neurons may be functionally relevant in mediating anti-social actions like stress-related behaviors while attenuating the activation of the HPG axis and sexual behavior in males. These results also demonstrate a robust dimorphic difference in the role of NK3RMeA neurons in socio-sexual behaviors from our previous evidence in females, which mostly mediate reproductive competence.

Disclosures: D.J. Paulis: None. **E. Torres Jimenez:** None. **M. Silva:** None. **V.M. Navarro:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.14/II19

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant DA055169 Awarded to MP

Title: Activation of maternally-responsive estrogen receptor alpha-expressing cells in the medial preoptic area is reduced in mother rats exhibiting a depression-like phenotype

Authors: *A. A. ANDERSON, A. CONTRERAS, K. A. COPELAS, E. ROBINSON, M. PEREIRA;
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Abstract: Mothers who experience postpartum depression often exhibit deficits in parenting. However, the brain mechanisms by which postpartum depression impact parenting abilities are not well understood. Our prior work using the Wistar-Kyoto (WKY) well-validated animal model of depression revealed that, consistent with clinical reports, deficits in maternal behavior in WKY mothers are associated with increased peripartum levels of the steroid hormone estradiol. In addition, our prior research showed that chemogenetic activation of the medial preoptic area (mPOA), a brain region essential for maternal behavior, in WKY mothers ameliorates deficits in parenting and biases choice toward pup-associated cues in a pup-induced conditioned place preference (CPP). The goal of the present study was to examine whether hyporesponsiveness to the stimulatory effects of estradiol in the mPOA underlies the deficits in maternal motivation in WKY mothers. To this aim, we used immunohistochemistry for estrogen receptor alpha (ERα) and cFos (a marker of neuronal activity) to examine the expression levels of ERα in maternally-responsive neurons in the mPOA of WKY and control Sprague-Dawley

(SD) mother. An additional experiment examined the expression of ERα in mPOA cells that are activated when WKY mothers expressed a pup-CPP versus when they did not. As expected, and consistent with our prior results, WKY mothers exhibited severe deficits in parenting and reduced pup-CPP compared with control SD mother rats. In addition, these maternal motivation deficits in WKY mothers were associated with altered activation of mPOA cells containing ERα. Together, the results suggest that estradiol-mediated altered activity of the mPOA may underlie depression-related parenting deficits.

Disclosures: A.A. Anderson: None. **A. Contreras:** None. **K.A. Copelas:** None. **E. Robinson:** None. **M. Pereira:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.15/II20

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: NIH Grant R01 HD100007
NIH Grant R01 HD100007-03S1

Title: Expression of kisspeptin and kisspeptin 1 receptor in adult Syrian hamster

Authors: *M. HALL[1], J. M. BORLAND[2], R. L. MEISEL[3];
[1]Neurosci., [2]Univ. of Minnesota, Minneapolis, MN; [3]Univ. Minnesota, Minneapolis, MN

Abstract: Kisspeptin and Kisspeptin 1 Receptor (KP, K1R) are vital in regulating a variety of functions across many species, with their primary function in reproduction. There have been studies mapping the protein and mRNA of KP and K1R across the central nervous system of rodents, humans, monkeys, sheep, goats, and horses. Syrian hamsters are valuable models for sex behaviors regulated by kisspeptin and its receptors. Our current study maps the distribution of KP mRNA, KP protein, and K1R mRNA in the Syrian hamster telencephalon, diencephalon, and midbrain using dual-labeled RNAscope and immunocytochemistry. In our study, the distribution of kisspeptin and its receptor was mapped across adult males as well as estrous or diestrous females. This brings additional insights into the expression of kisspeptin across estrogen levels. A comparison of these findings to those from other species found that expression in Syrian hamsters was similar to that reported for other species, demonstrating conservation of expression. In addition, the combination of kisspeptin cellular localization along with receptor localization provides an initial view of kisspeptin circuitry in the Syrian hamster brain.

Disclosures: M. Hall: None. **J.M. Borland:** None. **R.L. Meisel:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.16/II22

Topic: F.02. Neuroendrocrine Processes and Behavior

Title: Anxiety-like behavior in crayfish exposed to blue wavelength

Authors: *S. Y. ABBAS, M. E. JACKSON;
Biol., Central Connecticut State Univ., New Britain, CT

Abstract: High levels of blue light cause an increase in stress hormones. Overuse of blue color containing Light Emitting Diode (LED) bulbs and electronic devices could also affect psychological well-being. We hypothesized that a daily 12-hour exposure to blue (415 nm) wavelength LED lights (8-10 lux) for seven days could induce an anxiety-like behavior (ALB) in crayfish. The control group was treated with yellow (590 nm) wavelength LED lights under similar conditions. ALB was measured using a plus-maze equipped with a pair of illuminated and non-illuminated arms. In comparison to the control, a significantly greater percentage crayfish treated with 415 nm light made first entry into the non-illuminated arms rather than illuminated arms. They also dwelled inside the non-illuminated arms for a longer period compared to the control. This effect was more pronounced for female than male crayfish. To test if serotonin is in involved in blue light induced ALB, 415 nm crayfish were injected daily with serotonin antagonists. The control crayfish were injected daily with vehicle only. Overall, this study suggests that blue light exposure could potentially induce anxiety-like behavior in crayfish, and serotonin may play a role in this phenomenon.

Disclosures: S.Y. Abbas: None. **M.E. Jackson:** None.

Poster

PSTR353. Sexual Behaviors

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR353.17/II21

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: Norwegian Research Council Grant 251320

Title: Neuronal activity in the medial amygdala and bed nucleus of the stria terminalis during male rat sexual behavior

Authors: *P. T. HUIJGENS, R. HEIJKOOP, E. M. SNOEREN;
Dept. of Psychology, UiT The Arctic Univ. of Norway, Tromsø, Norway

Abstract: Both the medial amygdala (MeA) and the bed nucleus of the stria terminalis (BNST) have a role in male rat copulation. Most prominently, inhibition of these brain areas impairs ejaculation, albeit through different behavioral mechanisms. In the current study, we looked at real-time neuronal activity in the MeA and BNST simultaneously in the same male rats, in search of a better understanding of the specific contributions of both these areas to the orchestration of sexual behavior and their relation to each other. In 12 male rats, 500 nL AAV5-hSyn-GCaMP6s was infused into the posterodorsal part of the MeA and into the posterior portion of the BNST, and 2 separate optical fibers were implanted in these regions, directly above the viral infusion location. Neuronal activity in the MeA and BNST was followed by means of fiber photometry over multiple sexual incentive motivation and copulation tests, from sexually naïve to experienced state. First results show that both the MeA and BNST increase their activity after mounts, intromissions, ejaculations, and genital grooming. This indicates a role in sensory processing of stimulation to the genitals, corresponding to previous theories. We hypothesize that the magnitude of the increase in neuronal activity in the MeA reflects the magnitude of penile stimulation as well as approach of ejaculation threshold. Neuronal activity in the BNST is hypothesized to predict inter-copulatory interval duration and state of sexual satiety. In addition, we hypothesize that neuronal activity in both areas may change over time during gain of sexual experience, possibly indicating a role for the MeA and BNST in shaping sexual experience-associated gain of copulatory efficiency. Further data analysis will allow us to explore these hypotheses in depth through our elaborate behavioral annotation and extensive study design.

Disclosures: P.T. Huijgens: None. **R. Heijkoop:** None. **E.M. Snoeren:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.01/II23

Topic: F.03. Stress and the Brain

Support: CONAHCyT (CB-2016-01) 0284867
PAPIIT-UNAM IN203820
CONAHCyT 966823

Title: Understanding the long-term behavioral effects of starvation in C. elegans

Authors: *L. GUTIÉRREZ-CHÁVEZ, F. PINTA-CASTRO, N. CANO-DOMINGUEZ, J. VALDES;
Developmental and Cell. Biol., Univ. Nacional Autonoma de Mexico, Coyoacán, Mexico

Abstract: During the life cycle of *C. elegans*, the nematodes may be exposed to hostile environments that trigger phenotypic changes to favor their survival. For example, long periods of starvation induce developmental arrests, in which worms of all larval stages pause or opt for alternate trajectories until environmental conditions improve. However, little has been

investigated regarding the long-term behavioral changes caused by the starvation-induced arrest in *C. elegans*.
Our study found that transient periods of starvation during larval stages or adults can cause changes in odorants preferences. We explored possible mechanisms that operate in the establishment of starvation-induced behavioral changes. Our results showed that DAF-16/FOXO, the master transcriptional factor of the insulin pathway, is involved in the behavioral change after an arrest. DAF-16 is one of the master regulators of arrests and diapauses described in *C. elegans* and can regulate odor perception in worms by regulating receptors expression. In fact, we detected overexpression of the odorant receptor ODR-10 during the arrest. Moreover, we found that some players of the endogenous RNAi silencing pathway are fundamental for the establishment of behavior observed in young adult nematodes following an arrest, since lack of the dsRNA transporter SID-1, the HRDE-1 argonaut protein, as well as PRDE-1 of the piRNA synthesis pathway are unable to establish the starvation-induced behavioral change.
Finally, we explored the participation of the transcription factor CREB, a master regulator of long-term memory and stress responses in animals and found that its activity is present in *C. elegans* neurons as well as intestine cells during starvation but remained only in the intestine after re-feeding of the animals. Transcriptional profiling of starvation-recovered worms showed important dysregulation in gene expression, especially in metabolic pathways. We hypothesize that CREB activity may play an important role in maintaining long-term transcriptional changes at neurons and intestine cells. Our results provide information to understanding how environmental stress can stably modify animal's responses to the environment, and shade light to understand how different signaling pathways participate in diverse tissues to modulate the behavior of an animal.

Disclosures: L. Gutiérrez-Chávez: None. **F. Pinta-Castro:** None. **N. Cano-Dominguez:** None. **J. Valdes:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.02/II24

Topic: F.03. Stress and the Brain

Support: ANR
FRM
ICM

Title: Perinatal fluoxetine exposure affects prefrontal cortical network activity of adult mice through alteration of a specific neuron type

Authors: *A. M. DE STASI[1], J. ZORILLA DE SAN MARTIN[1], N. SOTO[1], A. AGUIRRE[1], J. OLUSAKIN[2], J. LOURENCO[1], P. GASPAR[1], A. BACCI[1];
[1]Inst. du Cerveau, ICM, Paris, France; [2]Inst. du Fer à Moulin, Paris, France

Abstract: The Prefrontal Cortex (PFC) plays a key role in high-level cognitive functions and emotional behaviors, and PFC alterations correlate with different brain disorders including major depression and anxiety. In mice the first two postnatal weeks represent a critical period of high sensitivity to environmental changes. In this particular temporal window, serotonin (5- HT) levels are tightly regulated as they control the wiring of PFC cortical neurons. Early life insults and perinatal exposure to the selective serotonin reuptake inhibitor fluoxetine (FLX) affect PFC development leading to depressive and anxiety-like phenotypes in the adult. However, the mechanisms responsible for these dysfunctions remain obscure. We found that perinatal FLX exposure (PNFLX) results in reduced firing of putative pyramidal neurons (PNs) of deep layers of the Prefrontal Cortex of adult mice in vivo. Moreover, ex-vivo, patch-clamp recordings revealed altered firing properties of a specific subpopulation of PNs, which transiently expresses the serotonin transporter SERT during the PFC critical period (SERT+ PNs), and exhibits distinct morpho-functional properties. Conversely, the excitability of SERT- PNs, and parvalbumin-expressing interneurons was not affected by PNFLX. Surprisingly, spontaneous glutamatergic and GABAergic neurotransmission onto PFC PNs was unaltered in mice that were exposed to PNFLX treatment. Previous transcriptomic experiments indicated that 5HT-7 receptor expression in the PFC overlaps with SERT during early postnatal life. Accordingly, genetic and pharmacological experiments both in vivo and in acute slices indicate that the hypoexcitability of PFC PNs mainly depended on 5HT-7 receptor. Our results suggest potential novel neurobiological mechanisms, underlying detrimental neurodevelopmental consequences of early-life insult.

Disclosures: A.M. De Stasi: None. **J. Zorilla de san Martin:** None. **N. Soto:** None. **A. Aguirre:** None. **J. Olusakin:** None. **J. Lourenco:** None. **P. Gaspar:** None. **A. Bacci:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.03/II25

Topic: F.03. Stress and the Brain

Support: Internal funding

Title: Postnatal maternal antibiotics dictate neonatal microbiome depletion in the offspring and affect behavior in C57Bl/6 mice in a sex-specific manner

Authors: *B. B. NANKOVA[1], F. HU[1], E. F. LA GAMMA[2];
[2]Pediatrics, [1]New York Med. Col., Valhalla, NY

Abstract: Emerging evidence suggest that exposure to antibiotics (Abx) at birth may affect long term health by disrupting the normal neonatal gut colonization at a critical point of development. In the current study C57Bl6 dams were given a broad-spectrum Abx (ampicillin, gentamicin, vancomycin, neomycin, and erythromycin) in the drinking water from postnatal day one until

weaning. Controls received sterile water. At weaning the offspring were subjected to a battery of behavioral tests, including Open Field (OF) and Elevated Plus Maze (EPM) to evaluate potential changes in locomotor activity and anxiety-like behaviors and sacrificed after exposure to acute metabolic stress (insulin-induced hypoglycemia). Individual fecal samples from each cohort were collected for whole genome shotgun taxonomic profiling and predictive functionality. As mood disorders and neurodevelopmental disorders occur at different rates between sexes, we investigated the effects of postnatal Abx exposure in both male and female pups. Baseline physiological markers in the offspring were not altered between the cohorts and between the genders. Maternal oral Abx administration caused neonatal microbiome depletion (evident by markedly enlarged ceca and no detectable by-products of bacterial fermentation, SCFA). The rise in urinary epinephrine levels in response to hypoglycemia was reduced only in male pups. Fecal DNA sequencing confirmed the dramatic changes in microbial composition and diversity in the offspring: significantly reduced alpha (Chao1, $p<0.004$ and $p<0.005$ resp.) and beta (Bray-Curtis, $p<0.003$) diversity, compared to their respective controls; with subtle gender differences in the relative abundance of microbial features. The analysis of the OF data revealed that the offspring of Abx treated dams had significantly fewer number of entries into the center compared to the controls ($p< 0.0014$; 0.0058). The time spent in the center of the arena was similar between the groups and sexes. However, the total distance traveled (a measure of locomotor activity) was significantly different between the groups (significantly decreased in Abx vs control, $p<0.0001$; 0.043) and genders (higher in male vs female controls, $p<0.035$). Further, on the EPM test female offspring of Abx exposed mothers spent significantly less time in the open arms compared to respective controls ($p<0.0029$), while measures for males were similar in Abx and control groups. In conclusion, maternal oral Abx during lactation depletes gut microbiome in the offspring and affects locomotor activity, anxiety-like behavior and sympathoadrenal epinephrine responses to acute stress in a sex-specific manner.

Disclosures: **B.B. Nankova:** None. **F. Hu:** None. **E.F. La Gamma:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.04/II26

Topic: F.03. Stress and the Brain

Support: 1 DP2 MH126377

Title: Vulnerability throughout the maternal experience: Electrical network and behavioral assessments of the maternal mouse brain

Authors: *S. MITCHELL, M. EBERLE, M. JOHNSON, A. JIMENEZ, M. MATKOVICH, R. VELAMURI, B. HING, H. STEVENS, R. HULTMAN;
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Abstract: The maternal brain experiences a cascade of changes over pregnancy and postpartum (PP) to the benefit of offspring. Yet this plastic state may yield heightened potential for the development of negative maternal outcomes. Adverse experiences, such as exposure to early life stress (ELS), are known to increase such vulnerability through lasting alterations to depression and anxiety associated neural pathways. These brain-wide adjustments have also been linked to the level of displayed maternal care. The aim of this investigation is to elucidate changes of electrical dynamics in the maternal brain relating to previously identified stress and vulnerability-associated electrical brain networks known as "*Electome Factors*" (*EFs*). This study also examines correlations of network activity with observed maternal care behavior and assesses alterations according to ELS history. A combination of maternal separation with early weaning and limited nesting material was used as a model of ELS in CD1 mice (n = 19-21 litters/group) to increase vulnerability. Female mice were surgically implanted with multi-site *in vivo* recording electrodes prior to breeding for the continued observation of brain-wide electrical dynamics. Recordings of network activity were captured in the home-cage at multiple time points from pre-gestation (PG) through weaning to capture the full course of maternal brain change (n = 12-14/group). In line with previous studies of stress vulnerability networks and in consideration of parental network literature, the electrodes targeted the following regions: prelimbic and infralimbic cortices, nucleus accumbens, basolateral, medial, and central amygdala, ventral hippocampus, ventral tegmental area. Maternal behaviors were recorded at parturition and during all assessment time points for evaluation alongside electrical dynamics. Initial findings from home-cage recordings and a task designed to elicit a negative affect response show that PP animals have trending heightened activity of multiple stress-associated networks as compared to PG, which also differed by ELS history. Activation of such networks, including *EF6*, was further seen to be dependent on the maternal engagement state of the dam (on nest vs. off) regardless of ELS history ($p = 0.012$, $n = 7$), with overall maternal care correlating with stress vulnerability network *(EF1*) activity ($p = 0.029$, $n = 7$) when off the nest. Moreover, dams with a history of ELS show evidence of disrupted maternal care through shorter nest visits ($p = 0.026$, n=6/group). These findings suggest intriguing overlaps between neural circuit correlates of maternal brain adaptations and changes with early adversity.

Disclosures: S. Mitchell: None. **M. Eberle:** None. **M. Johnson:** None. **A. Jimenez:** None. **M. Matkovich:** None. **R. Velamuri:** None. **B. Hing:** None. **H. Stevens:** None. **R. Hultman:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.05/II27

Topic: F.03. Stress and the Brain

Support: Academy of Finland Grant #1333341

Title: Early-life stress leads to hyperactivity of developing basolateral amygdala neurons

Authors: *A. DONATI, F. VEDELE, A. KALMENOVA, H. HARTUNG;
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Abstract: Early-life stress (ELS) such as maternal maltreatment, neglect, or abuse predispose an individual to develop mental disorders. Disease hallmarks include heightened amygdala reactivity and impaired prefrontal-amygdala functional interactions, already during childhood and adolescence. However, which cellular and circuit mechanisms underlie these hallmarks, as well as the altered developmental trajectory of prefrontal-amygdala networks, is not known.
Aims: To test the effect of ELS on the firing activity of developing basolateral amygdala (BLA) neurons.
Methods: ELS was induced in mice with the limited bedding and nesting (LBN) model between P4-P14 and combined with maternal separation for one hour at P8, P10, and P12. *In vivo* multi-unit activity (MUA) and local-field potential (LFP) recordings were performed in the BLA of urethane-anaesthetized pups at P18-P20. Furthermore, immunohistochemistry against ΔFosB, a marker of persistent neuronal activation by chronic stress, was carried out and quantified in the BLA at P14 and P18.
Results: Our data show no significative differences of the mean firing rates between control and ELS mice for both genders. However, plotting the logarithmic frequency distribution of the firing rates, revealed a bimodal distribution in ELS mice only, suggesting that a distinct population of BLA neurons showed increased neuronal firing activity after ELS. To confirm and further characterize the population of activated neurons in the BLA, we quantified ΔFosB expression. ΔFosB expression was significantly increased at P14 immediately following ELS, and to a lesser extent still at P18. In addition, the valley-to-peak time of the action potential waveform (corresponding to the medium afterhyperpolarization) was significantly decreased in ELS males, while females were not affected. This could be caused by a compromised expression of SK2 channels after ELS.
Conclusion: Our findings further our knowledge on the effects of ELS on the developing BLA. Our *in vivo* electrophysiological and immunohistochemical data suggest that ELS leads to a persistent activation of a distinct population of developing BLA neurons in a sex dependent manner, in males only. Efforts to further characterize this population are currently undertaken.

Disclosures: A. Donati: None. **F. Vedele:** None. **A. Kalmenova:** None. **H. Hartung:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.06/II28

Topic: F.03. Stress and the Brain

Support: Bowdoin College Paller Award
Maine IDeA Network for Biomedical Research Excellence (INBRE) by the National Institute of General Medical Sciences of the National

Institutes of Health P20GM103423
Surdna Foundation Undergraduate Research Fellowship Program

Title: Corticolimbic neural recruitment and behavioral changes in response to early life adversity are sex-specific in rats

Authors: *Z. D. REYNOLDS[1], K. N. PATEL[1], K. PATEL[1], J. A. HONEYCUTT[2];
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Abstract: Excessive stress at any stage of life increases the likelihood that a person may develop mental health problems in the future. However, extensive stress during developmental periods such as juvenility or adolescence predisposes individuals to develop mental health disorders such as anxiety and depression in the future, and disproportionally impacts women. The early-life adversity (ELA) caused by extensive stress can contribute to maladaptive outcomes through the disruption of the development of brain regions directly related to stress and emotional regulation such as the circuit linking the prefrontal cortex (PFC) and basolateral amygdala (BLA). Sex works as a biological variable in the development of these regions in both humans and model systems. In response to ELA, the connections from the BLA to the PFC develop at sex-specific rates, with females experiencing precocial maturation of axonal innervation earlier than their male counterparts. However, the overall local functionality and cell-type specific integration of this maturation - as well as concomitant behavioral manifestation - is unclear and remains to be investigated. Using stereotaxic electrical stimulation via bipolar electrode, we introduced physiologically relevant stimulation to the BLA of adolescent rats (postnatal day 28) to activate the region and enable the quantification and characterization of downstream circuit-specific effects. Within the PFC, we use immunohistochemistry to label cells recruited by this activation through upregulation of the immediate early gene, *c-Fos,* and leveraged cell-specific markers for inhibition (i.e., parvalbumin; PV) as a proxy to determine changes in local excitatory: inhibitory balance. Immunohistochemistry results suggest differences in overall neural, as well as cell-type, recruitment across sex and rearing condition within the PFC. To connect the neurological results to larger scale changes, rat behavior in response to an aversive social stimulus (22kHz ultrasonic vocalization (USV) alarm calls) were assessed. Rats were placed in the open field for a total of 10 minutes, with a 5 minute baseline followed by 5 minute exposure to 22kHz rat USV playback to assess environmental vigilance within the context of potential threat. Center duration, latency to center, average velocity, and maximum velocity were compared for all conditions across both 5-minute time bins. Sex and rearing condition differences in these measures suggest an impact of sex on behaviors. Overall, these findings provide compelling evidence that point to a sex-specific effect of ELA on the neural circuitry responsible for behavioral regulation during ambiguous threat.

Disclosures: **Z.D. Reynolds:** None. **K.N. Patel:** None. **K. Patel:** None. **J.A. Honeycutt:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.07/JJ1

Topic: F.03. Stress and the Brain

Support: College of Arts and Sciences, Quinnipiac University

Title: Enhanced sex differences in double-hit stress effects during early life and adolescence: impact on rodent behavior, adrenal function, meningeal activity, and serum tryptophan signaling.

Authors: H. JACK[1], J. MIRRA[2], E. METJAHIC[1], G. BURMAN[2], L. O'CONNOR[3], S. AZERRAD[4], M. CHO[2], G. M. ANDERSON[6], *A. BETZ[5];
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Abstract: It is known that early life stress (ELS) in humans contributes to the development of psychiatric mood disorders in adulthood. Further, sex differences in stress responses have been extensively studied suggesting males and females exhibit distinct patterns of physiological and behavioral differences following stress exposure. Consistent with these findings, animal models of ELS have demonstrated long-term behavioral, physiological, and neuroendocrine changes. Maternal separation in rodents is a widely used model for simulating ELS. We used a double-hit model of ELS with the addition of mild chronic unpredictable stress (CUS). Traditionally the first hit is in the critical period of development with a second hit later in life. However, there is a paucity of data looking at the double-hit within the juvenile to adolescent period. Male and female rodents were subjected to maternal separation (PND 2-14) and adolescence unpredictable chronic mild stress (PND21- 37). Control groups underwent standard facility handling. Following stress, rodent behavior was assessed. We found that males and females were differently affected by stress as measured by elevated plus maze (EPM) and tests of anhedonia. Both males and females spent more time in closed arms in EPM. Male offspring exposed to CUS alone displayed increased bouts of play and activation suggesting hyperresponsivity to novel and mild threats. Additionally, we analyzed tryptophan metabolism in the serum and inflammation signaling in the adrenal glands and meninges. We found altered levels of 5-HT but not IAA or UNK in the serum. Further, we found changes in NfkB protein expression in the adrenal glands and meninges. Together, this suggests complex interactions between sex, stress and neuroendocrine signaling. Studies such as these can contribute to the development of targeted interventions and therapeutic strategies for stress-related conditions in humans.

Disclosures: H. Jack: None. **J. Mirra:** None. **E. Metjahic:** None. **G. Burman:** None. **L. O'Connor:** None. **S. Azerrad:** None. **M. Cho:** None. **G.M. Anderson:** None. **A. Betz:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.08/JJ2

Topic: F.03. Stress and the Brain

Support: CIHR Grant PJT162376

Title: Sex-dependent effects of early life stress on medial prefrontal cortex function during fear conditioning in juvenile rats

Authors: *J. SONG[1], H. LONG[3], A. GRATTON[3,2], T. WONG[3,2], C.-D. WALKER[2,3];
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Abstract: Exposure to early life stress (ELS) can exert long-lasting impacts on emotional regulation. The medial prefrontal cortex (mPFC) plays a key role in fear learning and is highly sensitive to environmental stressors throughout development. Using the limited bedding paradigm (LB) between postnatal days (PND)1-10 as a model of ELS, we examined the functional consequences of ELS on excitatory and inhibitory tone in the prelimbic (PL) mPFC of juvenile rats. We measured glutamate concentrations during fear conditioning using *in vivo* microdialysis in freely behaving juvenile (PND28-32) and adult rats. In adults, LB exposure enhanced glutamate release in the PL mPFC during fear conditioning in male, but not female offspring. In contrast, glutamate response to fear conditioning tended to be diminished in LB-exposed juvenile males, but not females. To confirm ELS-induced attenuation in presynaptic glutamate neurotransmission, we examined the paired pulse ratio of evoked field excitatory postsynaptic potentials (fEPSPs) in the PL mPFC of juvenile males. Preliminary data showed paired-pulse facilitation of fEPSPs in the PL mPFC of LB-exposed male offspring and paired-pulse depression in normally raised control animals. To estimate ELS effects on fear-induced activity of parvalbumin (PV) and somatostatin (SST) interneurons, we determined the density of cFos and PV or SST co-expression after fear conditioning using immunohistochemistry. We reported that cFos activation in SST, but not PV interneurons in the PL mPFC was elevated after fear conditioning only in juvenile males exposed to the LB condition, but not in control animals. These results suggest that ELS modified the excitatory/inhibitory balance in the PL mPFC during fear conditioning in a sex- (primarily in males) and age- dependent fashion.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

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Program #/Poster #: PSTR354.09/JJ3

Topic: F.03. Stress and the Brain

Support: Magellan Scholar Grant #111100-23-64098
NIH Grant AT010903

Title: Different wavelengths of light at night alter the medial amygdala and social behavior among adolescent mice in a sex-specific manner

Authors: *J. KARTIK, A. SHANKS, P. BONILLA, A. PORCU;
Drug Discovery and Biomed. Sci., Univ. of South Carolina, Columbia, SC

Abstract: Light serves as a sensory stimulus that has significant biological effects on brain circuits and physiological processes. Chronic exposure to light at night has been linked to various health problems, including emotional disorders. However, the influence of interindividual traits, such as sex differences, on responses to different light parameters has yet to be fully understood. In our study, we aimed to investigate potential sex differences in the response to different wavelengths of light at night in adolescent mice, focusing on their impact on social behavior and neuronal responses in the medial amygdala.To achieve this, we designed a novel light paradigm that aimed to mimic the light exposure patterns commonly observed in human adolescents. The model involved a prolonged light phase of 19 hours per day, with light exposure occurring during the biological night phase of the mice. Adolescent mice at post-natal day 30 were exposed to three different light conditions for a duration of four weeks: 12 hours of darkness and 12 hours of LED light (control group), 5 hours of darkness and 19 hours of LED light with a blue wavelength (blue light at night group), and 5 hours of darkness, 7 hours of ambient light, and 12 hours of LED light without the blue component (reduced blue light group). Following the exposure period, mice were tested for social behaviors and brains were processed to perform RNAscope and immunofluorescence analysis.We found that male adolescent mice exposed to blue light at night exhibited a reduction in social interactions compared to the control group. However, female mice exposed to blue light at night or ambient light, as well as males exposed to ambient light, did not demonstrate the same decrease in social interactions. Moreover, we further explored the underlying neuronal activity within the medial amygdala by quantifying the expression of the cFOS marker, which indicates neuronal activation, as well as the expression of sex hormone receptors. We found that female mice exposed to blue light at night displayed increased expression of the androgen receptor and estrogen type 2 receptor in the medial amygdala. Conversely, male mice exposed to blue light at night exhibited decreased expression of these receptors compared to the control condition.Taken together, our data suggests the presence of sex differences in response to different wavelengths of light at night, which may play a pivotal role in the regulation of social behavior during adolescence.

Disclosures: J. Kartik: None. **A. Shanks:** None. **P. Bonilla:** None. **A. Porcu:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

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Program #/Poster #: PSTR354.10/JJ4

Topic: F.03. Stress and the Brain

Support: T32MH125786
P50MH115874

Title: Adolescent chronic social defeat produces robust social avoidance and changes in neural activation in the adult mouse

Authors: *E. HISEY[1], E. NEWMAN[2], K. J. RESSLER[3];
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Abstract: Adolescence is a time of monumental cognitive and neurobiological changes. Trauma during this time period can in turn alter normal brain development and result in lifelong psychiatric disorders. While models of early life neglect in rodents do exist, models of early life trauma are nearly absent. In order to better understand the neurobiological underpinnings of early life trauma on the adult brain, we have developed a model of adolescent chronic social defeat in male mice. After chronic defeat exposure in early adolescence, mice display robust social avoidance that is maintained throughout late adolescence and adulthood. This social avoidance behavior also generalizes to female mice of the same strain as those used as aggressors during defeat (reductions in following and contact, increases in defensive posturing and freezing in defeated mice compared to controls). Re-exposure to a nonaggressive male mouse during adulthood drives dramatic increases in c-fos activity, with changes in network connectivity, in the brains of male mice defeated as adolescents, as revealed by whole brain c-fos imaging. Significant increases in c-fos expression in comparison to control mice were found in hippocampus (CA3), medial and lateral entorhinal cortex and anterior insula (AI). Of these areas, we were most interested in AI, given its implications in salience processing and its dysregulation in PTSD and mood disorders. We performed a viral manipulation in adults defeated as juveniles to reversibly silence AI. Chemogenetic silencing of AI produced significant increases in social interaction and a reduction of fear-related behaviors during social interaction in susceptible adults defeated as juveniles. We next plan to further dissect which projection types within AI underlie this deficit as well as to perform RNAseq on AI in adults defeated as juveniles to identify molecular targets that could be modulated to shape aberrant social behavior. Overall, our mouse model of early life trauma can robustly capture both behavioral and neural alterations in adults traumatized as juveniles in hopes of better understanding the neural circuit basis and treating the effects of early life trauma on the brain.

Disclosures: E. Hisey: None. **E. Newman:** None. **K.J. Ressler:** 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.; Takeda, Brainsway and Alto Neuroscience. F. Consulting Fees (e.g., advisory boards); Bioxcel, Bionomics, Acer, Takeda, and Jazz Pharma.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.11/JJ5

Topic: F.03. Stress and the Brain

Support: Seaver Foundation

Title: The impact of social isolation on social behavior and VTA-DA neuron activity

Authors: *M. KIM, M. BARBIER, H. HARONY-NICOLAS;
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Abstract: Social interactions during development are crucial for establishing adult social behavior. An important facet of social behavior is the rewarding properties of social interaction, and in both humans and animals, social reward undergoes a shift in salience during adolescence. However, there is a gap in understanding how juvenile social isolation (jSI) may impact social rewardseeking and processing in adulthood. Previous studies have found NAc-projecting VTA dopamine (DA) neuron activity encodes social behavior but not interactions with a novel object, and VTA-DA neurons show increased firing rate during interactions with social stimuli. Together, these findings suggest that VTA-DA neurons encode the positive value of social interaction, and our study uses *in vivo* fiber photometry to gain insight into how VTA-DA neuron activity is affected by jSI. At weaning age, rats are assigned to either jSI or group-housing (GH) for 3 weeks. Immediately following isolation, we inject a TH-Cre virus with a Cre-dependent GCaMP virus into the VTA of all rats before rats are re-housed and re-socialized with a novel age- and sex-matched rat until adulthood (P60). At adulthood, we assess social preference and social reward-seeking behavior while recording from VTA-DA neurons. For this purpose, we use a social preference assay and a social vs. food task, respectively. The latter task consists of presenting a rat with both a novel social stimulus along with a competing non-social reward (food). We found male rats raised in jSI ($n = 7$) do not show any significant differences in social preference or social reward-seeking behavior compared to GH rats ($n = 8$). However, VTA-DA neurons in GH rats show increased activity during social interaction in a social preference assay and the social vs. food task while VTA-DA neurons in jSI rats did not display this increase. Area under the curve was calculated to compare changes in VTA-DA activity during social interaction, and the increase in VTA-DA activity during social interaction seen in GH rats is significantly higher than VTA-DA activity seen in jSI rats during both the social preference assay (unpaired ttest, $p < 0.05$) and the social vs. food task (unpaired t-test, $p < 0.05$). Our findings indicate that compared to VTA-DA neurons in GH rats, VTA-DA neurons in jSI rats show significantly lower activity during interaction with a social stimulus, suggesting a potential link between jSI and deficits in processing the value of social interaction despite no deficits observed in social interaction. Ongoing fiber photometry recording experiments in female rats will shed light on potential sex differences in how jSI impacts the activity of VTA-DA neurons.

Disclosures: M. Kim: None. **M. Barbier:** None. **H. Harony-Nicolas:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.12/JJ6

Topic: F.03. Stress and the Brain

Support: NARSAD Young Investigator Grant from the Brain & Behavior Research Foundation

Title: Determining the effects of early adolescent stress on psychiatric-related behaviors of C57BL/6J adult mice

Authors: A. R. BIDDLE, **K. R. SAIKALY**, C. E. HAMMOND, N. P. SHENOY, S. PARK, H. VANDRASI, K. E. QUINN, S. M. VERNADAKIS, *C. A. VADNIE;
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Abstract: Anxiety and mood disorders are highly prevalent and often diagnosed in late adolescence or early adulthood, with stress being a major risk factor. However, limited studies have focused on the long-term neurological and behavioral effects of early adolescent or juvenile stress in mouse models, which are a powerful model system for basic neuroscience research. In this study, male and female C57BL/6J mice were separated into stressed and unstressed control groups (n = 7-8). The stressed mice experienced 3 days of stressors from postnatal day (PND) 25-27 during the light phase. Control non-stressed mice remained undisturbed in the animal facility. The stressors used were forced swim, restraint, and elevated platform stress. We hypothesized that stress during early adolescence would cause anxiety- and depressive-like behaviors, as well as cognitive deficits in male and female adult mice. To test this hypothesis, behavioral testing was carried out starting at PND 60. We used the open field (OF) and elevated plus maze (EPM) as measures of anxiety-like behavior, the sucrose-preference test (SPT) and tail-suspension test (TST) as measures of depressive-like behavior, and the Y-maze (YM) to test spatial working memory. Results thus far show that early adolescent stress increases avoidance behavior in adult mice in the OF as measured by a decrease in center entries and center time ($*p < 0.05$). However, stress had no impact on behaviors in the EPM, SPT, TST, and YM. Thus, similar to previous findings (Brydges et al. 2014), we observed that stress during early adolescence increases some anxiety-like behaviors in adult C57BL/6J mice. Early life stress has been shown to affect the expression of subunits of the $GABA_A$ receptor in rodents but results have been inconsistent (Jacobson-Pick et al. 2012, Jacobson-Pick and Richter-Levin 2012, Tzanoulinou et al. 2014). Therefore, we are currently determining the effects of early adolescent stress on the expression of $GABA_A$ receptor subunits in brain regions known to play an important role in regulating psychiatric-related behaviors. We hope that our work in combination with previous studies will contribute to the understanding of the long-term impacts of early life stress on adult behavior and neurobiology.

Disclosures: A.R. Biddle: None. **K.R. Saikaly:** None. **C.E. Hammond:** None. **N.P. Shenoy:** None. **S. Park:** None. **H. Vandrasi:** None. **K.E. Quinn:** None. **S.M. Vernadakis:** None. **C.A. Vadnie:** None.

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PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.13/JJ7

Topic: F.03. Stress and the Brain

Support: Academy of Finland Grant #1333341

Title: Early-life stress impacts the functional development of Prefrontal-Amygdala networks

Authors: *F. VEDELE, A. DONATI, H. HARTUNG;
Univ. Helsinki, Helsinki, Finland

Abstract: Early-life stress (ELS) exerts a strong effect onto brain development, perturbating its normal progress, and bearing consequences that extend into adult life. The greatest contributors to adverse outcomes for the offspring are scarce access to resources, including but not limited to proper nutrition, maternal maltreatment and neglect, and physical and psychological abuse. These vastly different forms of ELS converge in their biological response: a premature activation of the stress axis during this sensitive time period. However, how ELS impacts the functional development of networks underlying emotional behaviors, such as prefrontal-amygdala networks, is still poorly understood.
Our aim was to determine if ELS alters the development of oscillatory coupling between the basolateral amygdala (BLA) and the prefrontal cortex (mPFC), two brain regions whose function is consistently affected after ELS in humans.
To do so, we employed an affirmed mouse model of ELS, the limited bedding and nesting model (LBN), with the stress period carried out between P4 and P14 and combined with maternal separation at P8, P10, and P12. We performed *in vivo* multi-unit activity and local-field potential (LFP) recordings simultaneously in the prelimbic (PL) and infralimbic (IL) subdivisions of mPFC and the BLA of urethane anesthetized pups at P18-P20 and P43-P47.
We found a sex-specific impairment in the development of functional interactions within mPFC and BLA networks. Male ELS mice showed an increase in LFP coherence between 10-20 Hz between superficial layers of PL and BLA at P18, while ELS females were similar to controls at this age. At P45, changes appear to be diametrically opposite to P18, with only ELS females showing an increase in LFP coherence between 1-20 Hz, but between the deep layers of IL and BLA. Our results were confirmed with phase-phase coupling and wavelet analyses. Interestingly, none of these changes were associated with differences in oscillatory power.
These data highlight a previously unknown effect of ELS onto brain development, which could be a result of an altered functional connectivity between these brain regions. Experiments to reveal if an altered structural connectivity forms the substrate of these changes in functional interactions are currently underway.

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Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.14/JJ8

Topic: F.03. Stress and the Brain

Support: MH091844 (AD)
MH115215(AD)

Title: Early life stress induces working memory deficit via dysregulation of cholinergic signaling in the dentate gyrus

Authors: *M. BOMPOLAKI[1,2], C.-Y. CHANG[1,2], H. GHONEIMA[2], J. NYE[3], P. ATSAK[1,2], A. Z. HARRIS[1,2], E. D. LEONARDO[1,2], A. DRANOVSKY[1,2];
[1]New York State Psychiatric Inst., New York, NY; [2]Psychiatry, [3]Biomed. Engin., Columbia Univ., New York, NY

Abstract: A staggering proportion of the population experiences adversity in early life, including inadequate caregiving, poverty, exposure to natural disasters or other traumatic events. These experiences, even if short-lived, occur during sensitive developmental periods and have pervasive consequences for mental health. The limited nesting and bedding paradigm (LBN) emulates fragmented care due to limited resources and has been used to study the extensive effects of early life stress (ELS) in rodents. We previously used LBN to find that ELS exposure delays maturation of the hippocampal dentate gyrus (DG), establishing the DG as a target brain system in this model. Furthermore, we recently showed that DG neurogenesis maintains local cholinergic innervation and working memory. Both the cholinergic system and memory functions are disrupted in animals exposed to ELS, however, an integrated systems level understanding of how this occurs and how the DG is involved is lacking. Here, we begin to fill that gap by simultaneously examining neural, neurotransmitter, and behavioral responses in control and ELS mice. We studied working memory using the spontaneous alternation task, which engages innate, non-reinforced behavioral programs. We found that ELS produced an enduring deficit in this task that was evident as early as 7 weeks and persisted until 18 months of age. We were able to restore normal performance with systemic administration of cholinesterase inhibitors, indicating that cholinergic dysfunction underlies working memory deficits in ELS. We then simultaneously measured ACh dynamics using the genetically encoded sensor GRAB-ACh3.0 and local field potentials (LFP) recordings in the DG of control and ELS mice during the spontaneous alternation task. ELS animals exhibited greater theta power at baseline, reminiscent of similar findings in schizophrenia and autism studies. In the maze, ELS animals had significantly reduced activation of theta and ACh, correlating with maze performance. These findings indicate that ELS disrupts cholinergic innervation of the DG leading to deficits in the local theta oscillations and working memory. We repeated these recordings in the presence of cholinergic agonists to establish the role of specific ACh receptors underlying ELS disruption of both theta oscillations and working memory. Our findings provide a systems level explanation for memory deficits associated with ELS and have treatment implications for individuals with a history of childhood trauma. These studies are designed to provide insights into the mechanisms underlying childhood adversity related risk of pathophysiology associated with cognitive deficits.

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Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.15/JJ9

Topic: F.03. Stress and the Brain

Support: NINDS R01 NS104436
Advance Clinical Translational Network AWD12047
URI College of Pharmacy Seed Grant

Title: Examining the development of Alzheimer's disease-like symptoms in a rabbit model of cerebral palsy

Authors: L. T. GENRY, E. J. REEDICH, E. MENA AVILA, K. GEVER, *K. QUINLAN; Univ. of Rhode Island, Kingston, RI

Abstract: Cerebral Palsy (CP) is the most common motor disability in children and affects 1.5 - 2.5 out of every 1,000 live births. As adults, people with CP have poorer general cardiovascular health and higher total cholesterol levels than those without CP. These and other modifiable lifestyle factors could contribute to an increased risk for Alzheimer's disease (AD). Recent evidence confirms that CP patients are at a higher risk for developing Alzheimer's disease and related dementias as they age. However, the nature of this link is still unclear: does early life stressors/environmental insults, like those leading to the development of CP, cause structural and functional changes in the brain that may lead to the development of disease later in life, or is it simply the presence of modifiable lifestyle factors that increases risk? We hypothesize that prenatal brain injuries exacerbate the development of AD-like symptoms, and we pursued this question using the high-cholesterol-fed rabbit model of AD combined with the prenatal hypoxia-ischemia (HI) model of CP. At 70-80% gestation, blood flow was restricted to the uterus of a pregnant dam for 40 minutes causing HI -mediated brain injury in the rabbit kits in utero as has been described in previous studies on CP. Rabbit kits were born naturally at term. At the time of weaning, rabbits were placed on a normal diet or a high cholesterol diet with copper in the drinking water as has been described in previous studies on AD. After approximately 6 months on the diet, trace eyeblink conditioning, open field, novel objection recognition, and novel object placement assays were performed to assess cognitive function. We found a significant positive correlation between serum cholesterol levels and the length of time it took the rabbits to reach the threshold for learning in trace eyeblink conditioning. Additionally, there was a significant effect of the prenatal brain injury on the number of conditioned responses at the conclusion of trace eyeblink conditioning. No significant differences were present in open field and novel object recognition/placement in this preliminary data set. More work needs to be done to clarify the relationship between prenatal brain injury and susceptibility to AD.

Disclosures: L.T. Genry: None. **E.J. Reedich:** None. **E. Mena avila:** None. **K. Gever:** None. **K. Quinlan:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.16/JJ10

Topic: F.03. Stress and the Brain

Support: NIH Grant 5T32MH018870

Title: Effects of early life adversity on alcohol consumption: compounding effects of secondary stress

Authors: *J. BRETON[1], B. WILLIAMS[2], K. G. BATH[1,3];
[1]Psychiatry, [2]Cell. and Mol. Physiol. and Biophysics, Columbia Univ., New York, NY; [3]New York State Psychiatric Inst., New York, NY

Abstract: Experiencing early life adversity (ELA) increases the risk for substance use, including alcohol consumption. ELA is also highly predictive of future adversity, which may have compounding effects, further increasing risk for use and abuse. However, little is known regarding how ELA interacts with later life stressors to increase drug seeking. Animal models of ELA, such as the limited bedding and nesting (LBN) paradigm, provide a tractable model to study how environmental conditions, including secondary stress, may interact with ELA to contribute to risk for alcohol use. Here, we assessed the impact of various secondary stressors on alcohol consumption in control and LBN-reared mice. Multiple experiments were conducted, each testing a different environmental condition. In the first, we tested alcohol consumption in group housed LBN or control reared mice. In the second, mice were socially isolated for one week prior to alcohol testing to examine potential compounding effects of social isolation stress. In the last experiment, group-housed mice received a series of high intensity shocks in a stress-enhanced fear learning (SEFL) paradigm prior to starting alcohol testing. For all experiments, adult male and female mice underwent two weeks of voluntary alcohol consumption using a two-bottle choice continuous access paradigm. In group housed mice, LBN females showed reduced alcohol intake relative to controls, with no difference in males. In contrast, in both socially isolated mice and mice that underwent SEFL, LBN males showed increased alcohol intake relative to controls. After two weeks of continuous access, group-housed and socially isolated mice were challenged with an acute foot shock stressor and alcohol intake was measured for an additional week. Acute foot shock stress did not impact alcohol consumption in group-housed mice; however, in socially isolated animals, alcohol consumption was elevated in LBN females for one day following the acute stressor; an effect not observed in male groups or control females. Together, this work suggests that housing conditions, sex and rearing condition all interact to influence stress-induced alcohol consumption. Future work will investigate potential neural mechanisms that may underlie observed changes in stress-induced alcohol seeking following LBN. Overall, these studies provide critical insight into the effects of ELA on stress-related alcohol intake and have important implications for conditions such as addiction.

Disclosures: **J. Breton:** None. **B. Williams:** None. **K.G. Bath:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.17/Web Only

Topic: F.03. Stress and the Brain

Support: KAKENHI18H00994
KAKENHI22K06851

Title: Effect of Poly(I:C) on perinatal body movements view from neural activity and behavioral analysis

Authors: *Y. KOSAKA[1], N. MASUTANI[1], C. SHIMIZU-OKABE[2], K. NAKAHARA[3], S. MOROKUMA[4], A. ARATA[1];
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Abstract: The fetal movement has a great influence on fetal development and survival rate. It has been reported that maternal viral infection increases the risk of psychiatric disorders such as schizophrenia, mental retardation, and autism, however, the effects of fetal infection on motor function and differences in symptoms depending on the timing of inflammatory exposure have not been clarified. In this study, we investigated the effects of Poly ((I:C): PIC) as an indicator of body movement activity. PIC is an immunostimulant in viral infections, and our previous studied that administration of PIC reduced fetal movements and increased peristaltic movement. These results suggested that infection may reduce fetal movements. Next, we examined whether there is a difference in the motor coordination function and behavior of rat pups after birth. On the 2nd and 3rd days after birth, the time required for rolling over was longer in all groups administered with PIC than in the control group. However, the effects of PIC on the medulla oblongata and spinal cord during the perinatal period are still unknown. The data were recorded from the C8 and L4 ventral roots of the spinal cord isolated from the embryonic 17-19-day-old and postnatal 0-2-day-old rat isolated brainstem-whole spinal cord preparation and recorded as an upper and lower limb, respectively. In embryonic preparations, 5-HT was applied to stimulate body movement activity; then PIC was applied under 5-HT. In the neonatal period, the preparations were applied with strychnine as a glycine antagonist to remove the blockade of body movement, and PIC was applied. We examined the effects of PIC on body movement activity during the embryonic and neonatal stages. In the embryonic period, small movement activity accompanied by body movement activity was observed when PIC was applied under 5-HT. Moreover, during the neonatal stage, the application of PIC under strychnine induced significant body movement followed by a small activity, which was more critical in the P0 than in the P2. These results

showed that PIC changed normal body movement to body movement with shaking action, a sort of movement with PIC-treated embryonic rat in the pregnant rat.

Disclosures: **Y. Kosaka:** None. **N. Masutani:** None. **C. Shimizu-okabe:** None. **K. Nakahara:** None. **S. Morokuma:** None. **A. Arata:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.18/JJ11

Topic: F.03. Stress and the Brain

Support: NIH DA052909
NIH DA038588
NIH MH078105
Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term effects of early life adversity on adult brain dopamine and serotonin receptor systems involved in cocaine reinforcement: a nonhuman primate study

Authors: *J. H. ACEVEDO-POLO[1], E. R. SIEBERT[1], K. A. JENKINS[1], R. J. VOLL[1], L. N. CHAVAN[1], M. M. GOODMAN[1], M. STYNER[2], L. L. HOWELL[1], J. A. NYE[1,3], M. A. NADER[4], M. M. SANCHEZ[1];
[1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina, Chapel Hill, NC; [3]Med. Univ. of South Carolina, Charleston, SC; [4]Wake Forest Univ. Sch. Med., Winston-Salem, NC

Abstract: Early life adverse experiences such as child maltreatment (MALT) are associated with the development of psychopathology, including anxiety and substance use disorders (SUDs). Understanding the neurobiological mechanisms involved is still unclear due to limitations of studies in humans. We have used a longitudinal, translational macaque model of infant MALT and reported long-term alterations in stress and emotional reactivity throughout development and cocaine (COC) self-administration (SA) during adolescence. This study examined the long-term effects of infant MALT on adult (1) brain serotonin (5HT) 1A and 2A receptors and dopamine (DA) D2/D3 receptors in cortico-limbic regions involved in emotional and reward control, using positron emission tomography (PET) imaging; and (2) whether those neurochemical alterations were associated with rates of COC SA. For the PET studies we examined differences in receptor binding potential (BP) in several brain regions between adult MALT (n=13, 7M, 6F) and Control animals (n=9, 5M, 4F) using Two-Way ANOVA. When MALT effects were detected, Pearson correlations were run to examine associations between PET BP and COC SA data. We found long-term effects of infant MALT on adult brain 5HT, but not DA receptors in cortico-limbic circuits. Specifically, MALT animals had lower 5HT1A BP in the anterior cingulate cortex - ACC- ($F(1,18)=5.159$, $p=0.036$, $\eta_p^2=0.327$), medial PFC ($F(1,18)=6.132$, $p=0.023$, $\eta_p^2=0.254$)

and hippocampus ($F(1,18)=4.649$, $p=0.045$, $\eta_p^2=0.015$) compared with Controls. A MALT by Sex interaction effect was also detected in 5HT2A BP in the orbital frontal cortex -OFC- ($F(1,18)=5.159$, $p=0.036$, $\eta_p^2=0.327$), with lower levels in MALT than Control males, but not in females. In addition, Sex effects were found for D2/D3 receptor BP in the ventrolateral PFC -vlPFC- ($F(1,18)=5.052$, $p=0.037$, $\eta_p^2=0.219$), with lower levels in males than females. Finally, in preliminary studies, when males were given access to COC (0.001-0.3 mg/kg/injection) under a progressive-ratio (PR) schedule of reinforcement to measure COC reinforcing strength, MALT animals (n=3) required doses about 1.0 log-units higher than Controls (n=3) to reach peak break points. Because there were no differences in sensitivity of COC to be reinforcing, but higher doses were needed for peak effects, these findings may provide evidence for long-term alterations in reward circuitry in MALT monkeys. Overall, these findings suggest long-term effects of infant MALT on adult brain 5HT, but not DA, receptors in cortico-limbic regions regulating emotional and reward processes, particularly in the ACC, medial PFC, OFC and hippocampus, and impacts on COC SA.

Disclosures: **J.H. Acevedo-Polo:** None. **E.R. Siebert:** None. **K.A. Jenkins:** None. **R.J. Voll:** None. **L.N. Chavan:** None. **M.M. Goodman:** None. **M. Styner:** None. **L.L. Howell:** None. **J.A. Nye:** None. **M.A. Nader:** None. **M.M. Sanchez:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.19/JJ12

Topic: F.03. Stress and the Brain

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Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term effects of social status and postnatal diet on adult prefrontal cortex structure and cognitive function in female macaques

Authors: Z. KOVACS-BALINT[1], T. JONESTELLER[1], K. BAILEY[1], A. GRAY[1], A. GOPAKUMAR[1], K. SHABBIR[1], A. WANG[1], R. KIM[1], R. VLASOVA[2], M. STYNER[2], J. RAPER[1], J. BACHEVALIER[1], M. C. ALVARADO[1], ***M. SANCHEZ**[1];
[1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: There is a strong link between chronic psychosocial stress, consumption of obesogenic high calorie diets (HCD) and cognitive impairments in human populations with low socioeconomic status, although the underlying neurobiological mechanisms remain unclear. This study used a translational rhesus monkey model to examine the long-term effects of low social status on adult prefrontal cortex (PFC), hippocampus (HIPPO) and related cognitive function.For this, 27 adult female rhesus monkeys (13 dominant (DOM), 14 subordinate (SUB)) received brain T1- and T2-weighted MRI scans to examine the volume of the HIPPO, PFC (total, grey matter (GM), white matter (WM)) and PFC subregions important for executive function, working memory, reward monitoring (dorsolateral PFC(dlPFC) -Area 46-; orbitofrontal cortex(OFC) -Area 13-; medial PFC(mPFC) -Areas 14, 25-). Total intracranial volume (ICV) was included as a covariate in the ANOVA statistical models to control for individual differences in brain size. Because this study also assessed the potential synergistic effects of postnatal obesogenic diets and social status on adult PFC structure and function, half of the animals in each social rank were assigned to a low-calorie diet (LCD) and the other half to a Choice between LCD and HCD (DOM: 8 LCD, 5 Choice; SUB: 6 LCD, 8 Choice) from birth through menarche. All animals consumed an LCD diet afterwards and diet was a covariate in the statistical models. Our preliminary results show region-specific social status x laterality effects in OFC ($F(1,23)=5.905$, $p=0.023$, $\eta_p^2=0.204$) and mPFC (Area 25: $F(1,24)=4.964$, $p=0.036$, $\eta_p^2=0.171$), driven by bigger right hemisphere volumes in SUB than DOM animals. Social Rank also affected the HIPPO volume ($F(1,24)=4.824$, $p=0.038$, $\eta_p^2=0.167$) which was bigger in SUB than DOM animals, even after controlling for postnatal diet. A diet x laterality interaction effect emerged on dlPFC volume ($F(1,23)=4.791$, $p=0.036$, $\eta_p^2=0.171$) when diet was included as a covariate in the model. Overall, these findings suggest long-term effects of social status on adult prefrontal and hippocampal structure, with bigger orbitofrontal, medial prefrontal and hippocampal volumes in SUB than DOM animals. Minor long-term effects of postnatal HCD consumption were observed. Cognitive testing (attention set-shifting task, ID/ED) is underway, and we are examining functional connectivity in these neurocircuits.

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Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.20/JJ13

Topic: F.03. Stress and the Brain

Support: NIH DA052909
NIH DA038588
NIH MH078105

Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term structural effects of infant maltreatment on neurocircuits underlying adult emotional regulation: a nonhuman primate study

Authors: *R. LEBOVIC[1,2], Z. A. KOVACS-BALINT[2], B. BEESLEY[1,2], E. R. SIEBERT[2], R. VLASOVA[3], M. A. STYNER[3], L. HOWELL[2], M. A. NADER[4], A. M. KAZAMA[1,2], M. SANCHEZ[5];
[1]Emory Univ., Atlanta, GA; [2]Emory Natl. Primate Res. Ctr., Atlanta, GA; [3]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [4]Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [5]Emory Univ. Sch. of Med., Atlanta, GA

Abstract: Early life adversity, such as child maltreatment (MALT), is a risk factor for later onset of psychopathology including anxiety and substance use disorders. Understanding the underlying neurobiological mechanisms and how they unfold has been a challenge due to limitations of prospective, longitudinal studies in humans. Using a translational macaque model of infant MALT our group has reported long-term and persistent alterations in emotional reactivity during development and elevated amplitude of the startle response in adolescence and adulthood, suggesting that infant MALT affects cortico-limbic circuits underlying emotional control. The present study examined: (1) the long-term structural effects of infant MALT on adult amygdala, hippocampus and subregions of the prefrontal cortex (PFC) involved in emotional regulation; (2) whether those structural alterations were associated with the elevated startle response previously reported by our group. Brain structural MRI was used to examine volumetric differences in those brain regions between adult MALT (n=13, 7M, 6F) and Control animals (n=9, 5M, 4F) using repeated measures ANOVA. When MALT effects were detected, Pearson correlations examined associations between regional volumes and adult anxiety (measured as baseline acoustic startle amplitude). We found long-term structural effects of infant MALT on adult PFC circuits. In particular, significant Group x Sex x Laterality volumetric effects were observed in the medial PFC (mPFC; area 25 -subgenual cingulate cortex- ($F(1,18)=8.200$, $p=0.010$)) where MALT males had smaller left hemisphere volumes than controls, and in orbital frontal cortex (OFC, area 13; $F(1,18)=5.828$, $p=0.027$) and orbital periallocortex (OPAI; ($F(1,18)=6.034$, $p=0.024$)) where MALT females had larger left hemisphere volumes than Controls. A negative correlation was found between the volume of the mPFC (area 25/subgenual cingulate cortex) and baseline startle amplitude, a proxy for anxiety ($r=-0.435$, $p=0.043$), consistent with reports of smaller volumes in subgenual cingulate cortex and elevated anxiety in humans. The mPFC has been implicated in emotional regulation through mechanisms such as its inhibitory regulation of the amygdala (Likhtik et al, 2005). Overall, these findings suggest long-term, persistent effects of infant MALT on specific medial and orbital PFC regions of adult rhesus monkeys, some associated with heightened measures of anxiety. Parallel studies in the lab are examining MALT effects on functional connectivity in these neurocircuits on the same adult animals.

Disclosures: R. Lebovic: None. **Z.A. Kovacs-Balint:** None. **B. Beesley:** None. **E.R. Siebert:** None. **R. Vlasova:** None. **M.A. Styner:** None. **L. Howell:** None. **M.A. Nader:** None. **A.M. Kazama:** None. **M. Sanchez:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.21/JJ14

Topic: F.03. Stress and the Brain

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NIH Grant MH078105-01S1
NIH Grant MH091645
NIH Grant MH086633
NIH Grant U54 HD079124
Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term structural effects of chronic psychosocial stress and postnatal obesogenic diets on cortico-limbic circuits involved in social and emotional regulation in adult female macaques.

Authors: *A. GOPAKUMAR[1], A. WANG[1], Z. A. KOVACS-BALINT[1], T. JONESTELLER[1], K. BAILEY[1], A. GRAY[1], M. KYLE[1], J. GODFREY[1], K. SHABBIR[1], R. KIM[1], R. VLASOVA[2], M. STYNER[2], K. ETHUN[1], M. WILSON[1], J. BACHEVALIER[1], J. RAPER[1], M. ALVARADO[1], M. SANCHEZ[1];
[1]Emory Univ., Atlanta, GA; [2]Univ. of North Carolina, Chapel Hill, NC

Abstract: Despite the strong link between chronic psychosocial stress, consumption of obesogenic diets and mental illness in children from low socioeconomic status, the neurobiological mechanisms remain unclear. This study used a translational macaque model of social subordination to examine its long-term structural impact and potential synergistic effects of postnatal obesogenic diets on brain regions involved in social and emotional regulation. In previous reports by our lab, 41 female rhesus monkeys from Dominant (DOM) or Subordinate (SUB) social status/ranks and fed either a low-calorie diet (LCD) or a Choice diet with access to both the LCD and a high-calorie diet (HCD) received brain MRI scans longitudinally from birth through the juvenile period, and showed drastic diet-related brain size increases, though only very localized social rank effects. In the present study, a subset of 27 macaques (DOM: n=13, 7 LCD, 6 Choice; SUB: n=14, 6 LCD, 8 Choice) that were assigned to a LCD-only diet from menarche were re-scanned as adults to examine long-term structural effects of social rank and whether the postnatal obesogenic diet effects were transient, or persisted through adulthood. We found that the impact of the postnatal Choice diet on brain size did not persist in adulthood. However, more social rank effects emerged in adults, including larger volumes of social processing cortical regions such as the superior temporal sulcus -STS- ($F_{(1, 19)}=6.797$, $p=0.017$), temporo-parieto-occipital rostral -TPOr- cortex ($F_{(1, 19)}=6.932$, $p=0.016$), and the temporal auditory -Ta- cortex ($F_{(1, 19)}=5.592$, $p=0.029$) in SUB than DOM animals. Structural effects of social rank were also detected in subcortical regions important for stress/emotional regulation, including larger hippocampal volumes in SUB than DOM macaques ($F_{(1,24)}=4.824$, $p=0.038$),

and similar trend -although non-significant- in amygdala. These findings highlight the potential of brain rescue mechanisms that offset lasting effects of early-life obesogenic diets. With respect to social status, region-specific effects were detected, with larger regions involved in socioemotional processing and monitoring of faces in adult SUB than DOM animals, suggesting potentially cumulative effects of social and stressful experiences. We are currently examining physiological measures of chronic stress, inflammation and activation of the kynurenine neurotoxic metabolic pathway that may underlie the brain structural impact of social subordination. Our findings suggest the possibility of long-term neurostructural adaptations in primates to increase survival in challenging social environments.

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Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: F.03. Stress and the Brain

Support: NIH DA052909
NIH DA038588
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Emory National Primate Research Center (ENPRC) Base Grant OD P51OD011132

Title: Long-term effects of early life adversity on functional connectivity of emotional and cognitive neurocircuits in adult macaques

Authors: *V. REDDY[1,2], Z. KOVACS-BALINT[2], A. BEESLEY[2], E. R. SIEBERT[2], E. FECZKO[3], E. EARL[4], M. STYNER[5], L. HOWELL[2], M. NADER[6], D. FAIR[3], A. M. KAZAMA[2], M. SANCHEZ[2];
[1]Emory Col. of Arts and Sci., [2]Emory Univ., Atlanta, GA; [3]Univ. of Minnesota, Minneapolis, MN; [4]Oregon Hlth. & Sci. Univ., Portland, OR; [5]Univ. of North Carolina At Chapel Hill, Chapel Hill, NC; [6]Wake Forest Univ. Sch. of Med., Winston-Salem, NC

Abstract: Child Maltreatment (MALT), a devastating early life adverse experience, is a major risk factor for the development of anxiety, substance use disorder as well as cognitive deficits. Despite this link between child MALT and psychopathology, the underlying neurobiological mechanisms are poorly understood due to limitations of studies in humans. Using a longitudinal, translational rhesus monkey model of infant MALT, our lab has reported long-term and

persistent cognitive impairments and elevated baseline startle amplitude in adolescence and adulthood, suggesting that infant MALT impacts cortico-limbic circuits underlying emotional and cognitive control. This study examined the long-term effects of infant MALT on adult functional connectivity (FC) between critical regions for emotional and cognitive control - amygdala (AMY), hippocampus (HIPP), and prefrontal cortex (PFC) regions, including the medial (mPFC), orbitofrontal (OFC), dorsolateral (dlPFC), ventrolateral (vlPFC). Resting state functional MRI (rs-fMRI) scans were collected in 13 MALT animals (7M, 6F) and 9 Controls (5M, 4F) to examine FC between those regions (AMY-AMY, AMY-PFC, AMY-HIPP, and HIPP-PFC). Our findings suggest that infant MALT did not have a long-term effect on adult AMY FC (AMY-AMY, AMY-PFC, or AMY-HIPP). This contrasts with a previous publication from this cohort of animals during infancy and the juvenile periods, where weaker AMY-PFC FC was reported in MALT than Control animals; these results suggest transient effects of MALT on AMY FC underlying emotional regulation with recovery by adulthood. However, we found significant long-term effects of MALT in adult HIPP-PFC FC. Specifically, MALT by Sex interaction effects were found in HIPP-dlPFC(Area9) FC ($F(1,22)=5.365$, $p=0.033$), and a MALT by Sex by Hemisphere interaction in HIPP-vlPFC(Area 45) ($F(1,22)=5.703$, $p=0.028$); in both cases MALT females showed weaker negative FC than Control females, whereas the opposite directionality was observed in males. Surprisingly, elevated adult startle in MALT animals was not associated with differences in the cortico-limbic circuits studied. Our findings suggest long-term effects of infant MALT on specific HIPP-PFC circuits involved on executive cognitive control, but not on AMY-PFC FC circuits involved in emotional regulation of adult rhesus monkeys. Our studies to date suggest that some effects of infant MALT on functional connectivity of these cortico-limbic circuits are temporary/transient, while others (in PFC-HIPP cognitive control circuits) are long-term.

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Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.23/JJ16

Topic: F.03. Stress and the Brain

Support: NIMH R01 MH120065

Title: Childhood threat is associated with stressor-evoked paraventricular nucleus of the hypothalamus and ventral bed nucleus of the stria terminalis activity: a 7 tesla study

Authors: B. M. SIBBACH[1], H. T. KARIM[2], D. LO[1], *L. BANIHASHEMI[2];
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Abstract: Childhood adversity dysregulates stress reactivity, however underlying neural mechanisms are unclear. Subcortical regions proximal to stress control have been less studied with human MRI due to their size and location. The paraventricular nucleus of the hypothalamus (PVN) is uniquely capable of proximal control over autonomic and neuroendocrine stress responses. The bed nucleus of the stria terminalis (BNST) directly modulates PVN function and plays an important role in stress control itself. The dorsal (d) BNST is predominantly preautonomic, while the ventral (v) BNST is predominantly viscerosensory/noradrenergic. Our goal was to identify the dBNST, vBNST and PVN using probabilistic atlases, and examine relationships between threat and deprivation dimensions of childhood adversity and stressor-evoked activity. Participants were young adults (mean age=26.4) from a transdiagnostic, abuse-enriched sample. Probabilistic atlases were derived from manual segmentations using 7 Tesla structural (MPRAGE) and susceptibility-weighted imaging (n=25). Stressor-evoked activity was elicited via an adapted, performance-titrated Multisource Interference Task (mild cognitive stress) and parameter estimates were extracted with the probabilistic atlases (20% threshold) inverse normalized (n=99). Multiple hierarchical regression analyses were performed covarying for age, sex and race, examining childhood threat (Childhood Trauma Questionnaire abuse) and socioeconomic deprivation (parental education level reverse coded). We also evaluated whether findings survived adulthood variables (traumatic events, socioeconomic status, negative life events). Curvilinear relationships were examined using squared terms for threat and deprivation variables. Regression analyses revealed a significant curvilinear relationship between childhood threat and PVN stressor-evoked activity (standardized Beta = 1.236, $p = 0.026$), remaining significant when adulthood variables were included in the model (st B = 1.213, $p = 0.028$). A significant linear relationship was found between childhood threat and vBNST stressor-evoked activity (st B = 0.248, $p = 0.039$) in the full model including adulthood variables. No relationships were found with socioeconomic deprivation. Childhood threat was associated with PVN (curvilinear, with midpoint being moderate severity) and vBNST (linear) stressor-evoked activity, indicating potential thresholds at which childhood threat exposures may contribute to adaptive or maladaptive stress responses. These findings may provide novel neural insights into childhood adversity-related risks for affective symptoms.

Disclosures: B.M. Sibbach: None. **H.T. Karim:** None. **D. Lo:** None. **L. Banihashemi:** None.

Poster

PSTR354. Effects of Early-Life Stress, Adversity, and Insults on the Brain and Behavior

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR354.24/JJ17

Topic: F.03. Stress and the Brain

Support: A. James & Alice B. Clark Foundation

Title: The effects of a prenatal mindfulness intervention on maternal distress reduction, infant brain and behavioral development during the COVID-19 pandemic

Authors: *S. WEINER[1,2], Y. WU[2], N. ANDESCAVAGE[2], D. HENDERSON[2], C. LIMPEROPOULOS[2,3];
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Abstract: Introduction: Prenatal maternal psychological distress has been on the rise since the onset of the COVID-19 pandemic and has been associated with impaired maternal-infant outcomes. This study examined the impact of a novel virtual prenatal stress reduction intervention on maternal stress symptoms in pregnancy and subsequent neonatal development during the COVID-19 pandemic.
Methods: We prospectively enrolled pregnant women during the COVID-19 pandemic into a randomized controlled trial to assess the efficacy of mindfulness interventions in decreasing maternal distress. Participants were randomized to a virtual weekly prenatal yoga and guided mindfulness intervention for 5 versus 10 weeks. Prenatal maternal anxiety, depression, and stress were measured using the Spielberger State-Trait Anxiety Inventory (SSAI/STAI), the Center for Epidemiological Studies-Depression, and the Perceived Stress Scale (PSS). biweekly from enrollment to birth. Neonates underwent non-sedated brain MRI (3 Tesla MRI scanner -GE Discovery MR750). Neonatal brain images were segmented using a 3D U-Net-based method followed by manual correction to derive global and tissue-specific volumes. The NNNS and the Ages and Stages Questionnaire (ASQ) were utilized to measure infant neurodevelopmental outcomes.
Results: We studied 94 pregnant women during the pandemic for maternal distress throughout their pregnancy. Maternal anxiety and stress scores decreased over time as measured by the SSAI, STAI, and PSS ($p<0.001$, $p=0.002$, and $p=0.007$), respectively, regardless of the intervention group. Compared to the 5-week group, infants of mothers in the 10-week group showed increased volumes in total brain ($p=0.01$) and cortical gray matter ($p=0.01$). Infants of mothers in the 10-week group showed increased attention ($p=0.0035$) and self-regulation ($p=0.0007$) on the NNNS and decreased arousal ($p=0.0015$) and excitability ($p<0.0001$). Infants from the 10-week cohort also showed higher communication and problem-solving scores at the 6-month ASQ ($p=0.0077$ and $p=0.04$) vs. the 5-week group.
Conclusion: We report that prenatal maternal psychological distress during the COVID-19 pandemic was reduced by implementing mindfulness/prenatal yoga activities and had a significant impact on infant brain and neurobehavioral development.

Disclosures: S. Weiner: None. **Y. Wu:** None. **N. Andescavage:** None. **D. Henderson:** None. **C. Limperopoulos:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.01/JJ18

Topic: B.07. Network Interactions

Support: R21 DA055166
R01 NS126816

Title: Lateralized vagus nerve stimulation activates differential neuromodulatory networks .

Authors: *N. KOPCHENKO[1], C. THORN[2];
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Abstract: Left vagus nerve stimulation (VNS) has been clinically approved for the treatment of several neurological disorders including epilepsy, major depressive disorder, stroke, and spinal cord injury. Previous studies have shown that stimulation of the right or left cervical vagus nerve differentially promotes the activation of pro-plasticity neuromodulatory networks affected by these disorders. It has been shown that right, but not left, VNS activates midbrain dopaminergic nuclei. Precisely timed activation of these differential neuromodulatory networks could potentially be used to treat different disorders related to dopamine dysregulation. To begin to assess the functional relevance of lateralized VNS-driven midbrain activation, we tested whether right or left VNS resulted in changes in locomotor activity in an open field. We implanted adult male (n = 8) and female (n = 8) Long Evans rats with VNS cuff electrodes around either the right or left cervical vagus nerve and administered brief bursts of stimulation (train duration = 0.5 sec; amplitude = 0.8 mA; pulse frequency = 30 Hz) as animals freely explored an open field. We then performed immunohistochemical analyses to examine neuronal activation patterns in the locus coeruleus and in the midbrain. Brains were stained for c-Fos and tyrosine hydroxylase and c-Fos expression was compared between right and left VNS treatment groups. Understanding the differential functional effects of right vs. left VNS on locomotor activity and midbrain dopaminergic signaling will inform the development of improved VNS strategies to modulate these networks to treat neurological disorders.

Disclosures: N. kopchenko: None. **C. Thorn:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.02/JJ19

Topic: B.07. Network Interactions

Support: BRAIN initiative Grant U19 NS107464-01
DIRP, NIMH, USA, ZIAMH002797

Title: Ketamine administration in mouse model for depression elicits a delayed rescue of critical dynamics

Authors: *P. KELLS, Y. BIBINEYSHVILI, V. SINFUEGO, T. RIBEIRO, D. PLENZ;
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Abstract: Treatment resistant depression (TRD) is a mental disorder affecting an estimated 90 million people worldwide,. As an effective treatment for TRD, the anesthetic Ketamine received breakthrough therapy status in 2019. However, the neural mechanisms by which ketamine alleviates depression are unknown. Here, using the corticosterone (CORT) animal model for depression, we assess the effects of ketamine on depressed mice through the lens of critical dynamics. We infected adult male mice (N=10; >P35; C57BL/6) with a viral vector to express the red-shifted GECI jRGECO1a in medial prefrontal cortex (mPFC) neurons. A dorsal cranial window combined with a chronically implanted microprism was used to image across the midline in the contralateral mPFC. We used 2-photon imaging (2PI) to record neurons in layer 2/3 of mPFC (45Hz frame rate). Raw 2PI movies were motion corrected, denoised, and subsequent neuropil subtracted fluorescence traces were deconvolved to obtain spike probabilities.Animals were divided equally into 2 cohorts. After 4-5 baseline recordings animals received ad libitum access to either fresh drinking water (control, CONT) or water containing CORT at 0.1mg/mL+1% ethanol (depressed, DEP). Recordings were conducted over 3-weeks of CORT treatment after which a 10mg/kg subanesthetic dose of ketamine was administered and animals were recorded 1hr, 1d, 3d, and 7d post-injection. Initial assessment of network spiking statistics revealed normal spike rates (lognormally distributed around 1 spike/s) and mean pairwise correlations (~0.02±0.01). Avalanche statistics were quantified by slope analysis of avalanche size and duration distributions (alpha and beta, respectively) as well as <size> vs. duration scaling exponent (chi).Ongoing activity revealed neuronal avalanche characterized by scale-invariant distributions of synchronized neuronal populations with significant numbers of system-wide population events. CONT animals displayed stable spikerate, mean pairwise correlations, and avalanche statistics (alpha -3/2, beta -2, and chi 2) across all recordings. In contrast, DEP animals displayed stable mean pairwise correlation and avalanche statistics across baseline recordings, which decreased across CORT recordings (alpha<-3/2, beta<-2, chi<2) suggesting a reduction in correlated population events. Avalanche statistics and correlations were rescued at 3- and 7-days after ketamine injections. Our results demonstrate that a single subanesthetic dose of ketamine can rescue avalanche dynamics in the prefrontal cortex of depressed animals. We suggest that ketamine improves TRD by re-establishing a critical state in frontal cortex.

Disclosures: P. Kells: None. **Y. Bibineyshvili:** None. **V. Sinfuego:** None. **T. Ribeiro:** None. **D. Plenz:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.03/JJ20

Topic: F.03. Stress and the Brain

Support: NIMH RO1MH096093
Harvey Family Endowment

Title: Reconfiguration of adult brain-state dynamics in response to acute threat after ELA

Authors: *T. W. USELMAN[1], R. E. JACOBS[2], E. L. BEARER[1,3];
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Abstract: Early life adversity (ELA) increases vulnerability to neuropsychiatric disorders. How fragmented maternal care, a type of ELA, affects the brain to lead to mental illness or addiction in adulthood is an area of intense investigation. Manganese-enhanced magnetic resonance imaging (MEMRI) offers an opportunity to define brain states and their dynamics in adults who have experienced ELA and compare them to normal rearing. Mn(II) is a paramagnetic ion that produces a hyperintense signal by T_1-weighted MRI. Systemic Mn(II) enters active neurons of awake-behaving mice via voltage-gated calcium channels and is imaged retrospectively. Enhancements from an accumulation of Mn(II) represent a composite picture of neural activity occurring between systemic delivery and imaging. Combined with advanced data-driven computational processing, MEMRI reveals regional activity brain-wide within and between networks. To model ELA, dams were deprived of adequate bedding from P2-9 (n=12). Adult pups (10 weeks) received $MnCl_2$ (0.3 mmol/kg, i.p.) and were returned to their home cage. Mice were imaged before $MnCl_2$, at 22h, short and long-term after an acute ethological threat (predator odor with TMT, 2,3,5-Trimethyl-3-thiazoline). After 8 days, the pre-Mn-post-Mn imaging sequence was repeated without a threat to rule out any residual Mn(II) and detect ongoing activity. MR images were preprocessed with our skull-stripping/registration pipeline and aligned images were analyzed using a suite of existing and novel data-driven voxel- and segment-wise algorithms. Segment-wise values were extracted based on our digital InVivo Atlas. Relative amounts of activation volumes within 96 segments at each condition, “brain state”, showed dynamic changes between segments within networks that differed in ELA compared to normally reared mice. Many theories of ELA effects on subregions and networks have been proposed. We further tested these theories by Mn(II) enhancements in defensive and reward circuitry with structural equation modeling (SEM). Compared to normal rearing, prior to threat, ELA increased signal in stress- and reward-related regions. After threat, new hyperactivity emerged in the locus coeruleus (LC). At 9 days post-threat, the prefrontal cortex (PFC) after ELA was hypoactive compared to normals. Data-driven coactivation analysis found differences in the amount of signal across multiple brain systems in ELA compared to normals across conditions. Thus, adult brain states and their dynamics in response to threat are reconfigured by ELA.

Disclosures: T.W. Uselman: None. **R.E. Jacobs:** None. **E.L. Bearer:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.04/JJ21

Topic: F.03. Stress and the Brain

Support: NIMH RO1MH096093
Harvey Family Endowment

Title: Acute Noradrenergic Stimulation Influences Brain-States

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Abstract: Brain-wide neural activity adapts to conditions and is influenced by the central noradrenergic system (CNA). CNA neurons in locus coeruleus (LC) modulate activity in distant brain regions within overlapping threat, reward, and arousal circuits. CNA is a target of cocaine and psychopharmaceuticals. Our hypothesis is that brief LC activation and increased CNA modulatory tone increases activity acutely in threat-mediating regions and promotes a longer-term shift in brain-state. Here, we utilize longitudinal manganese-enhanced MRI (MEMRI) coupled with chemogenetic LC activation (hM3Dq) to investigate short and longer-term brain states in response to shifting CNA modulation. Paramagnetic Mn(II) (0.3 mmol/kg, i.p.) highlights brain activity of awake normally moving mice by T1-weighted MRI. Naïve C57BL6J mice underwent stereotactic injection of CAV2-PRS-hM3D(Gq)-mCherry targeting bilateral LC (AP,-5.4; ML,±0.80; DV,-3.80). After injection (4-6w) mice were imaged for Mn(II) accumulation before and immediately after two doses 3h apart of clozapine-n-oxide (CNO)(5 mg/kg, i.p.), an activator of hM3Dq. To capture longer-term shifts in brain state, hM3Dq mice were imaged again nine days later. A control group was imaged using similar MEMRI procedures to examine acute effects of CNO without hM3Dq transfection. The effect of LC activation was monitored by pupillary light response to CNO. Microscopy of brains sectioned through LC confirmed bilateral neuron-specific mCherry expression. We tested pupillary light response and exploration after CNO±hM3Dq transfection. Statistical parametric mapping of our MEMRI images showed CNO increased brain-wide Mn(II)-enhanced intensity in specific subregions. hM3Dq transfection heightened activity in limbic regions and in LC, confirming stimulation. By day 9, activity persisted within many regions, accompanied by a newly heightened signal in the hippocampus. Segmentation and bootstrap resampled cross-correlation analysis of 96 brain regions revealed increased integration of basal brain state subnetworks. These initial results emphasize the broader significance of acute LC activation, as it influences both immediate brain-state changes and sets the stage for longer-term evolution within deep brain structures.

Disclosures: E.L. Bearer: None. **R.E. Jacobs:** None. **T.W. Uselman:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.05/JJ22

Topic: B.02. Transmitter Receptors and Ligand-Gated Ion Channels

Support: NSF 2015276

Title: Beta1-adrenergic receptormediated activation of fast-spiking putative inhibitory neurons impairs delay-related firing

Authors: *S. YANG[1], M. JOYCE[2], K. MORIN[2], D. DATTA[2], J. ARELLANO[2], A. DUQUE[3], M. WANG[5], A. F. ARNSTEN[4];
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Abstract: Title: Beta1-adrenergic receptor mediated activation of fast-spiking putative inhibitory neurons may impair delay-related firingAuthors: Yang S, Joyce MKP, Morin K, Datta D, Arellano J, Duque A, Wang M, Arnsten AFT Abstract: The dorsolateral prefrontal cortex (dlPFC) is necessary for working memory and contains layer III pyramidal cells that fire persistently during working memory delays. Working memory behavior and delay-related firing are sensitive to circulating stress neuromodulators, and high levels of dopamine and norepinephrine (NE) rapidly silence dlPFC pyramidal neuron activity and impair working memory performance. In pyramidal neurons, stress-related effects are triggered by metabotropic receptor-induced calcium-mediated opening of potassium channels that weaken delay-related firing. However, the extent to which catecholamines also interact with inhibitory neurons in layer III dlPFC microcircuitry remains an open question particularly the parvalbumin (PV) neurons that are thought to play a major role in spatial tuning of pyramidal neurons that exhibit delay-related firing. One receptor that mediates the stress response is the beta1-adrenergic receptor (β1-AR), but it is unknown if β1-ARs are expressed on interneurons as well as pyramidal cells, and how β1-ARs affect fast-spiking putative PV inhibitory neuron activity in layer III dlPFC *in vivo* during a spatial working memory task. Here, we addressed these questions using immunohistochemistry paired with confocal and electron microscopy to examine the distribution of β1-AR in layer III dlPFC inhibitory neurons of two female macaques (aged 8-10 years). The calcium-binding proteins PV, calbindin (CB), and calretinin (CR) are largely non-overlapping and label almost all cortical inhibitory neurons in dlPFC. We found that β1-AR are robustly expressed in the somata of PV neurons, and also in CB and CR neurons of layer III dlPFC. Single label immunoelectron microscopy evidence confirmed robust β1-AR expression in aspiny inhibitory-like dendritic shafts on layer III dlPFC. These results were consistent with iontophoretic, single unit recordings from the dlPFC of monkeys performing a working memory task. These results showed that fast-spiking, putative PV inhibitory neurons showed increased firing with local application of a β1-AR agonist. As high levels of NE engage β1-AR during stress, β1-AR activation of fast-spiking putative PV neurons in dlPFC may contribute to attenuation of delay-related firing in pyramidal neuron assemblies, and rapid silencing of neuronal assemblies that subserve cognitive functions.

Disclosures: S. Yang: None. **M. Joyce:** None. **K. Morin:** None. **D. Datta:** None. **J. Arellano:** None. **A. Duque:** None. **M. Wang:** None. **A.F. Arnsten:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.06/JJ23

Topic: F.03. Stress and the Brain

Support: Taiwan MOST 108-2320-B-003 -006 -MY3

Title: Enhancing GABAergic efficacy for adolescent anxiety through NKCC1 inhibition

Authors: *W.-H. LIN[1], Y.-H. TUNG[1], Z.-S. WU[1], P.-K. CHANG[1], S.-T. YANG[1], Y.-L. YANG[2], K.-T. LU[1];
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Abstract: Enhancing GABAergic efficacy by activating GABA receptors and their inhibitory signaling was a commonly used strategy for confronting anxiety disorders. However, the existing prescriptions, such as benzodiazepine, were often reported to be low efficacy and have been linked to numerous side effects and addiction potential. It's an urgent need to improve approaches to achieve anxiety relief. Our research revealed the underlying mechanism of neural hyperexcitability and GABAergic system dysfunction in the anxiety-like animal model. We further proved the combining usage of NKCC1 inhibitors with GABAergic treatment could lead to more effective treatment outcomes for anxiety disorders. Adolescent anxiety poses a significant challenge, which was in the developmental stage and was particularly sensitive with susceptible to long-lasting effects. In this study, we utilized an anxiety-like animal model, the juvenile immobilization treatment (J_IMO), in male C57BJ/6 mice (5 weeks old) and which was observed abnormalities in learning and memory. Following J_IMO treatment, mice exhibited anxiety-like behaviors in open field tests (OFT), and aberrant learning in extinction blockage was observed using inhibitory avoidance (IA). Electrophysiological recordings in the hippocampal Schaffer-collateral pathway revealed significantly increased long-term potentiation (LTP) signals in J_IMO-treated mice compared to the control group. Following the input/output (I/O) curve examination, it suggested post-synaptic dysregulation was involved. Subsequent qPCR and western blot analyses demonstrated no excitatory glutamatergic (*Grin2a* and *Gria1*) or inhibitory GABAergic, (*Gabra1* and *Slc12a5)* but only *Slc12a2* expression showing significant upregulation. To validate NKCC1 (*Slc12a2*) as a therapeutic target for anxiety, we administered NKCC1 inhibitors, bumetanide or furosemide, then confirmed its effectiveness in reducing hippocampal hyperexcitability (LTP), restoring anxiety behaviors in the OFT, and normalizing abnormal extinction blockage in IA. Furthermore, with the administration of a GABA agonist, isoguvacine, we revealed significantly attenuated GABAergic inhibitory effects in the hippocampus of J_IMO-treated mice and that could be restored by NKCC1 inhibition, which explained the limited efficacy of only benzodiazepine treatment. By targeting NKCC1, we not only rescued anxiety-like behaviors but also reduced neural hyperexcitability by restoring GABAergic inhibitory signaling. Combining NKCC1 inhibitors with enhancing GABAergic treatment could lead to more effective treatment outcomes for anxiety disorders.

Disclosures: **W. Lin:** None. **Y. Tung:** None. **Z. Wu:** None. **P. Chang:** None. **S. Yang:** None. **Y. Yang:** None. **K. Lu:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.07/JJ24

Topic: F.03. Stress and the Brain

Support: CONACYT national scholarship 1159644

Title: Effect of the hypercaloric diet during pregnancy and lactation on the anxiety-like behavior of adult male offspring

Authors: *D. RENDÓN CORIA[1], G. A. CORIA-AVILA[2], M. E. HERNÁNDEZ-AGUILAR[2], G. E. ARANDA-ABREU[2], D. HERRERA-COVARRUBIAS[2];
[1]Univ. of Veracruz, Xalapa, Mexico; [2]Univ. Veracruzana, Xalapa, Mexico

Abstract: The diet we consume plays a vital role in maintaining our health. Given the current prevalence of high-calorie food consumption, it is important to consider the long-term effects of hypercaloric diet during critical periods of brain development on motivated behavior of the offspring, particularly during the transition into adulthood. Accordingly, we explored the effect of a hypercaloric diet during pregnancy and lactation on the anxiety index of puberal male offspring. For this study two groups of Wistar females were formed (n=5): 1) females fed with a cafeteria-type hypercaloric diet (HD, with +80% increase in calorie intake) and 2) control females that received standard laboratory feed (SD). Both groups received their diet throughout an 8-week period that included 2 weeks prior to gestation, 3 weeks during gestation and 3 weeks of lactation. Body weight of the dams and their male pups was recorded weekly during 12 postnatal weeks (PW). Then on PW6 (45 days) we assessed the males' anxiety index in the elevated plus-maze. Paradoxically, the results showed that both groups of females gained equivalent body weight during pregnancy. In addition, they both delivered similar litter size (SD mean=10) (HD mean=10.2). Interestingly, male pups from SD mothers expressed significantly more body weight at PW6, 7, 11 and 12 and displayed higher anxiety index. Our findings suggest that, maternal hypercaloric diet modulates the offspring body weight and the expression of adult offspring behavior. We discuss the possible role on reward and effects.

Disclosures: **D. Rendón Coria:** None. **G.A. Coria-Avila:** None. **M.E. Hernández-Aguilar:** None. **G.E. Aranda-Abreu:** None. **D. Herrera-Covarrubias:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.08/JJ25

Topic: H.08. Learning and Memory

Support: NRF Grant 2017R1D1A1B05036195
NRF Grant RS-2023-00245605

Title: Impact of 5/6 nephrectomy-induced chronic kidney disease on emotional function and cognitive behavior

Authors: *H. IM, Y. YU, G. KIM, Y. LEE, D. PARK, D. KIM;
Soonchunhyang university, Cheonan, Korea, Republic of

Abstract: Chronic kidney disease (CKD) is affected the structure and function of the kidney, decreased glomerular filtration rate or increased albumin excretion in urine. Psychological distress and depression in patients with CKD are serious problems, and neurological disorders are prevalent in patients with CKD. Vascular factors and uremic toxins are associated with cognitive impairment in CKD. Additionally, structural and functional changes in the hippocampus induced by vascular dementia may lead to defects in hippocampal synaptic plasticity and cognitive function. However, the pathophysiology of emotional deterioration and cognitive impairment in patients with CKD is not fully understood. Therefore, this study aims to identify emotional and cognitive dysfunctions caused by CKD. First of all, We generated CKD rat models by performing 5/6 nephrectomy, and established the model based on hematological level and renal histological results. To determine whether CKD affects to the locomotion, the anxiety disorders and the cognitive deficits, We performed behavioral analyzes to related to these in a CKD rat model, which was tested using various in vivo electrophysiological parameters. In addition, histological experiments was performed to confirm the functional changes of uremic toxin-induced acidosis and blood-brain barrier (BBB) damage in the hippocampus of CKD rats. Moreover, We measured hippocampal volume based on ESRD patients with cognitive impairment. Therefore, this findings indicate that uremia through declined kidney function may cause destruction BBB as acidosis in the brain, leading to the cognitive changes, the anxiety behaviors and the depression in the hippocampus. These abnormal symptoms can be worsened as the onset of CKD was prolonged.

Disclosures: H. Im: None. **Y. Yu:** None. **G. Kim:** None. **Y. Lee:** None. **D. Park:** None. **D. kim:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.09/KK1

Topic: F.03. Stress and the Brain

Support: Grants-in-Aid for Scientific Research No. 20K07948

Title: Social function recoveries in refractory psychiatric disorders: Behavior/neural circuit variation analysis using stem cells and Kampo medicine kamikihito

Authors: *W. UKAI[1], K. DERIHA[1,4], E. NISHIMURA[1], E. HASHIMOTO[1], M. YAMADA[5], H. HASHIGUCHI[1], S. HIROSE[1,4], M. MOCHIZUKI[1,6], Y. MOMOKI[1], K. FURUSE[1,6], T. ISHII[1,2], K. NAGAISHI[3], M. A. RIVA[7], C. KAWANISHI[1];
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Abstract: The brain pathophysiology of social dysfunctions in depression and autism spectrum disorder are complex, and there is a growing need to understand the relevant neuronal circuit alterations and to find direct and effective treatments for their recoveries. In this study, we have investigated the influences of bone marrow-derived mesenchymal stem cell (MSC) transplantation and Japanese Kampo medicine, Kamikihito on social behaviors as a novel treatment options for intractable psychiatric disorders, using refractory depression animal model related to stress vulnerability, we had preliminary established, induced by dual stress during embryonic/juvenile period (Furuse et al., 2019). We explored the possibility that MSC transplantation, which we have demonstrated the recovery effect on behavioral abnormalities in refractory depression model (Kigawa et al., 2014), and Kamikihito that has been used for anxiety and nervousness in clinics and recently reported to induce changes in emotional and social functions via oxytocinergic neuron enhancement (Tsukada et al., 2021), may promote neural repair and regeneration in brain regions related to social cognition and empathy, which in turn may enhance recovery for social dysfunctions. In the refractory depression model, active social interaction behavior, as indicated in the social interaction test, was reduced by approximately 50%. In contrast, treatments of MSC alone or MSC and kamikihito significantly improved social interaction behavior, which was reduced in the model animal. On the other hand, no such effects were observed in the group treated with kamikihito alone. In the model group, contact time with the restrained individual and empathy-like voluntary behavior as indicated by rescue behavior were reduced by approximately 40%. Interestingly, the reduction of empathy-like behavior in the model group was significantly improved in the groups treated with kamikihito alone and in the group treated with kamikihito and MSC. On the other hand, MSC monotreatment group could not show any effect. We would add some data of analyzing changes of dynamics of oxytocinergic neurons from the hypothalamic paraventricular nucleus, Parvalbumin (PV)/somatostatin (SS) positive GABA interneuron changes in brain regions including the cingulate cortex, amygdala, and hippocampus, and excitatory/inhibitory neuron balance changes in each animal group, and identify brain circuit mutations that lead to social cognitive behavioral abnormalities for revealing the mechanisms of their recoveries.

Disclosures: W. Ukai: None. **K. Deriha:** None. **E. Nishimura:** None. **E. Hashimoto:** None. **M. Yamada:** None. **H. Hashiguchi:** None. **S. Hirose:** None. **M. Mochizuki:** None. **Y. Momoki:** None. **K. Furuse:** None. **T. Ishii:** None. **K. Nagaishi:** None. **M.A. Riva:** None. **C. Kawanishi:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.10/KK2

Topic: F.03. Stress and the Brain

Support: Emmanuel College

Title: Male mice are more susceptible to developmental stress in a two-hit paradigm: effects on adult mood-related behavior, social behavior and glucocorticoid receptor expression

Authors: *A. P. GEMOS, C. E. DONAHOE, S. G. DANIEL, I. CRAUS, Z. M. PALERMO, N. LUPU, S. T. ZDON, M. P. LEUSSIS;
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Abstract: Early adversity is known to increase the likelihood of developing mental illness later in life, but there is less research examining the possible cumulative impact of multiple early adverse events. This study focused on the long-term effects of a two-hit model composed of stressors during childhood and adolescent development and evaluated behaviors related to mood and sociability in adulthood in both males and females. Maternal separation was used to model early adversity and stressful events in childhood, while social isolation was used as a stressor in adolescence. Male and female CD-1 mice were exposed to either maternal separation (MS; PND 2-12), adolescent social isolation (ASI; PND 35-56), a combination of both (MS-ASI), while controls experienced no stress CON. Overall, results suggest males are more susceptible to developmental stress exposure than females across multiple behavioral tests. For example, we found higher anxiety in all three developmentally stressed male groups compared to controls in the elevated plus maze (EPM total time in open arms, $p=0.003$) with a similar but not significant pattern exhibited in the novelty suppressed feeding paradigm for latency to eat. In the social preference test, once again males were more affected, as developmentally stressed male groups showed higher preference for social contact in a three-chamber test compared to controls, while in the females there was no effect of treatment condition (treatment effect on time near social cup, $p=0.037$; sex effect for time near social cup, $p<0.001$). Treatments did not appear to impact measures of depression in either the forced swim test or sucrose preference test. Further, there was no overall impact of treatment on locomotor behavior in an open field. Ongoing analyses seek to assess potential changes in glucocorticoid receptor protein levels in the hippocampus following the developmental stress treatments to assess whether similar sex differences can be detected in the brain.

Disclosures: A.P. Gemos: None. **C.E. Donahoe:** None. **S.G. Daniel:** None. **I. Craus:** None. **Z.M. Palermo:** None. **N. Lupu:** None. **S.T. Zdon:** None. **M.P. Leussis:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.11/KK3

Topic: F.03. Stress and the Brain

Support: Medical Research Council (MRC) DTP QIBIOMED studentship grant

Title: The atypical antidepressant tianeptine reverses the impairment of sociability and social recognition memory caused by acute & chronic stress in mice

Authors: *F. J. MCENANEY, J. J. LAMBERT, S. J. MARTIN;
Cell. and Systems Med., Univ. of Dundee, Dundee, United Kingdom

Abstract: Tianeptine is an atypical antidepressant and opioid receptor agonist that can improve depressive behaviours in humans and animals after chronic administration for as little as 1 week. Acute administration can reverse stress-induced impairments in synaptic plasticity and memory formation. As chronic or early-life stress can cause social anhedonia in humans, we examined how tianeptine impacts the decrease in sociability caused by early life adversity (ELA) in mice using the limited bedding/nesting model. We also examined the ability of tianeptine to prevent the deficit in long-term social recognition memory caused by acute post-training stress. From P2-9, C57BL6/J dams and pups were placed in cages with reduced bedding on a metal mesh platform (ELA group; n=9 dams) or in standard cages (control group; n = 5 dams). Dam sortie count and pup weight during development were measured. ELA dams had significantly increased sortie numbers and ELA pups had significantly decreased weight until adulthood. At 10 weeks, male and female offspring were given subcutaneous tianeptine (30 mg/kg) or vehicle (saline) injections 2x daily for 7 days (control + tianeptine: n=13; ELA + tianeptine: n=14; control + vehicle: n=11; ELA + vehicle: n=13). Additional ELA groups received tianeptine (n=12) or tianeptine + cyprodime (a μ-opioid receptor (μ-OR)-selective antagonist; 10 mg/kg; n=13). ELA reduced social interaction and social recognition memory in a 3-chamber sociability task, actions that were reversed by tianeptine; this effect was blocked when tianeptine was co-administered with cyprodime. No sex differences were observed. For the acute stress experiments, adult male and female mice (8-12 weeks) received a single 10mg/kg dose injection of tianeptine (n=10), vehicle (n=10), tianeptine + cyprodime (n=10) or cyprodime (n=10) 15 minutes before the sample phase of a social recognition memory task. Mice were then placed on an elevated platform (15 x 15cm; height = 1m) for 30 min. Platform stress caused a reduction in social recognition memory tested 24 h later. Tianeptine restored social recognition memory to control levels, an action that was blocked by cyprodime co-administration. No sex differences were observed. All data were analysed using unpaired t-tests or repeated measures ANOVA. The experimenter was blind to the drug administered. These results indicate that tianeptine administration can ameliorate the impact of both early-life and acute stress on social behaviour in mice. These findings may have implications for our understanding of the role of opioid-receptor signalling in social anhedonia and resilience in the face of environmental stressors.

Disclosures: F.J. McEnaney: None. **J.J. Lambert:** None. **S.J. Martin:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.12/KK4

Topic: F.04. Neuroimmunology

Support: Brain & Behavior Research Foundation Young Investigator Grant # 29185 (BC)
(NIMH) Grant No. NIH R01- MH111751-01 (SB)
R01MH111751 (SB)

Title: A sexually dimorphic CRISPR-based rat model of stress vulnerability

Authors: *B. CORBETT[1], S. M. LUZ[2], V. ESTELA-PRO[3], S. BHATNAGAR[4];
[1]Rutgers-Camden: Rutgers Univ. Camden, Camden, NJ; [3]Critical Care and Anesthesiol., [4]Anesthesiol. and Critical Care, [2]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: It is well established that glucocorticoid receptors (GRs) bind DNA, regulate gene expression, reduce inflammatory processes, and modulate behavior. However, the precise loci bound by GRs that are necessary for these effects *in vivo* are not fully understood. In previous work, we showed that the expression of sphingosine-1-phosphate receptor 3 (S1PR3) was increased in the medial prefrontal cortex of resilient rats and promoted behavioral resilience. The expression of S1PR3 was increased by GRs. Here, we deleted the GR binding site near the sphingosine-1-phosphate receptor 3 gene using a CRISPR/Cas9 approach ($S1PR3^{GR-/GR-}$ rats). Defeated $S1PR3^{GR-/GR-}$ males displayed increased inflammatory markers and social anxiety-like behavior. Similar effects were observed in non-stressed females, indicating a greater dependence for GR-induced S1PR3 in females. Coherent neural activity between the locus coeruleus (LC) and medial prefrontal cortex (mPFC) was increased in $S1PR3^{GR-/GR-}$ males following 7 defeats. Chemogenetically inhibiting mPFC-projecting LC neurons during defeat increased subsequent social interaction in wild-type and $S1PR3^{GR-/GR-}$ males. Together, these findings demonstrate that GR-induced S1PR3 promotes resilience by mitigating stress-induced inflammatory processes and LC-mPFC coherence and indicate a specific role for GRs in regulating S1PR3 expression.

Disclosures: B. Corbett: None. **S.M. Luz:** None. **V. Estela-Pro:** None. **S. Bhatnagar:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.13/KK5

Topic: F.03. Stress and the Brain

Title: Differential effects between two early life stress exposure models and SSRIs on Depressive-like Behaviors and Pentylenetetrazole-Induced Seizures.

Authors: *S. SIMON;
Lab. de Neurofisiología del Control y la Regulación, Dirección de Neurociencias., Inst. Nacional de Psiquiatría Ramón de la Fuente Muñiz, México, Mexico

Abstract: Simon-Medrano Stephanie, Castillo-Villegas, Diana Larissa Loe-Martínez Malery, Valdés-Cruz Alejandro.Simon-Medrano Stephanie, Castillo-Villegas, Diana Larissa Loe-Martínez Malery, Romero-Elizalde Ilse Julia, Valdés-Cruz Alejandro. Depression-epilepsy is a complex and bidirectional comorbidity, leading severe seizures and a lower quality of life. Selective serotonin reuptake inhibitors (SSRIs), such as Fluoxetine (FLX) and Citalopram (CIT), are primary treatment for depression. Controversy surrounds SSRIs in epilepsy due to conflicting reports on their impact on seizure severity and frequency. Animal models like chronic unpredictable stress (CUS) and early-life social isolation (SI) induce depressive-like behavior in rodents. CUS exposes animals to stressors simulating daily adversities, while SI replicates adverse experiences during early life, such as neglect and trauma. Pentylenetetrazole (PTZ) administration, which induces generalized tonic-clonic seizures (GTCS) and status epilepticus, is a model of epileptogenesis. By concurrently using models of GTCS and depressive-like behavior induced an ecological model of comorbidity. This study aims to compare the effects of CUS and SI models, as well as treatment with saline solution (SS), FLX, and CIT, on depressive behavior induction and epileptogenesis. For measure the depressive behaviors, anhedonia, and hopelessness, we used sucrose preference test (SPT) and the forced swim test (FST). Stainless-steel electrodes were placed in dorsal dentate gyrus area of the hippocampus (HipD), prefrontal cortex (PFC) and occipital cortex bilaterally. PTZ administered at 20 mg/kg initially, followed by 10 mg/kg every 10 minutes until SE. EEG recorded from HipD and PFC. Results indicated that CUS increased GTCS severity and latency to status epilepticus, resulting in longer cumulative seizure duration but a lower seizure count. SI, on the other hand, led to a higher number and longer duration of GTCS. FLX treatment not only alleviated depressive behaviors but also exerted a preventive effect on seizure severity and latency to status epilepticus in both models. In contrast, CIT administration increased seizure latency and the number of paroxysms while decreasing the number of GTCS. However, FLX administration in rats without depressive model induction resulted in increased GTCS duration. These findings suggest differential effects of CUS and SI on GTCS duration and maintenance processes. Moreover, FLX exhibited a protective effect against the onset of status epilepticus in the presence of depressive processes, potentially by interfering with the mechanisms underlying GTCS maintenance.

Disclosures: S. Simon: None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.14/KK6

Topic: F.02. Neuroendrocrine Processes and Behavior

Support: The Abramson Fund
The ABCD Charitable Trust

Title: Ankyrin-g heterozygous knockout mice with social defeat stress as a model for gene-environment interaction in depression

Authors: *J. LI[1], J. KIM[2], K. BASHIRI[3], E. HSIEH[3], R. L. MARGOLIS[3], C. A. ROSS[4];
[1]Johns Hopkins Med. Institutions, Baltimore, MD; [2]Korea Brain Res. Inst. (KBRI), Daegu, Korea, Republic of; [3]Johns Hopkins Univ., Baltimore, MD; [4]Div. of Neurobio., Johns Hopkins Med. Sch., Baltimore, MD

Abstract: Ankyrin-G is a scaffolding protein located in the axon initial segment of neurons, supporting voltage-gated ion channels and forming inhibitory synapses with Chandelier cells. Genetic alterations of ankyrin-G have been found to be associated with multiple psychiatric disorders, and we previously reported the behavioral alterations in conditional ankyrin-G forebrain-specific heterozygous or homozygous knockout mice compared to control mice. In the present study, we investigated whether Ankyrin-G conditional forebrain-specific heterozygous mice (AnkG-Het) show behavioral differences compared to control mice at baseline and after social defeat stress, a known trigger of a “depression-like” behavioral state in mice. For behavioral phenotyping, we performed the sucrose preference test (SPT) and the elevated plus maze test (EPM) before and after chronic social defeat stress (CSDS) for 10 days. In the basal condition, AnkG-Het and control mice did not show any differences. However, after 10 days of CSDS, both AnkG-Het and control mice showed reduced sucrose consumption. There was a trend towards a greater reduction in AnkG-Het mice. Moreover, Ank-G Het-mice showed significantly shorter time and distance traveled in open arms compared to control mice in the EPM test after 10 days of CSDS. These data suggest that AnkG-Het plus CSDS would be a useful mouse model system for studying the neurobiology of “depression”-like behavior as a result of combined genetic and environmental factors.

Disclosures: J. Li: None. **J. Kim:** None. **K. Bashiri:** None. **E. Hsieh:** None. **R.L. Margolis:** None. **C.A. Ross:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.15/KK7

Topic: F.03. Stress and the Brain

Title: Effect of chronic administration of Tibolone on anxiety-like behavior and cognitive performance in aging mice

Authors: *R. GARCIA[1], A. G. MARTINEZ[2], G. MANJARREZ- GUTIÉRREZ[3], T. NERI-GOMEZ[4];
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City, Mexico; [3]Lab. de Patología Mol. de la Unidad de Investigación en Biomolecular en Cardiología, Hosp. de Cardiología. Ctr. Médico SXXI, IMSS, Mexico city, Mexico; [4]UNAM, Mexico, Mexico

Abstract: During aging gonadal steroids, pregnenolone, dihydrotestosterone and testosterone decreases. In the central nervous system (CNS), these hormones preserve neural function and promote neuronal survival. Therefore, the age-related decreases of these gonadal steroids have a negative impact on neural function, leading to a memory deficit. Testosterone through the conversion to estradiol prevents neuronal loss in the CNS in different male experimental animal models of neurodegeneration. However, hormone replacement therapy may increase the incidence of prostate and breast cancer in male. A strategy to reduce these latter is the use of safety molecules that exert similar effects as gonadal steroids, such as Tibolone (TIB), which has estrogenic and androgenic metabolites. However, the effect of TIB on memory and learning is poorly understood. The aim of this work was to evaluate the long-term effects of TIB (0.1 mg/kg and 1 mg/kg, orally for 12 weeks) on cognitive task and anxiety-like behavior in aging mice. The mice were divided into the following groups: a) Vehicle (water), b) Tibolone (0.01 mg/Kg) and c) Tibolone 1.0 mg/Kg, with n=15 per group. The cognitive capacity of the rodents was evaluated at 3 and 18 months of age and after the administration of TIB (21 months of age), the task on anxiety-like behavior was the T-maze (TM) and cognitive task was the object recognition (OR) to evaluate short (1h) and long memory (24h). In TM, a significant decrease in latency was observed in the training sesión test on old mice when compared to young mice, this indicates that the aging mice were unable to learn the avoidance response passive inhibition. Even so, mice with TIB a significant increase in latency was shown. In the object recognition test, administration of TIB increased the percentage of time spent in the novel object, the groups treated with vehicle did not show preference for any object, indicating that the TIB improves short- and long-term memory in old mice. These data suggest that TIB improves memory and learning in male mice aged.

Disclosures: **R. Garcia:** Other; Principal investigator. **A.G. Martinez:** Other; co-author. **G. Manjarrez- Gutiérrez:** Other; Chief Research. **T. Neri-Gomez:** 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.; Laboratorio de Patología Molecular de la Unidad de Investigación en Biomolecular en Cardiología, Hospital de Cardiología. Centro Médico SXXI, IMSS.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.16/KK8

Topic: F.03. Stress and the Brain

Support: URSP Award Fall 2021
URSP Award Spring 2022

Title: Effects of foraging crumbles on aggression in laboratory mice

Authors: *M. BENNETT[1], R. E. BARKEY[1], A. PERLBERG[2], A. B. BOOTH[1], E. Q. MURDOCH[1], J. M. FLINN[1];
[1]George Mason Univ., Fairfax, VA; [2]Psychology, George Mason Univ., Chantilly, VA

Abstract: Affective and predatory aggression is exhibited in laboratory mice (Donkelaar et al, 2020). Aggressive behavior has been shown to impact data validity (Sherwin, 2004), and reducing aggressive behavior aligns with section 2143 of the Animal Welfare Act, wherein animal pain and distress are to be minimized (Animal Welfare Act, 1966). Environmental enrichment is frequently used in attempts to both reduce aggression and generally promote laboratory animals' welfare (Howerton et al., 2008) and is meant to simulate a more natural habitat. Ideally, this decreases the stress of captivity and provides an outlet for activity (Lidster et al., 2004). Empirical evidence for the effect of enrichment is contradictory in the field as several studies found a higher prevalence of aggression (Howerton et al., 2008, Lidster et al., 2004). Thus, there is concern that enrichment materials introduce a source of competition and increase activity in a way that provokes fighting. The effect of foraging crumbles on mitigating aggression remains an open question. These nutritionally complete food bits are used to allow mice to seek their food in a more natural manner than traditional baskets. This study compared the instances and severity of aggression between male laboratory mice with and without crumbles for 4 months. The mouse strain used was tau-/-, a strain that exhibits heightened aggression. A teaspoon of crumbles was administered to the experimental group 3 times a week. While there was no significant difference in the total instances of aggression, a chi-square goodness of fit test showed that there were significantly fewer instances that led to injury ($X^{2(1)}$= 5.44, p=.020) and a trend of fewer cases that led to isolation ($X^{2(1)}$= 3.00, p=.083) in the group that received crumbles. This test included the first 50 days of data, as after that, most cages were already separated. Fewer aggression cases, resulting in less isolation, lead to reduced housing costs and may improve behavioral outcomes. Behavioral studies were performed and are currently being analyzed. While this study should be replicated for other strains of laboratory mice, it appears that administering crumbles prevents severe aggression in laboratory mice.

Disclosures: M. Bennett: None. **R.E. Barkey:** None. **A. Perlberg:** None. **A.B. Booth:** None. **E.Q. Murdoch:** None. **J.M. Flinn:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.17/KK9

Topic: A.04. Transplantation and Regeneration

Support: NIH Grant 1F31NS127541
NIH Grant RO1NS082645

Title: Specification of Lef1-dependent hypothalamic neurons that modulate the stress response

Authors: *P. FIGUEROA, D. BRITO DE ANDRADE, C. KARTCHNER, J. CHENG, R. DORSKY;
Neurobio., Univ. of Utah, Salt Lake City, UT

Abstract: Hypothalamic development requires Wnt/β-catenin signaling through the transcriptional regulator Lef1 to assist in proper neurogenesis, in order to modulate physiological and behavioral responses to environmental stimuli. Failure to modulate appropriate responses to external stimuli can manifest as psychiatric diseases and behavioral disorders. We previously observed increased stress-related behavior in *lef1* mutant zebrafish when exposed to a novel environment, and a significant decrease in expression of *crhbp*, which encodes a negative regulator of the stress response signal Crh. However, we do not understand the molecular and cellular processes that contribute to the specification of *crhbp*-expressing neurons in the hypothalamus that modulate stress-related behavior. Additionally, we found that null mutants for the Lef1-dependent gene *otpb* also exhibited increased stress-related behavior and a decrease in *crhbp* expression in the hypothalamus. To elucidate the fate and function of *crhbp*+ neurons I created a zebrafish transgenic reporter containing a small regulatory region upstream of *crhbp* to drive GFP expression in hypothalamic neurons. Transgene expression is absent in the hypothalamus of *lef1* mutants, which is consistent with the role of Lef1 in neurogenesis. In contrast, GFP+ neurons are present in *otpb* mutants, even though *crhbp* mRNA is absent. This suggests that another regulatory region is required for *otpb*-dependent specification of the *crhbp*+ neuronal subtype. I am using this reporter line to label cells transplanted from wild-type donor embryos into *lef1* and *otbp* mutant hosts, to determine whether restoring gene function is sufficient to rescue transgene expression, *crhbp* expression, or normal behavior. To determine the necessity of *crhbp*+ neurons in regulating stress-related behavior I am using the same regulatory region and the Gal4/UAS system to express Nitroreductase and performing ablation experiments with the addition of Metronidazole, followed by behavioral analysis. Collectively this work will assist in identifying which Lef1-dependent hypothalamic neuronal subtypes are involved in physiological and behavioral modulation of the stress response.

Disclosures: P. Figueroa: None. **D. Brito De Andrade:** None. **C. Kartchner:** None. **J. Cheng:** None. **R. Dorsky:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.18/KK10

Topic: F.03. Stress and the Brain

Support: AMED JP23dm0207116

Title: Stress-impaired reward pathway promotes distinct feeding behavior patterns

Authors: *Y. FUJIOKA[1], K. KAWAI[2], G. SOBUE[3], S. ISHIGAKI[1];
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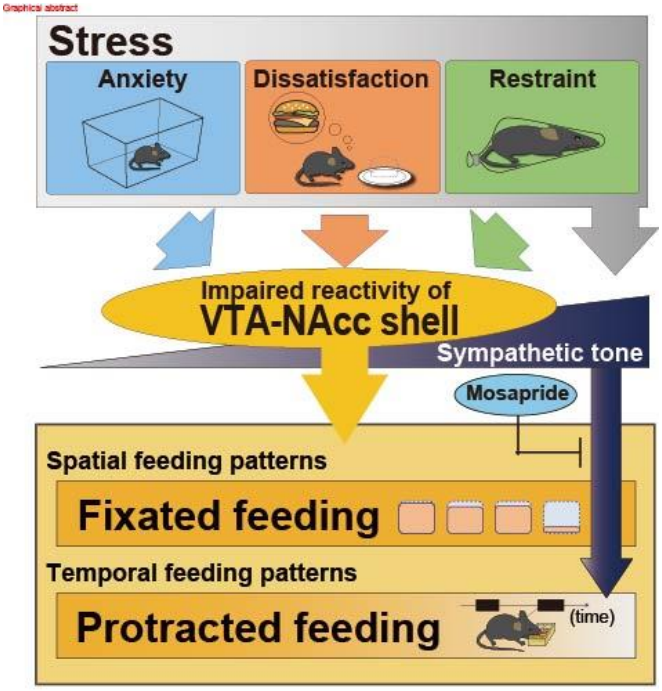
Abstract: Although dietary behaviors are affected by neuropsychiatric disorders, various environmental conditions can have strong effects as well. Quantitative aspects of feeding behaviors are defined by the degree of food consumed and are mainly controlled by hypothalamic circuits. In contrast, qualitative aspects depend on the identification of feeding patterns, the underlying biological processes of which remain to be fully clarified. In this study, we developed a real-time monitoring system to assess the spatial and temporal aspects of feeding behavior patterns as a means to more accurately detect the physiological state of subjects. We found that mice under multiple psychosocial stresses, including social isolation, intermittent high-fat diet, and physical restraint, developed feeding behavior patterns characterized by a deviated bait approach (fixated feeding) and a prolonged uninterrupted feeding interval (protracted feeding). All the tested stressors affected dopamine release at the nucleus accumbens (NAcc) shell and dopamine normalization reversed the feeding defects. Moreover, inhibition of dopaminergic activity in the ventral tegmental area that projects into the NAcc shell with the Designer Receptors Exclusively Activated by Designer Drugs (DREADD) system caused similar feeding pattern aberrations. Given that the deviations were not consistently accompanied by changes in the amount consumed or metabolic factors, the alterations in feeding behaviors likely reflect perturbations to a critical stress-associated pathway in the mesolimbic dopamine system. Thus, deviations in feeding behavior patterns that reflect reward system abnormalities can be sensitive biomarkers of psychosocial stress.



Disclosures: **Y. Fujioka:** None. **K. Kawai:** None. **G. Sobue:** None. **S. Ishigaki:** None.

Poster

PSTR355. Studies of Stress, Anxiety, and Depression in Animal Models

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR355.19/KK12

Topic: F.03. Stress and the Brain

Title: Rapamycin causes dysfunctional light-seeking behaviors in zebrafish larvae

Authors: *Y.-C. CHEN[1], P. A. PANULA[2];
[1]HiLIFE Univ. of Helsinki, Helsinki, Finland; [2]Univy Helsinki, Univy Helsinki, U Helsinki, Finland

Abstract: The mechanistic target of rapamycin (mTOR) complex is an evolutionarily conserved signaling hub that participates in multiple signaling pathways, including cell proliferation, autophagy, and apoptosis. Rapamycin (Rapamune or Sirolimus) is a macrolide compound from *Streptomyces hygroscopicus*. In addition to its well-known clinical significance as an immunosuppressant and anti-cancer agent, growing evidence shows that inhibition of mTOR complex 1 with rapamycin significantly improves lifespan and aging-related diseases. Research mainly addresses the effect of rapamycin on mouse models of lifespans, cancer, and cardiac diseases. Still, little is known about how it affects the central nervous system in normal and disease conditions. In this study, we investigated the rapamycin effects on neurodevelopment in zebrafish larvae. Two-dpf zebrafish embryos were treated with one μM rapamycin, which was replaced every second day until six dpf. The rapamycin-treated larvae grew normally without gross phenotype. At seven dpf, the sleep-like behavior, the light-dark flash response, and the tapping startle response were analyzed. Following the behavioral analysis, the brain dopaminergic, histaminergic, and serotonin systems, as well as the epithalamus structure, were studied by immunostaining. Compared with the vehicle-treated larvae, the rapamycin-treated group showed hypermotility during the dark period and recovery to normal locomotion during lights-on periods. This hyperactive movement following the lights-off stimulus also appeared in one-month-old rapamycin-treated zebrafish. On the other hand, the rapamycin-treated larvae displayed normal startle responses to repetitive vibrational stimulation similar with the vehicle-treated group. Notably, morphological abnormality of the pineal gland and a decreased number of histaminergic cells were found in the rapamycin-treated zebrafish brain. Our result reveals that rapamycin influences photomotor responses and the epithalamus development in zebrafish, which may contribute to the pre-clinical studies when considering the possible consequence of rapamycin as a potential treatment for aging-related diseases.

Disclosures: **Y. Chen:** None. **P.A. Panula:** None.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.01

Topic: F.03. Stress and the Brain

Support: HDRF 203840-01
NIMH 5R01MH118451-05
NIH 1S10OD032415-01

Title: Opencortex: an integrated platform for cortex-wide imaging, electrophysiology, and network analysis in behaving mouse models of neuropsychiatric disorders

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Abstract: Cognitive functions in both healthy and disordered states involve intricate rearrangements of neuronal networks spanning the entirety of the cortex and its interactions with subcortical structures. However, the precise mechanisms underlying large-scale network interactions remain uncertain due to the absence of comprehensive recording methods that offer simultaneous access to cortical and subcortical activities. To address this limitation, we devised a technique involving an open-skull cranial window preparation in mice, which allows for optical imaging of the complete dorsal cortical surface. In addition, micro-prisms were incorporated to enable access to medial and lateral cortical regions. To comprehensively examine network dynamics at both the meso-scale and single-neuron levels, we employed a multiscale imaging approach, combining one-photon and 2-photon microscopy. To further enhance our recording capabilities, we integrated high-density silicon multi-electrodes into the preparation, enabling large-scale recording from multiple subcortical regions while maintaining access for cortex-wide imaging. This integrated system, referred to as the OpenCortex platform, permits simultaneous cortex-wide imaging and subcortical electrophysiology during behavioral tasks performed by head-fixed animals in mixed-reality environments. Overall, the OpenCortex platform facilitates the acquisition and open dissemination of longitudinal multi-modal data, which are crucial for elucidating the brain-wide reconfigurations of networks underlying the progression of neuropsychiatric disorders, including stress, anxiety, and depression, in mouse models.

Disclosures: B.S. Huang: None. **Y. Kageyama:** None. **A. Buch:** None. **G. Manzano Nieves:** None. **R. Rahn:** None. **A. Singh:** None. **A. Polat:** None. **A. Qureshi:** None. **S. Huang:** None. **D. Meyer:** None. **A. Bellafard:** None. **P. Golshani:** None. **A. Vaziri:** None. **L. Grosenick:** None. **C. Liston:** None.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.02/KK13

Topic: F.03. Stress and the Brain

Title: Impacts of pregestational maternal social isolation on offspring anxiety and cognitive function

Authors: A. L. LUMLEY, T. PROVOST, *S. M. KEESOM;
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Abstract: Social isolation is an increasing global issue, exacerbated by social distancing measures employed to reduce disease transmission during the COVID-19 pandemic. While there is mounting evidence to support that isolated individuals are more likely to develop mental health disorders, the possible transgenerational effects of reduced social contact remain underexplored. Previous research suggests that stressors experienced by mothers prior to gestation have adverse effects on offspring anxiety and memory, with underlying neural correlates. In contrast, it is unknown whether maternal social deprivation before conception affects mental well-being of the offspring. We therefore conducted a study to test the hypothesis that pregestational maternal social isolation increases anxiety-like behaviors and impairs cognitive function in offspring. To do this, we bred female Swiss-Webster mice after they experienced one of four housing treatments (n = 3 females per category): short-term individual (IND-ST), long-term individual (IND-LT), short-term group (GR-ST), and long-term group (GR-LT). At postnatal day 70, we tested female offspring from the following mothers: GR-LT (n = 4), IND-ST (n = 7), and IND-LT (n = 9), noting that GR-ST mothers did not have viable litters. We investigated anxiety-related behavior using the open field test, with observers blind to maternal treatment. Our preliminary analysis suggests that offspring from mothers of all treatments did not differ in total duration of wall-hugging behavior. However, IND-LT and IND-ST offspring displayed greater variation in the number of fecal boli deposited compared to GR-LT offspring, which deposited consistently few fecal boli in the open field, suggesting some effect of preconception social environment on offspring anxiety. Following this, we assessed offspring cognition using the novel object recognition test, with an observer blind to maternal treatment and object novelty. Here, GR-LT offspring spent more time investigating the new object, and IND-ST offspring had a slight preference for the new object. Interestingly, IND-LT offspring did not display a consistent preference for the new object, indicating an impairment in memory. Taken together, our initial findings suggest that maternal isolation prior to conception may differentially impact anxiety and cognitive function of offspring, depending on the duration of social deprivation. More broadly, this preliminary research highlights the importance of the pregestational time period for the mental health of future offspring.

Disclosures: A.L. Lumley: None. **T. Provost:** None. **S.M. Keesom:** None.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.03/KK14

Topic: G.03. Motivation

Title: Examining Behavioral and Neural Effects of Exposure to Early Life Trauma on Maternal Care

Authors: *L. ROTHI, M. NGUYEN, L. HALLADAY;
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Abstract: Early-life stress (ELS) during critical periods of development can lead to lifelong adverse behavioral consequences, including one's ability to properly care for offspring. Prior research from our lab identified the bed nucleus of the stria terminalis (BNST), a brain region that integrates reward, anxiety, and social behavior, as being a key mediator of ELS-induced social behavioral dysfunction. The BNST and downstream medial preoptic area (mPOA), have been implicated in maternal behaviors, yet it is still unknown if deficits in maternal care behaviors following ELS can be attributed to altered neural activity in those two regions. Therefore, we hypothesized that deficits in maternal care incited by ELS are mediated by the BNST, the mPOA, or both. To test this, we used a combination of mouse behavior models and physiological techniques. First, we induced ELS using an established mouse model of childhood neglect, maternal separation with early weaning (MSEW); this procedure induces behavioral deficits, including anxiety and social impairments. We then used in vivo electrophysiology and immunohistochemistry techniques to study maternal behavior and neural activity in adult female MSEW and control mice during the first days after their pups were born. We also simultaneously conducted recordings of the pups' distress vocalizations, which triggered maternal care from the dam. This combination of methods allows us to understand whether neural activity in the BNST and mPOA 1) differs in ELS mice versus controls, and 2) corresponds with instances of pup distress calls and/or drives maternal behaviors observed. Results showed that ELS altered both behavior and neural responsiveness to maternal care, highlighting the BNST and mPOA as potential therapeutic targets for maternal behavior deficiencies in mothers with a history of early childhood trauma.

Disclosures: L. Rothi: None. **M. Nguyen:** None. **L. Halladay:** None.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR356.04/KK15

Topic: F.03. Stress and the Brain

Support: DFG EXC-2049 – 390688087

Title: Impact of Early-Life Adversity on Brain Aging and Neurodegeneration in Adult Women

Authors: L. FLECK[1,2], M. STEIN[1,3,4,5], M. BAUER[1,2], J. RAMLER[1,2], C. BUSS[1,2,7], S. ENTRINGER[1,2,7], M. ENDRES[1,3,6], *C. HEIM[1,2,6,8];
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Abstract: Problem Statement: Despite the overwhelming evidence of the impact of early-life stress (ELS) on brain structure and immune activation, there is no knowledge regarding the effects of ELS in humans on accelerated brain aging and neurodegenerative disease. In our study, we assessed the effects of ELS on brain aging in adult women. **Methods**: We recruited N=180 women aged 30 to 60 years, including 83 women without ELS exposure (Mean Age (SD)=40.4 (9.03) years) and 97 women with ELS exposure (Mean Age (SD)=41.6 (9.43) years; t=-.858, p=.392). Women in the ELS group had experienced maltreatment or loss before the onset of puberty. Severity of maltreatment was assessed using standardized instruments. Brain volumetrics were measured by structural MRI. The Cambridge Neuropsychological Automated Test Battery (CANTAB) was used to assess cognitive functioning in domains indicative of prodromal Alzheimer's Disease. Furthermore, we investigated peripheral biomarkers of neurodegeneration, i.e., plasma β-amyloid 42/40. **Results:** General linear model (GLM) analysis revealed a significant effect of age (β=-.3, t(2)=-4.07, p<.001) and a significant interaction effect of age and ELS exposure (β=-.28, t(3)=-2.52, p=.013, DR^2=.03) in predicting subcortical grey matter volume, indicating a more pronounced volume loss with increasing age in individuals exposed to ELS. There was a specific interaction effect of age and maltreatment expsoure on hippocampal volume loss (β=-.196, t(3)=-1.981, p=.049, DR^2=.023). Severity of maltreatment was associated with poorer episodic memory performance (r=.21, p=.005). The interaction of age and maltreatment severity predicted visual recognition memory performance (r=-.177, p=.019). The protein, β-amyloid 42, which reflects senile plaque deposits, was more frequently detectable in women with ELS exposure as compared to women without ELS (χ^2(2)=5.57, p=.018). **Conclusion:** We here provide novel evidence that early-life adversity promotes accelerated brain aging and may contribute to the risk of neurodegenerative disorders later in life. A precise understanding of the early factors and mechanisms that lead to pathological brain aging will enable novel strategies to promote healthy brain aging.

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Poster

PSTR356. Stress and Cognition

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.05/KK16

Topic: F.03. Stress and the Brain

Support: NIMH: R01MH123686

Title: Persistent effects of adolescent stress exposure on adult cognitive processes.

Authors: *A. Y. FLORES;
Neurobio. and Behavior, Univ. of California, Irvine, Irvine, CA

Abstract: Persistent effects of adolescent stress exposure on adult cognitive processes.
Abigail Yap Flores, Adolfo Torres, Adeline Cheng, Gyorgy Lur. Department of Neurobiology and Behavior, University of California, Irvine, 1215 McGaugh Hall, Irvine, CA92697. Abstract: Stress is known to induce short and long-term alterations in neural circuits which coincide with changes in behavior. We have previously shown that repeated exposure to multiple concurrent stressors (RMS) during adolescence reduces both excitatory and inhibitory synapse density in the mouse posterior parietal cortex. However, the long-term effects of adolescent (p30-40) RMS exposure on adult cognitive performance remains unknown. Our current study addresses this gap in knowledge by analyzing behavioral performance in adult (p75-150) mice. We used a head-fixed, visual discrimination task based on the go-nogo framework, to assess the effect of adolescent stress exposure on multiple cognitive processes. We found that associative learning and working memory performance in adulthood are impacted by adolescent RMS in a sex specific manner. Stressed females obtained task proficiency slower than controls while males learned at the same rate, but their performance plateaued at a lower level. In contrast, performance on a well-rehearsed delayed discrimination task was impaired in males but not in females. We have also found sex specific effects of RMS on distractibility. Overall, our results suggest that adolescent stress exposure results in sex-specific deficits in adult task acquisition, adaptability to uncertainty, working memory, and task improvement upon multiple iterations.

Disclosures: A.Y. Flores: None.

Poster

PSTR356. Stress and Cognition

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.06/KK17

Topic: F.03. Stress and the Brain

Support: AFOSR Grant 20RHCOR04

Title: Characterization of sleep restricted stress-induced cognitive impairment: Neurobehavioral sex-specific differences, melatonin production, and hippocampal neuroinflammation

Authors: *L. OLSEN[1], R. J. MOORE[2], N. GARGAS[1], J. STRICKER[1], H. MCCUBBINS[2], J. ROHAN[1], C. HATCHER-SOLIS[2];
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Abstract: Critical for healthy cognitive function, sleep modulates learning and memory across species. Restricted sleep (RS) can impair cognition and increase susceptibility to neurodegenerative conditions. The purpose of this study is to characterize the behavioral and physiological consequences of RS, including sex-specific differences, melatonin sleep hormone production, and hippocampal neuroinflammation. All animal activities were approved under IACUC protocol F-WA-2019-0184. Male and female Sprague-Dawley rats (N = 40-50) underwent 96-120 h of RS. Novel Object Recognition (NOR) and Passive-Avoidance Task (PAT) assessed cognitive performance (n = 6-12). Sex-specific effects found RS males to have increased locomotor activity ($p<0.01$) and impaired performance in NOR ($p<0.05$) and PAT ($p<0.01$), while RS females had no change across all behaviors when compared to control females ($p>0.05$). However, estrus cycle stage (determined by wet smear cytology) mediated PAT performance with females in estrus, not diestrus, exhibiting impaired PAT performance after RS ($p<0.05$). Tissue was collected following humane euthanization. Plasma melatonin production as measured by Enzo enzyme-linked immunoassay (ELISA, n = 4-5) found no significant effect of 96 h RS among males ($p>0.05$). Hippocampal protein analysis was performed using immunohistochemistry (n = 3-6) and ELISA (n = 8-13). Quantified using ImageJ, 120 h RS increased the number of Iba-1+ identified microglial in the stratum radiatum of hippocampal subregions CA1 ($p<0.05$) and CA2 ($p<0.05$) in males. ELISA V-PLEX assay panel of whole hippocampus tissue found higher levels of pro-inflammatory chemokine ligand 1 (CXCL1, $p<0.05$) among RS males. Sex-specific differences in neurobehavior after RS suggest that males are more susceptible to RS stress-induced cognitive impairment; albeit, estrus cycle stage may mediate fear aggravated learning in females during RS. Further research is needed to determine the influence of estrus cycle stage on learning and memory during RS. An increase in microgliosis and CXCL1 in the hippocampus suggests that neuroinflammation in regions that mediate declarative memory may underlie RS stress-induced cognitive impairment in males. No DoD endorsement implied. This study was supported by AFOSR grant 20RHCOR04.

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Poster

PSTR356. Stress and Cognition

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Program #/Poster #: PSTR356.07/KK18

Topic: F.03. Stress and the Brain

Support: R00 DA045795
P30 DA033934

Title: Histone H1x in the Ventral Hippocampus Correlates With, But Does Not Cause Susceptibility to Social Stress

Authors: *R. K. KIM[1], N. L. TRUBY[1], J. A. PICONE[1], G. M. SILVA[1], X. CUI[2], R. L. NEVE[3], P. J. HAMILTON[2];
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Abstract: Chronic stress is a risk factor for developing many neuropsychiatric syndromes including post-traumatic stress disorder and major depressive disorder. A better understanding of the neurobiological mechanisms that engender vulnerability to chronic stress may guide the development of novel therapeutics. Prior work discovered linker histone H1x is differentially expressed within the ventral hippocampus (vHipp) of stress-susceptible and resilient mice[1]. Additionally, the stress-resilient phenotype is known to be a biologically active state, involving many unique transcriptional, protein, and metabolic adaptations[2]. Existing data demonstrates linker histones are responsible for coordinating and maintaining condensed heterochromatin[3,4]. Further, using Western blots, we demonstrated that stress-susceptible mice express significantly increased levels of H1x in the vHipp compared to both resilient mice and unstressed controls (n=30 stressed, n=15 unstressed). Thus, we hypothesized this elevated level of H1x in the vHipp may lead to transcriptional silencing of pro-resilience gene networks and impede the neuroadaptations necessary for developing a stress resilient phenotype. To test this, male C57BL/6J mice (8-10 wks old) were randomly assigned to stressed and unstressed groups. Stressed animals underwent a 10-day chronic social defeat stress (CSDS) paradigm[5], while unstressed animals were single housed for the same period. Following CSDS, animals were defined as susceptible versus resilient by social interaction (SI) testing as previously described[5]. Following Herpes virus-mediated overexpression of H1x within vHipp, we subjected experimental mice to a battery of social behavior, anxiety-like behavior, and memory assessments. Although the elevated plus maze (EPM) showed some initial results that suggested H1x overexpression (OE) may be anxiogenic, this was not replicated across alternative behavior modalities including SI, novelty suppressed feeding, and fear potentiated startle. Further, upon further powering of the EPM experiment, no multiple comparisons across conditions survived post-hoc testing. In sum, we present a comprehensive behavioral analysis following linker histone H1x OE, a protein shown to be correlated with susceptibility to social stress. Although we were able to recapitulate the protein level alterations of H1x associated with stress susceptibility, we did not observe a significant behavioral consequence of H1x OE. Thus, we conclude elevated levels of H1x correlate with the susceptible phenotype, but does not causally contribute to the development of stress susceptibility.

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Poster

PSTR356. Stress and Cognition

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.08/KK19

Topic: F.03. Stress and the Brain

Support: NARSAD
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NIH
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Title: Big Potassium Channel Knockout Mouse as the Behavior Switch Model to Study Bipolar Disorder

Authors: *R. SRIVASTAVA, K. ISHIZUKA, A. SAWA;
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Abstract: Bipolar disorder is a major mental disorder characterized by a mood-switch between depressive and manic episodes. The mechanistic understanding remains challenging due to complex genetic and environmental factors contributing towards its etiology and etiopathology. In addition, the animal models that could mimic the characteristic 'switch' process remain challenging to generate. Based on our previous unpublished studies, we have nailed down calcium and voltage activated big potassium (BK) channels as important players in cellular deficits from neurons derived from the bipolar disorder patients. In this study, we utilized genetic knockout (KO) of the BK channel in combination with the environmental stressor of sleep deprivation to mimic the 'switch' process. Our results showed that the BK KO heterozygous (het) mice have higher immobility during the forced swim test (FST) at the baseline. When these mice are exposed to four hours of sleep deprivation, they 'switch' to lower immobility. This behavior goes back to its default state when the mice are rested for a few weeks. The sleep deprivation effect persisted for at least several days, as the mice showed lesser immobility even after five days of sleep deprivation. Of note, the 'switch' was rescued by the injection of a BK channel agonist. Our study has characterized one important mechanism-based mouse model system for bipolar disorder demonstrating 'switch' behavior. The BK channel could be an important target for potential use in the bipolar disorder treatment.

Disclosures: R. Srivastava: None. **K. ishizuka:** None. **A. Sawa:** None.

Poster

PSTR356. Stress and Cognition

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Topic: F.03. Stress and the Brain

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VIEP-BUAP 2021-2022 to CA in Neuroendocrinología (BUAP-CA-288)
CONACYT Doctorate fellowship No. 926368.

Title: Fear acquisition by two fear conditioning paradigms in a rat model of anxiety

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Abstract: Fear conditioning is an association between one neutral conditioned stimulus (CS) and one unconditioned often an aversive stimulus (US). Once this association is consolidated, an unconditioned response is formed, which in rodents express as freezing behavior. Most of the subjects can break this association over time, however subjects that are more susceptible to anxiety-like behaviors maintain this association, thus has been proposed as a model for post-traumatic stress disorder (PTSD). We have selectively bred two sublines from Sprague-Dawley (SD) rats that differs in their spontaneous yawning frequency. The high-yawning (HY) rats have a mean of 20 yawns/h whereas low-yawning (LY) rats have a mean of just 2 yawns/h. Additionally, HY rats have shown resilience behavior and LY rats have shown an anxious trait when evaluated in different anxiogenic tests. The aim of this study was to assess the effectiveness of two fear conditioning paradigms in SD, HY and LY rats and to assess the strength of the fear response acquired based on their innate resilience capabilities. We used four male rats of each HY and LY sublines and SD rats at 3 months of age. Subjects were exposed to a single 0.5 mA shock (US) paired to light and sound (CS) training session of an acute protocol; the shocks lasted for 1 second with an interval of one minute. In an overtraining protocol, shocks were delivered on the last two seconds of a 10 second exposure to light and sound with an interval of one minute. Re-exposure on the acute protocol took place the next day and for the next 4 weeks. For the overtraining protocol a context re-exposition with no CS took place on the next 48 and a cue re-exposition with a changed context and CS the next day. Another context and cue re-expositions were made after a 6-week interval. The acute protocol was the least effective in maintaining a fear response over the four weeks of re-exposure in all subjects. Overtraining was the most effective protocol, with a higher fear response across all subjects, evoking a higher fear response with cue re-exposition. LY and SD rats presented higher fear response with respect to HY rats ($P<0.05$). In conclusion, HY rats is a model of resilience and LY rats an anxiety model, because they are more susceptible to maintaining fear response. Both sublines are an adequate model for studying the behavioral mechanisms to PTSD due to their different responses.

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Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.10/LL1

Topic: F.03. Stress and the Brain

Title: Amyloban, extracted fromHericium erinaceus, recovers social deficit induced by social defeat stress by suppressing the dopaminergic system

Authors: *T. WANG[1,4,2], *T. WANG[3], K. TORIUMI[4], K. SUZUKI[6], M. MIYASHITA[4,5], M. ITOKAWA[2,4,7], M. ARAI[4];
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Abstract: Social symptoms are common in various psychiatric disorders, including depression, schizophrenia, and autism, and they are long-lasting and difficult to treat. The development of treatments for social impairment is critical for addressing many psychiatric disorders. Clinical studies have reported that the administration of Amyloban® 3399 (Sun Medica Co., Ltd.), a product extracted from the mushroom *Hericium erinaceus*, markedly improves social symptoms in patients with treatment-resistant schizophrenia and depression. The main components of amyloban are hericenones, which were discovered in 1991 as the first natural compounds reported to promote nerve growth factor (NGF) synthesis. However, the molecular mechanisms through which amyloban ameliorates social impairment remain unclear, making it currently difficult to identify patients for whom amyloban is effective and to use it as a therapeutic drug. In this study, we aimed to uncover the molecular mechanisms underlying the effect of amyloban by evaluating its therapeutic effects on social deficits and neurochemical dysfunction in a mouse model of social defeat stress (SDS). In the SDS mouse model, C57BL/6J mice were physically attacked by aggressive ICR mice once a day for 10 minutes and then exposed to sensory contact, such as smells and sounds, for the rest of the day through a transparent divider for 10 days. Mice were then assessed for sociability using social interaction test, and some of them were orally administered amyloban for 2 weeks. After the administration of amyloban, mice were reassessed for social recoveryusing the social interaction test. The results showed that amyloban exerts therapeutic effects on stress-induced social deficits. To clarify neurochemical changes through which amyloban recovered the behavioral impairments, monoamine contents and their metabolites in the prefrontal cortex (Pfc), the hippocampus (Hip), the nucleus accumbens (Nac), and the striatum (Str), which are deeply related to sociality, emotional motivation, and anxiety, were measured by HPLC. The results showed that the ameliorative effects of amyloban might be due to the suppression of the enhanced dopaminergic system in the Hip or Pfc. These findings suggest that amyloban alleviates social deficits by modulating the dopaminergic system and that it may be an effective treatment for social symptoms associated with many psychiatric disorders.

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Poster

PSTR356. Stress and Cognition

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Program #/Poster #: PSTR356.11/LL2

Topic: F.03. Stress and the Brain

Support: R01MH115020-04
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Title: Receptor-specific mechanisms underlying psychedelic effects on stress-induced compulsivity

Authors: *Y. JAQUES[1], M. KO[2], B. RORIE[3], R. KHERA[2], D. KAUFER[2];
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Abstract: Background: Compulsivity is the irresistible urge to perform purposeless repetitive acts. Patients with post-traumatic stress disorder (PTSD) exhibit co-morbid symptoms of severe compulsive behaviors. Recent psychedelic clinical trials show promising effects on PTSD, yet effects on compulsivity are unknown. Psychedelics act through serotonin and may be regulating these compulsive behaviors through specific receptor subtypes. We investigate these receptors as potential mechanisms underlying compulsive behaviors following a preclinical PTSD model.
Methods: First, 8~10-week-old male C57BL/6J mice underwent 10-day social defeat stress (SDS). Afterwards, all subjects' compulsivity was measured in the nestlet shredding test (NST), anxiety in the elevated plus maze (EPM), and social behavior in the social interaction test (SI). Secondly, a cohort received a 2,5-Dimethoxy-4-iodoamphetamine (DOI) injection at 0, 0.5, or 3 mg/kg, then underwent a behavioral battery of the forementioned tests. Thirdly, we tested DOI+M100907 (a serotonin receptor 2a antagonist) or DOI+RS102221 (a 2b antagonist) followed by the tests. In a last experiment, compulsivity was measured after SDS
Results: SDS increased amounts of repetitive shredding in the NST compared to control (n=10~17, t-test, p=0.206), indicating an increase in compulsivity. A correlation between NST and EPM, but not SI, was found (r=0.58,p=1.557e-004). Furthermore, an injection 0.5 or 3 mg/kg of DOI decreased measures in NST (n=10, one-way ANOVA, p=0.0325). Also, DOI+M100907 and DOI+RS102221 groups did not decrease NST measures whereas the DOI group did (n=5~15, one-way ANOVA, p=0.0138). Lastly, a DOI injection after SDS decreased NST measures compared to a vehicle group (n=13, on-way ANOVA, p=0.0063).
Conclusions: Our research shows that SDS induces compulsive behaviors whereas a psychedelic compound decreases compulsive behaviors as well as stress-induced compulsive behaviors. Serotonin antagonists reverse this behavior. This research indicates a potential for psychedelics as an integrated treatment for neuropsychiatric disorders.

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Poster

PSTR356. Stress and Cognition

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Topic: F.03. Stress and the Brain

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Title: Correlation between reduction of LF/HF and functional connectivity among brain regions in relation to mindfulness meditation

Authors: *I. KONDO, S. SHIMADA;
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Abstract: In recent years, mindfulness meditation has been reported to be effective in reducing stress and anxiety. However, many previous studies examined and objectively demonstrated the relationship between mindfulness meditation and stress. In this study, we carried out a four-week mindfulness meditation training program. We conducted the experiment before and after the training and measured heart rate variability and electroencephalography (EEG) analysis in each phase (First rest, Stress task, Meditation, and Second rest). In the Stress task, the participants performed a mental arithmetic task in which 13 was continuously subtracted from 1022 for 10 minutes, and in the Meditation, they performed breathing meditation for 7 minutes. Then, in each phase, LF/HF, which is generally used as a stress evaluation index, was calculated based on the heart rate variability, and EEG functional connectivity was calculated on prior selected regions of interest (ROIs). In this study, we examined the correlation between LF/HF and functional connectivity among brain regions. In total, 24 healthy university students participated in this study. However, the analysis of LF/HF excluded five participants whose LF/HF values were not larger than those at the First rest during the Stress task. Five participants were additionally removed who made a lot of EEG noise during the Stress task. The result of the correlation analysis showed that the meditation group had positive correlations between LF/HF and various brain networks from δ wave to γ wave frequency bands. In particular, we found positive correlations between the reduction of the LF/HF ratio and the functional connectivity attenuation in networks among the PCC, mPFC, ACC, insula, dlPFC, superior parietal lobule, and others, which are the brain regions related to mindfulness meditation, as in the LF/HF ratio and the functional connectivity ratio of left ACC and right insula in the θ-wave band ($r = 0.579$, $p < 0.01$). On the other hand, the control group showed negative correlations between the LF/HF ratio and the functional connectivity ratio among the aforementioned brain regions. These results showed that there is a relationship between LF/HF and the activity of functional connectivity among brain regions related to mindfulness meditation. Higher values of LF/HF indicate a state of stress, while lower values indicate a state of relaxation. This study suggested that the four-week mindfulness meditation training decreased functional connectivity among brain regions, including the default mode network, by reducing stresses.

Disclosures: I. Kondo: None. **S. Shimada:** None.

Poster

PSTR356. Stress and Cognition

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Topic: F.03. Stress and the Brain

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Title: Bidirectional alpha power EEG-Neurofeedback during a focused attention meditative practice in novices

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Abstract: Background. Neurofeedback and meditation practices are techniques aimed at enhancing awareness and regulation over the self. Training of alpha power has been found to increase mindfulness outcomes and increases in alpha power have been shown relatively consistent during focused attention meditation practices. In the current study, we examined the trainability of alpha power, delivering alpha neurofeedback in the context of a focused attention meditation, providing novice practitioners with a comprehensive training for improving self-regulation. **Methods.** Thirty-one young adults (25 women, aged 23.16, range 18-30 years) with no prior experience in meditation practices participated in our study. In a within-subject design, participants engaged in two runs of 6 trials each, aimed at up-regulation of global alpha absolute power (average of 19 scalp electrodes) recorded with electroencephalography (EEG). During neurofeedback training, participants were instructed to close their eyes and focus the attention on a point above the crown of the head, while perceiving continuous auditory feedback regarding their alpha power (increasing in volume with increasing alpha power). As an active control, participants also underwent two neurofeedback runs in which alpha power down-regulation was trained, with volume increases upon alpha power reductions. **Results.** Linear mixed-effect analyses revealed a significant main effect of *training condition* ($F(1,30) = 10.49$, $p < .001$), indicating higher alpha power across trials during the up-regulation compared to the down-regulation condition. Also a trend but non-significant main effect of *trial* ($F(5,30) = 2.16$; $p = .06$) as well as *trial by training interaction* ($F(18,30) = 2.05$; $p = .07$) were found. These results reflected a tendency that training was most pronounced for the last, compared to the first trials, divergently for the up- and down-regulation conditions. Separate analyses for each training condition revealed significant reductions in alpha absolute power across trials during down-regulation, ($F(5,30) = 2.46$; $p = .03$), and a non-significant increase during-up regulation ($F(5,30) = 1.77$; $p = .12$). **Discussion**. The alpha power neurofeedback protocol achieved distinct bidirectional results for the two conditions. However, only the down-regulation training was successful at significantly reducing alpha power. These results suggest that successful alpha power up-regulation might require more training trials/sessions. Additionally, future protocols would benefit from identification of neurofeedback non-responders to help elucidating eligibility for neurofeedback training and prediction of success.

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Poster

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Program #/Poster #: PSTR356.14/LL5

Topic: F.03. Stress and the Brain

Title: Underlying neuropharmacological mechanisms of the effects of acute stress on decision-making

Authors: *L. VAN HERK, F. P. M. SCHILDER, A. D. DE WEIJER, B. BRUINSMA, E. GEUZE;
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Abstract: Individuals might be exposed to intense acute stress while having to make decisions with far-reaching consequences. Acute stress impairs processes required for decision-making by activating different biological stress cascades that in turn affect the brain. By knowing which stress system, brain areas, and receptors are responsible for compromised decision-making processes, we can effectively find potential pharmaceutics that can prevent the deteriorating effects of acute stress. Our main objective was to collect data from experiments investigating the effects of acute stress on decision-making, and additionally, how pharmacological neuromodulation might counteract these effects. We collected data from 44 articles using a systematic literature search following PRISMA standards. Risk of bias of each article was identified using the Cochrane Risk-of-Bias tool for studies using human subjects and Systematic Review Centre for Laboratory animal Experimentation tool for animal studies. Decision-making processes could be subdivided into 4 domains (cognitive, motivational, affective, and uncertainty) and could be referenced to specific brain areas, while analysis of the data showed impairment by molecules associated with the sympathetic-adrenal-medullar (SAM) and hypothalamic-pituitary-adrenal (HPA) axes. Potential drugs to alleviate these effects included α_1 and β adrenoceptor antagonists, α_2 adrenoceptor agonists, and corticotropin releasing factor receptor$_{1/2}$ antagonists, while consistent stress-like effects were found with yohimbine, an α_2 adrenoceptor antagonist. Disentangling the mechanisms how these molecules, that are involved in the SAM and HPA axes, affect decision-making will be the initiation to promising future research.

Disclosures: **L. van Herk:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **F.P.M. Schilder:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **A.D. de Weijer:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **B. Bruinsma:** A. Employment/Salary (full or part-time):; UMC Utrecht,

Ministry of Defence. **E. Geuze:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.15/LL6

Topic: H.03. Decision Making

Title: Comparing the impact of biological and psychological stressors on decision-making performance and cortical activity

Authors: *C. LÓPEZ ESPARZA[1], F. IRIBE BURGOS[1], J. GARCÍA HERNÁNDEZ[2], P. CORTES ESPARZA[1], M. GUEVARA[1];
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Abstract: Stressors are stimuli that trigger physiological responses to restore homeostasis and can affect various cognitive processes, including decision-making (DM). Some authors have proposed classifying stressors into biological stimuli (those that immediately trigger the stress response by acting on sensory receptors) and psychological stimuli (those involving cognitive, emotional, and social components that determine whether they are perceived as threatening). However, there are few studies that directly compare these types of stressors' impact on behavior, especially in relation to decision-making. Therefore, the objective of this study was to compare the effects of biological and psychological stressors on the performance of a decision-making task. Twenty men voluntarily participated in the study, with 10 assigned to each group. The participants were required to solve a decision-making task aimed at accumulating as many points as possible. The participants were divided into two groups as follows: 1) The biological stressor group, where participants were exposed to an irritating sound before and during the decision-making task, and 2) The psychological stressor group, where participants were exposed to a video containing explicit violent content. The researchers analyzed the participants' reaction time, cumulative total score in the decision-making task, and the relative power (RP) of the prefrontal and temporal cortices related to the task. The results of the task execution showed that the biological stressor group achieved a lower score compared to the psychological stressor group. However, no differences were found regarding reaction times. In terms of electroencephalographic activity, it was observed that participants in the psychological stressor group exhibited higher RP in the frontal areas for the gamma and beta bands. On the other hand, the biological stressor group showed higher RP in the temporal areas for the delta, theta, and alpha bands. These results indicate that the biological stressor group is more susceptible to the effects of stress and elicits a stronger response from the temporal areas, suggesting an impulsive and emotional reaction. In contrast, the psychological stressor, which involves prefrontal areas, elicits a more reasoned response, potentially mitigating the effects of stress. These findings highlight the importance of considering the type of stressor when examining its influence on

decision-making processes. Further research in this area can contribute to a better understanding of how stressors affect cognitive functions and guide the development of strategies for stress management in various contexts.

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Poster

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Program #/Poster #: PSTR356.16/LL7

Topic: F.03. Stress and the Brain

Title: Impact of a stressor on Decision-Making Behavior and Brain Activity

Authors: *F. A. IRIBE BURGOS[1], P. CORTES ESPARZA[1], J. P. GARCÍA HERNÁNDEZ[2], C. LÓPEZ ESPARZA[1], M. HERNANDEZ[1], M. A. GUEVARA[1];
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Abstract: Stress refers to a set of biological, cognitive, and behavioral reactions that occur when an organism perceives a stimulus as a threat. When faced with a stressor, various organic mechanisms are activated, leading to changes in the functionality of different brain areas, such as the prefrontal and temporal cortex. These regions have been associated with specific EEG activity during decision-making (DM). Hence, this study aims to evaluate the effect of a stressor on both DM behavior and brain activity. Nineteen healthy, right-handed men between the ages of 20 and 35 voluntarily participated in the study. They completed a DM task both with and without exposure to a stressor, which involved watching videos of explicit violence and a man walking. The relative power (RP) in traditional EEG rhythms was recorded while performing the DM task. The results indicate that exposure to a stressor is associated with lower RP in the delta band and fast rhythms in both frontopolar cortices: left (Fp1) [delta: $t= -2.556$; $p\leq0.02$; beta1: $t= 2.562$; $p\leq0.02$; beta2: $t= 3.477$; $p\leq0.004$; gamma: $t= 3.341$; $p\leq0.004$] and right (Fp2) [delta: $t= -2.269$; $p\leq0.03$; beta2: $t= 2.659$; $p\leq0.01$; gamma: $t= 2.19$; $p\leq0.04$]. The dorsolateral prefrontal area showed similar results, as the stressor group exhibited lower RP in the beta1 and beta2 bands in both the left hemisphere (F3) [beta1: $t= 2.505$; $p\leq0.02$; beta2: $t= 2.288$; $p\leq0.03$] and the right hemisphere (F4) [beta1: $t= 2.18$; $p\leq0.04$; beta2: $t= 3.242$; $p\leq0.005$]. In contrast, for the temporal cortex, results were only observed in the left hemisphere (T3). The stressor group showed higher RP in the delta [delta: $t= -2.243$], while lower RP was found in the beta2 and gamma rhythms [beta2: $t= 2.176$; $p\leq0.04$; gamma: $t= 2.134$; $p\leq0.04$]. These findings demonstrate that exposure to a stressor alters EEG activity during DM in the prefrontal, frontopolar, and temporal cortices, particularly at fast frequencies, interestingly, despite similar performance on the DM task with and without exposure to the stressor. These results suggest the activation of a

modulation mechanism that allows for proper DM performance in the presence of a stressor, as evidenced by EEG activity.

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Poster

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Title: Emotional memory control under stress:augmented overnight retention, adaptive suppression and brain functional reorganization

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Abstract: Long-term stress can have a profound impact on human emotion and memory systems, with both adaptive and maladaptive outcomes. It is known that long-term stress results in a tendency to be more sensitive and unforgettable to emotional memories as seen in various stress-related mental disorders. However, the neurocognitive mechanisms underlying long-term stress-induced over-retention of emotional memories remain elusive. Here we investigate how long-term stress influences brain systems, representations and network connectivity changes involved in emotional memories before and after overnight reorganization. Behaviorally, we found that long-term stress group showed less decay of emotional memories over time, and this trend persisted even after repeated retrieval and could be intercepted by voluntary suppression. After a 24-hour overnight delay, retrieving emotional memories showed less deactivation over time in widespread brain regions involved in successful retrieval under long-term stress. The decrease in multivoxel neural distinctiveness and network efficiency over time was attenuated in the posterior neocortex under long-term stress, particularly in the posterior cingulate cortex and fusiform gyrus. Suppression of remote memories was associated with higher engagement in the right prefrontal cortex, but less disengagement in concomitant posterior neocortex in controls, but not in long-term stress group. The long-term stress group exhibited reduced time-dependent changes in the fusiform gyrus, which were associated with higher negative coupling between the inferior frontal cortex and fusiform gyrus during the suppression of remote memories. Time-dependent functional connectivity changes in the amygdala-left posterior cingulate cortex are

negatively correlated with psychological distress. Our findings suggest a neurocognitive model that explains how long-term stress affects the retention of emotional memories and the processes of memory suppression that are intertwined with it, as well as the potential psychological health risks. This model provides a potential basis that sheds light on the heightened sensitivity and over-retention of emotional memories observed in stress-related disorders.

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Poster

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Topic: F.03. Stress and the Brain

Support: JSPS KAKENHI 17H01758
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Title: Cognitive fatigue changes resting-state functional connectivity in the dorsal attention networks as well as default-mode network

Authors: *S. IWAKI[1,2], T. FUJIWARA[2];
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Abstract: Cognitive fatigue (CF) is defined as a decline of cognitive resources caused by sustained cognitive demands (Pattyn, 2018). The cognitive fatigue had been evaluated by behavioral measures such as the flicker test (Iwaki, 2013) which assesses the perception threshold of flickering lights. However, the neural mechanisms underlying the cognitive fatigue is still unclear. The current study employs functional MRI to evaluate resting-state functional connectivity between brain regions to investigate changes in brain networks under cognitive fatigue. Eighteen adults participated in the study which includes two days of lab visits, separated by more than a week. On each visit, the participants conducted two sessions of MRI data acquisition separated by either cognitive task sessions for fatigue loading (CF condition) or passive environmental video viewing sessions as control. The order of CF and control conditions was randomized between participants. The fatigue loading in the CF condition consisted of 4 sessions of 30-minutes continuous simple mental arithmetic task with 5 minutes of interval. MRI scans were conducted before and after the fatigue loading/control sessions, which include T1 structural scans and 12-minutes resting-state fMRI (rsfMRI) scans using 3 T scanner (Philips Ingenia 3.0T). CONN software (Whitfield-Gabrieli, 2012) was used to calculate the effective connectivity between brain regions.. This study was approved by the internal review board of National Institute of Advanced Industrial Science and Technology (AIST). The flicker perception threshold (FPT) after the fatigue loading session in the CF condition decreased significantly compared to the FPT measured before the session ($p < 0.05$) while FPT did not changed significantly in the control condition. Results of the connectivity analysis of the rsfMRI

data showed that (a) significant increase in effective connectivity between the posterior cingulate cortex (PCC), which is a part of the default mode network (DMN), and the bilateral inferior occipital gyrus (IOG), and (b) decrease in connectivity between the frontal eye field (FEF), one of the nodes included in the dorsal attention network (DAN), and the anterior cingulate cortex (ACC), in the CF condition compared to the control condition. The current results indicate the changes in resting-state brain network after the cognitive fatigue loading which might reflect the difficulty in allocating attentional resources to specific task performance under cognitive fatigue (Esterman, 2013).

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Poster

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Program #/Poster #: PSTR356.19/LL10

Topic: F.03. Stress and the Brain

Title: Exposure to the Trier Social Stress Test enhances central detail memory, reduces false memory, and results in intrusive memories that last for days

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Abstract: Recent work has used a modified version of the well-known laboratory stressor, the Trier Social Stress Test (TSST), to study participant memory for a stressful experience. The paradigm is useful because, unlike most studies examining stress effects on memory, it allows investigators to measure what participants remember about the stressor, not unrelated information. It also presents an opportunity to model other stress-related symptoms, such as intrusive memories, but these have yet to be assessed with this paradigm. Intrusive memories have been notoriously difficult to measure in laboratory settings; most of this research involves participants watching arousing videos and subsequently reporting any intrusions they experience. However, measuring intrusive memories that result from arousing videos is quite dissimilar from measuring intrusive memories that result from a stressful event. Thus, we aimed to replicate and extend previous work by examining the impact of TSST exposure on (1) participant memory for the stressful event, (2) false memories of the stressful event, and (3) intrusive memories related to the stressful event. Healthy undergraduate students were exposed to the TSST or the friendly-TSST (f-TSST). The TSST required participants to deliver a ten-minute speech in front of two lab panel members as part of a mock job interview; the f-TSST required participants to casually converse with panel members about their interests and hobbies. In both conditions, the panel members interacted with (central) or did not interact with (peripheral) several objects sitting on a desk in front of them. Participants' heart rate, blood pressure, and anxiety levels were assessed

before and after the TSST or f-TSST, and saliva samples were collected to assay for cortisol and alpha-amylase. The next day, participants' memory for the objects that were present on Day 1 was assessed with recall and recognition tests. We also quantified participants' intrusive memories for each task by having them complete an intrusive memory questionnaire on Days 2, 4, 6, and 8. Participants exposed to the TSST exhibited greater recall of central objects and fewer falsely recalled objects than participants exposed to the f-TSST. Most importantly, participants exposed to the TSST reported a greater number of intrusive memories related to the speech task. Some measures of intrusive memories in this group persisted for several days after stress exposure. Collectively, our work demonstrates that the modified TSST paradigm is a useful tool to not only study what participants remember about a stressful event but to also investigate characteristics of intrusive memories that may ensue.

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Poster

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Title: Spatiotemporal neural characteristics of human psychological resilience measured by simultaneous fMRI-EEG recordings.

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Abstract: Psychological resilience is an individual ability to adapt successfully to acute stress, trauma, or more chronic forms of adversity. Non-human animal studies have shown that several systems in the brain and peripheral organs involve in resilience. In these studies, individual differences in resilience have been assessed by the occurrence of depressive behaviors following

repeated stressful experiences. In humans, however, resilience is not simply assessed by depressive symptoms. Rather, human resilience requires highly cognitive mental processes, such as self-confidence and positive acceptance of challenges, to overcome experienced stressful events. Therefore, to understand the full picture of the underlying neurophysiological responses that contribute to psychological resilience, examining these responses in humans is essential. Here, we investigated acute stress-driven whole-brain dynamics (for 90 minutes) simultaneously with functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and peripheral responses, including the pupil, cardiac, respiration, and cortisol measurements in humans. Then brain and peripheral responses correlated with the CD-RISC, a popular human resilience questionnaire, were evaluated. fMRI and EEG data collected from 90 participants (68 males and 22 females, mean age was 20.13 years old) identified several brain activities corresponding to individual resilience differences ($P < 0.05$ *FDR corrected*). Functional connectivity (FC) analysis with fMRI detected two FC networks. One network was negatively and the other positively correlated with the individual resilience level (RFCn and RFCp, respectively). RFCn overlapped with the salience network, and RFCp overlapped with the default mode network. In addition, the fractional amplitude of low-frequency fluctuations (fALFF) analysis in the subcortical regions showed spontaneous brain fluctuations in the posterior hippocampus were increased in higher resilient individuals. EEG power spectrum density (PSD) at 26.5 Hz (high beta) and 43.0 Hz (gamma) negatively correlated with the individual resilience level. Interestingly, all of these identified brain activities were revealed approximately 70 minutes after the stress exposure but not immediately after (peak timing of stress-driven autonomic response) or 20 minutes after (peak timing of stress-driven hormonal response). Our experiment identified spatially and temporally novel neural dynamics associated with individual differences in human resilience.

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Poster

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Program #/Poster #: PSTR356.21/LL12

Topic: F.03. Stress and the Brain

Title: Cognitive characteristics that predict helpful thoughts during periods of stress

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Abstract: Prior research shows that individuals differ in the way they respond cognitively and behaviorally to stress. A better understanding of these differences - as well as demographic and trait factors that may moderate such responses - can lead to more effective stress management

strategies for various groups. Here we used Ecological Momentary Assessment (EMA) in an exploratory study to examine how individuals who report primarily helpful thoughts during stressful episodes differ in their everyday thinking profiles from those who report primarily unhelpful thoughts during similarly stressful episodes. Potentially moderating variables, including sociodemographic characteristics and trait-level factors such as personality, were also examined. Six times daily, EMA surveys assessed self-reported mood and several characteristics of momentary thought (such as perceived helpfulness, valence, intentionality, self-focus, etc.). Participants were included in the current analyses if they reported "stress," based on a combination of high arousal and low valence, during at least 5 EMA surveys. The included sample (n = 703) had an age range of 18-89 and 75.6% self-identified as female, 22.1% as male, and 2.1% as non-binary/declined to identify. Participants were then characterized as belonging to one of two groups based on the mean of their answers during stressful episodes to the question "Regarding your thoughts in the moments just before the notification, how productive or helpful do you think they were?". One group reported helpful thoughts when experiencing stress while the other group reported the opposite. Logistic regression indicated that having helpful thoughts during periods of stress was significantly predicted by higher ratings of positivity among thoughts in general. There were no statistical differences between groups with other selected thought characteristics, or sociodemographic or trait variables examined. In summary, individuals who are generally able to maintain positivity in their thoughts may find their thoughts to be more beneficial during periods of stress. Although correlational, these results suggest that interventions that emphasize practicing positive thinking may provide better stress management outcomes across diverse groups.

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Poster

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Program #/Poster #: PSTR356.22/LL13

Topic: F.03. Stress and the Brain

Title: The effect of virtual environments on objective and subjective stress parameters and cognition in healthy military personnel - A validation study

Authors: *F. P. M. SCHILDER[1], F. WITHAGEN[3], L. VAN HERK[5], A. DE WEIJER[4], B. BRUINSMA[2], E. GEUZE[6];
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Abstract: Military personnel engaged in military operations are increasingly confronted with acute stress arising from physical, environmental, social, and psychological stressors, which can negatively impact cognitive functions. Accordingly, modern-day military training incorporates these stressors in real-life training simulations to prepare the trainee for the effect of operation-related stressors on a physical and mental level. Military training, however, often emphasizes the multi-stressor environment by implementing a variety of mental and, mainly, physical stressors. Therefore, the effect of a distinctive stressor can hardly be disentangled from combined stressors using objective psychophysiological stress parameters (e.g., heart rate or blood pressure) as all combined stressors exert a simultaneous and significant effect on stress levels, mood, and cognition. These limitations have been increasingly addressed over the last decade now the development and implementation of computerized simulations is becoming more important. Unlike military field training, the versatility of virtual simulations creates reliable and controllable situations that enables real-time monitoring of psychophysiological parameters and precise control over distinctive stressors.
For that reason, a within-subject validation study (N = 20) was conducted in healthy military personnel to determine the efficacy of Virtual Battle Space, a renowned virtual military simulator, in eliciting a stress response. This was achieved by comparing the physiological and psychological effects of two military VBS scenarios designed to elicit a stress response, to the effect of a validated virtual reality stress task, the Narrow Beam Walking Test. Physiological data, including heart rate, heart rate variability and respiration rate, was collected to objectively quantify stress responses. Subjective stress levels were assessed using the Visual Analogue Scale and the State-Trait Anxiety Inventory. Additionally, we assessed cognitive functioning, namely attention, processing speed, reaction time, and vigilance, using the Stroop task and a psychomotor vigilance task.
We are currently in the final stages of data analysis and expect to present the conclusive results at the upcoming conference, offering valuable insights and contributions to the field.

Disclosures: **F.P.M. Schilder:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **F. Withagen:** A. Employment/Salary (full or part-time):; Ministery of Defence. **L. van Herk:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **A. De Weijer:** A. Employment/Salary (full or part-time):; Ministry of Defence. **B. Bruinsma:** A. Employment/Salary (full or part-time):; UMC Utrecht, Ministry of Defence. **E. Geuze:** A. Employment/Salary (full or part-time):; Ministry of Defence.

Poster

PSTR356. Stress and Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR356.23/LL14

Topic: F.03. Stress and the Brain

Title: Sleep problems during the confinement for COVID-19 in Mexico population

Authors: *Y. DEL RÍO-PORTILLA[1], L. V. ORTEGA-LEONARD[2], S. P. CAÑARTE-VARELA[3], M. CASTRO-GONZÁLEZ[4], A. FLORES-FLORES[4], B. LEYTE-MEDINA[4], D. VALDÉS-REYES[4];
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Abstract: During the COVID-19 pandemic in Mexico, social isolation began in March 2020, in which activities in schools and work were paralyzed and only people who needed to leave or health personnel, or transportation (essential) were touring the city. Therefore, we were interested in knowing what happened in the psychological aspects of people, especially during rest, if sleep had changed. Sleep is a natural process, necessary for living beings, it is a physiological process of vital importance, which involves different systems of the organism, for the integral health of human beings. Disturbed sleep and associated sleep-facilitated fever responses are known to be adaptive responses to infection. But we will not talk about patients with COVID-19 but what happens with isolation. For this reason, we carried out a survey in Google form that included questions about hours of sleep before and during the pandemic. In addition, the survey included sleep quality and behavioral symptoms. The survey was answered by 2,528 from different entities in Mexico, 1,911 women, 619 men; between 13 and 75 years old, on average it was 34 years.During lockdown or being isolated at home; or stay at home; people have increased the use of digital media; have changed bedtime, but this change did not affect sleep habits. However, during home confinement, sleep schedules have changed markedly, as people go to bed and wake up later, spend more time in bed, but paradoxically also report lower sleep quality. The increase in sleep difficulties was stronger for people with a higher level of depression, anxiety, and stress symptoms, and was associated with the feeling of lengthening time. Given that the lockdown is likely to continue for weeks, research data is urgently needed to support decision-making, raise public awareness, and provide timely and supportive psychosocial interventions (Sher, 2020). This is considering the beginning of the pandemic.

Disclosures: Y. del Río-Portilla: None. **L.V. Ortega-Leonard:** None. **S.P. Cañarte-Varela:** None. **M. Castro-González:** None. **A. Flores-Flores:** None. **B. Leyte-Medina:** None. **D. Valdés-Reyes:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.01/LL15

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant U19NS128613
NIH Grant R01NS078168

NIH Grant R01NS101353
NIH Grant T32NS115667

Title: Understanding the Influence of Cholinergic and Noradrenergic Modulation on Hemodynamics during Sleep

Authors: *M. HOSSAIN[1,2], K. L. TURNER[1,2,3], Q. ZHANG[2,3], P. J. DREW[1,2,3,4,5];
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Abstract: Cortical arteries undergo substantial dilations and constrictions during sleep, more prominent than those observed while awake. These arterial dynamics have been linked to periarterial pumping, the displacement of cerebrospinal fluid (CSF). However, the drivers of vascular dynamics during sleep remain poorly understood. To better understand the neuromodulatory effect of norepinephrine (NE) and acetylcholine (ACh) on vascular dynamics, we used fiber photometry to measure cerebral blood volume (CBV) and neuromodulators using fluorescent NE- and ACh-sensitive biosensors (GRAB-NE, GRAB-ACh) in the barrel cortex of head-fixed mice, during awake states, and NREM and REM sleep. Sensory stimulation of the whisker caused vasodilation and increases in ACh and NE. There was an increased blood volume during the awake-to-NREM sleep and NREM-to-REM sleep transitions. These blood volume increases were more prominent than sensory stimulus-induced changes. ACh levels decreased during awake -to- NREM sleep transition but slowly increased during the NREM-to-REM sleep transition. NE levels decreased during awake-to-NREM sleep and NREM-to-REM sleep transitions. Awakening from either sleep stage was associated with large increases in NE and ACh levels, tied to sharp decreases in CBV. Microarousals (MA), detected from brief EMG spikes during NREM sleep, were associated with a rise in NE and ACh levels and a brief decrease in blood volume. These results suggest that NE and ACh modulation may help shape vascular dynamics during sleep-wake stages.

Disclosures: **M. Hossain:** None. **K.L. Turner:** None. **Q. Zhang:** None. **P.J. Drew:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.02/LL16

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIA/NIH/IRP

Title: Early vascular aging in chronic kidney disease model is associated with cognitive dysfunction in aged male and female Sprague Dawley rats.

Authors: *C. ROCHA DOS SANTOS[1], Y. N. GRIGOROVA[1], R. A. MCDEVITT[2], J. LONG[3], M. HAGHKAR[1], W. WEI[1], V. ZERNETKINA[1], O. JUHASZ[1], C. H. MORRELL[1], P. R. RAPP[3],

K. SODHI[4], E. G. LAKATTA[1], O. V. FEDOROVA[1];
[1]Lab. of Cardiovasc. Sci., [2]Comparative Med. Section, [3]Lab. of Behavioral Neurosci., NIH, Natl. Inst. On Aging, Baltimore, MD; [4]Marshall Univ., Huntington, WV

Abstract: Early vascular aging (EVA) is associated with accelerated central arterial stiffening (CAS) and vascular remodeling, which develop earlier in life than expected and contribute to cardiovascular disease (CVD) and cognitive impairment. CVD and CAS accompany chronic kidney disease (CKD). Hypothesis: CKD will be accompanied by EVA and may differentially impact on cognition in aged male and female rats. Male and female 10-mo old Sprague-Dawley rats were fed a 0.25% adenine diet to induce CKD (n=10-12) or standard control diet (n=8-10) for 2-mo. Body weight (BW), systolic blood pressure (SBP), aortic pulse wave velocity (aPWV; an index of CAS), ejection fraction (EF; by echocardiography), plasma creatinine (pCr), blood urea nitrogen (BUN), hematocrit and tissue weights were assessed at 12-mo. Anxiety-like behavior was examined in an open field at 10- and 12-mo, and in an elevated plus maze (EPM) at 12-mo. Spatial memory was tested in water maze (MWM) and cross maze (CM) procedures at 12-mo. Data analysis by 2-way ANOVA, 2-tailed $p<0.05$ was considered significant. CKD had higher aPWV and aortic weight in both sexes vs. control groups, but only CKD females displayed lower EF. CKD in both sexes was accompanied by enlarged kidneys, higher BUN and pCr, and lower hematocrit. Kidney function was more compromised in CKD males than CKD females; CKD males unlike CKD females had lower BW and SBP vs. CNT group. Both sexes displayed numerically poorer spatial memory, i.e., trends toward greater path length to find the hidden platform in MWM than controls, while alternation rate in CM was lower selectively in CKD males, and greater change between 10- and 12-mo for time spent in the center of the open field. CKD females exhibited higher level of anxiety, spending less time in open arm of EPM vs. control females. CKD development in aged male and female rats was accompanied by EVA, increase in CAS and anxiety. In contrast, CKD-induced CV remodeling was more pronounced in females whereas cognitive impairment was more pronounced in males.Supported by NIA/NIH/IRP

Table 1. Physiological, cardiovascular, and behavioral parameters in aged males and females in a rat model of CKD

	Males		Females		Statistical analysis
	Control, n=11	CKD, n=10	Control, n=10	CKD, n=12	2-way ANOVA (significant effects are in bold)
Body weight, g	809 ± 121	601 ± 55 *	422 ± 106 #	339 ± 21 #	**Sex: p=0.02; CKD: p<0.01; CKD*Sex: p<0.01**
SBP, mmHg	135 ± 14	118 ± 12 *	120 ± 14 #	134 ± 16 #	Sex: p=0.99; CKD: p=0.73; **CKD*Sex: p<0.01**
DBP, mmHg	101 ± 12	90 ± 8 *	85 ± 9 #	94 ± 11	Sex: p=0.08; CKD: p=0.72; **CKD*Sex: p<0.01**
HR, bpm	319 ± 23	283 ± 21 *	349 ± 19 #	312 ± 25 *#	**Sex: p<0.01; CKD: p<0.01;** CKD*Sex: p=0.96
aPWV, m/s	3.5 ± 0.4	4.6 ± 0.8 *	3.5 ± 0.3	4.2 ± 0.4 *	Sex: p=0.28; **CKD: p<0.01;** CKD*Sex: p=0.16
Ejection fraction %	68.2 ± 6.0	69.7 ± 5.2	73.2 ± 5.7	62.0 ± 2.8*#	Sex: p=0.37; **CKD: p<0.01; CKD*Sex: p<0.01**
Hematocrit, %	47 ± 2	34 ± 5*	39 ± 2#	36 ± 2*	**Sex: p<0.01; CKD: p<0.01; CKD*Sex: p<0.01**
BUN, mg/dL	14.0 ± 2.8	89.1 ± 40.0*	14.1 ± 2.9	33.6 ± 13.0 #	**Sex: p<0.01; CKD: p<0.01; CKD*Sex: p<0.01**
Plasma Creatinine, mg/dL	0.4 ± 0.1	2.5 ± 1.1 *	0.3 ± 0.06	0.8 ± 0.3 *#	**Sex: p<0.01; CKD: p<0.01; CKD*Sex: p<0.01**
Kidney weight/BW, g/kg	4.7 ± 0.4	9.6 + 1.8 *	6.1 ± 1.2 #	9.7 ± 1.4 *	Sex: p=0.06; **CKD: p<0.01; CKD*Sex: p=0.11**
Heart weight/BW, g/kg	2.0 ± 0.1	2.2 + 0.2	2.6 ± 0.4 #	2.9 ± 0.3 #	**Sex: p<0.01; CKD: p=0.02;** CKD*Sex: p=0.47
Aortic weight/BW, mg/mm x kg	2.29 ± 0.49	2.94 ± 0.34 *	3.46 ± 0.84 #	4.18 ± 0.69 *#	**Sex: p<0.01; CKD: p<0.01**; CKD*Sex: p=0.83
OFT, Δ of time spent in the central zone, sec	-20 ± 23	-45 ± 48 *	8 ± 19	-4 ± 13 #	**Sex: p=0.02; CKD: p<0.01; CKD*Sex: p<0.05**
CM, % spontaneous alternation	36 ± 12	15 ± 13 *	36 ± 17	36 ± 12 #	**Sex: p=0.04; CKD: p=0.02;** CKD*Sex: p=0.23
MWM, path length, Δ hidden-visible platform, m	2.2 ± 4	3.7 ± 3.5	1.0 ± 2.4	3.8 ± 3.8	Sex: p=0.62; CKD: p=0.056; CKD*Sex: p=0.61
EPM, time spent in open arm	59 ± 43	18 ± 12	97 ± 60	46 ± 45 *	**Sex: p<0.01; CKD: p<0.02;** CKD*Sex: p=0.77

Data presented as mean ± SD. Aortic weight was normalized to aortic length and body weight; kidney and heart weights were normalized to body weight. Adenine does not cross blood brain barrier, for that, it does not affect brain function directly. Open field test (OFT) results are expressed as difference (delta, Δ) between 12-mo and 10-mo. Statistical analysis by 2-way ANOVA followed by Holm-Sidak's multiple comparison post hoc test: * p<0.05 vs. sex-matched control; # p<0.05, females vs. males.

Disclosures: **C. Rocha dos Santos:** None. **Y.N. Grigorova:** None. **R.A. McDevitt:** None. **J. Long:** None. **M. Haghkar:** None. **W. Wei:** None. **V. Zernetkina:** None. **O. Juhasz:** None. **C.H. Morrell:** None. **P.R. Rapp:** None. **K. Sodhi:** None. **E.G. Lakatta:** None. **O.V. Fedorova:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.03/LL17

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Cgrp regulates small vessels of the vasa nervorum in peripheral nerves

Authors: S. R. L. SVENSSON[1], H. NILSSON[1], ***I. HAMMAR**[2];
[1]Neurosci. and Physiol., Göteborg, Sweden; [2]Inst. Neurosci & Physiol, Goeteborg, Sweden

Abstract: The vasa nervorum form a microvascular network within peripheral nerves and are in turn regulated by sensory innervation. The aim of this study was to examine the vascularization of the sciatic nerve and the degree to which the vessels of the vasa nervorum are innervated by calcitonin gene-related peptide (CGRP). Secondly, we aimed to compare the vascularization and innervation of vasa nervorum in the sciatic (mixed) nerve with a motor (quadriceps) and a sensory (saphenous) nerve.
The sciatic, saphenous and quadriceps nerves were harvested from 4 adult female Sprague Dawley rats and cut into 15 µm transverse sections. The vascularization was visualized with tomato lectin and the number of vessels and their approximate lumen diameter measured on the fixed specimens and analyzed using Zen 3.3. Vessels positive or negative for a CGRP marker (#A-11032) were analyzed using the FIJI plug in BIOP JaCoP.
Of 384 (± 97) labelled vessels analyzed in a sciatic nerve, 53% (± 20%) were positive for CGRP. CGRP was almost exclusively detected in vessels with a diameter < 15 µm (57% ±20%) compared to larger vessels (5%±-0,8%). In comparison, a higher degree of vessels small diameter vessels were CGRP-positive in the saphenous nerve (63% ± 18%) while in quadriceps only 22% (± 17%) were positive.
The results suggest that within peripheral nerves CGRP primarily innervates smaller vessels while it is almost completely absent in vessels with a diameter >15 µm. Furthermore, the vasa nervorum supplying sensory nerve fibres seem to have a higher degree of CGRP innervation than those supplying motor nerve fibres. The results could have clinical implications in peripheral neuropathies affecting the blood flow of peripheral nerves and indicate possible clinical targets.

Disclosures: **S.R.L. Svensson:** None. **H. Nilsson:** None. **I. Hammar:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.04/LL18

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant EB028319

Title: Monitoring blood flow in a preclinical model of intraventricular hemorrhage using diffuse correlation spectroscopy

Authors: *J. V. JETHE[1], G. VINUKONDA[2], J. A. N. FISHER[1];
[1]Physiol., [2]Cell Biol., New York Med. Col., Valhalla, NY

Abstract: Monitoring blood flow in a preclinical model of intraventricular hemorrhage using diffuse correlation spectroscopy Jyoti V. Jethe, Govindaiah Vinukonda, Jonathan A. N. Fisher
Intraventricular hemorrhage (IVH) is a common condition in premature infants and is associated with white matter injury (WMI) as well as neurodevelopmental and cognitive disabilities. Transcranial Doppler ultrasound measurements can detect IVH by visualizing the presence of blood clotting in the lateral ventricles and within the parenchyma; however, the modality has limited sensitivity and cannot easily provide real-time measurements of cerebral blood flow (CBF). In this study, using a well-established premature rabbit model of glycerol induced intraventricular hemorrhage (Georgiadis et al., *Stroke,* 2008 and Vinukonda et al., *Stem Cells Transl. Med.* 2019), we examine the feasibility of using optical diffuse correlation spectroscopy (DCS) as a non-invasive technique for detecting microvascular blood flow fluctuations during the early stages of hemorrhage and after hemorrhage. DCS is a noninvasive diffuse optical modality that permits the measurement of blood flow changes in microvasculature and has already been validated for detecting a wide range of neurological conditions that affect CBF. We describe a DCS apparatus that is compatible with preclinical studies of the acute phase of IVH. To assess sensitivity, we used a premature rabbit pup model of IVH that features germinal matrix hemorrhage induced by glycerol. Future experiments will leverage this technical approach to provide noninvasive early-stage monitoring of IVH in order to facilitate the development of therapeutic approaches for IVH, which are currently lacking.

Disclosures: J.V. Jethe: None. **G. Vinukonda:** None. **J.A.N. Fisher:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.05/LL19

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01NS078168
R01NS079737
U19NS128613
T32GM108563
T32NS115667

Title: Long-range projecting cortical nNOS neurons modulate bilateral synchronization and neurovascular coupling

Authors: *K. TURNER[1,2], H. S. UNSAL[3], D. F. BROCKWAY[4], M. HOSSAIN[3], D. I. GREENAWALT[5], Q. ZHANG[2], Q. ZHANG[3], K. W. GHERES[2], N. A. CROWLEY[4], N. ZHANG[3], P. J. DREW[2];
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Abstract: Understanding the relationship between neural activity and vascular dynamics is important for interpreting local and global hemodynamic signals in the brain. Previous work has shown that the activity of neuronal nitric oxide synthase (nNOS) expressing neurons play an important role in neurovascular coupling (NVC) and setting the basal diameter of arterioles. To explore the role of these neurons in NVC and bilateral hemodynamic correlations, we selectively removed a subset of nNOS-expressing interneurons, known as long-range projecting interneurons or type-1 nNOS neurons, in the somatosensory cortex of one hemisphere. These neurons comprise ~0.2% of the neurons in the cortex, and are the only cell type in the cortex that express the substance P receptor. We unilaterally ablated these neurons in the somatosensory areas of mice with intracranial injections of saporin conjugated to substance P. We measured cerebral blood volume and neural activity in these mice during sleep and wake states using widefield imaging, electrophysiology, and 2-photon microscopy. Ablation decreased sustained vascular responses to stimulation and the bilateral correlations in gamma-band power and blood volume in the somatosensory cortex at ultra-low frequencies (~0.003-0.1 Hz). Our results suggest that a small subset of nNOS neurons may play a key role in neurovascular coupling and in synchronizing neural and vascular signals across hemispheres at ultra-low frequencies.

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Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Career Development Award from the American Heart Association 935961
NIH Grant R01NS078168
NIH Grant R01NS079737
NIH Grant U19NS128613

Title: Opposing effects of neuromodulation and local neural activity on cortical hemodynamic responses during behavior

Authors: *Q. ZHANG[1], K. W. GHERES[2], P. J. DREW[3];
[1]Dept. of Engin. Sci. and Mechanics, [2]Grad. Program in Mol. Cell. and Integrative Biosci., [3]Departments of Engin. Sci. and Mechanics, Biomed. Engineering, Biology, and Neurosurg., The Pennsylvania State Univ., University Park, PA

Abstract: While the local control of cerebrovascular dynamics (via signaling from neurons and astrocytes) has been extensively studied, brain-wide neuromodulatory inputs, particularly noradrenergic projections from the locus coeruleus (LC), may have an important role controlling the dynamics of neurovascular coupling. However, we do not know how noradrenergic modulatory drive from the LC influences cerebral responses, particularly during behaviors in which noradrenergic tone changes. Previous work has shown that during locomotion, neural activity in the frontal cortex increases without corresponding changes in blood volume. We sought to mechanistically understand this neurovascular decoupling. We hypothesized that neuromodulatory input, specifically a vasoconstrictory noradrenergic signal, interacts with neural activity-driven vasodilation to shape the hemodynamic response. Using fiber photometry and genetically encoded calcium indicators, we found that both pyramidal and nitric oxide synthase expressing neurons were robustly activated by locomotion in the frontal cortex. Using genetically encoded fluorescent biosensors, we found that cortical extracellular levels of noradrenaline increased during locomotion. Pharmacological manipulation of adrenoceptors revealed that locomotion-evoked blood volume responses are inversely related to noradrenergic tone. Selectively activating LC using optogenetics replicates the region-specific hemodynamic responses during locomotion. These results suggest that in addition to local vasodilatory signals released from neurons and astrocytes, changes in neuromodulatory tone (particularly noradrenaline) play an important vasoconstrictory role in shaping hemodynamic signals during behavior, contributing to a disassociation between neural activity levels and blood volume signals.

Disclosures: Q. Zhang: None. **K.W. Gheres:** None. **P.J. Drew:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

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Program #/Poster #: PSTR357.07/LL21

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: JSPS Kakenhi (21H05614, 22K06454, 22KK0140)
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AMED Brain/MINDS (JP21dm0207111)

Title: Visualization of murine cerebral circulation by fluorescent protein knock-in to the albumin locus.

Authors: M. VITTANI[1], P. A. G. KNAK[1], Y. HIRAOKA[2], A. B. LEE[1], X. WANG[1], A. KONNO[4], P. KUSK[1], M. NAGAO[1], A. ASIMINAS[1], T. MISHIMA[1], C. KJAERBY[1], M. TERUNUMA[5], M. FUKUDA[6,7], H. HIRAI[4], M. NEDERGAARD[1,8], K. TANAKA[3], ***H. HIRASE**[1,8];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Tokyo Med. and Dent. Univ., Tokyo, Japan; [3]Tokyo Med. and Dent. Univ., Bunkyo-ku, Japan; [4]Gunma Univ., Maebashi, Japan; [5]Niigata Univ., Niigata, Japan; [6]Duke-Nus Grad. Med. Sch. Singapore, Singapore, Singapore; [7]Kumamoto Univ., Kumamoto, Japan; [8]Univ. of Rochester Med. Ctr., Rochester, NY

Abstract: Albumin, a protein produced by liver hepatocytes, represents the most abundant protein in blood plasma and cerebrospinal fluid. We have previously engineered a liver-targeting adeno-associated viral vector (AAV) that expresses fluorescent protein-tagged albumin to visualize blood plasma (DOI: 10.1016/j.crmeth.2022.100302) in mice. While this approach was versatile for imaging in adult mice, a few weeks are needed before enough fluorescent plasma label is produced, making chronic imaging in infant or juvenile mice unattainable. Here, we use CRISPR/Cas9 genome editing to insert a fluorescent protein sequence to produce fluorescent protein-tagged albumin. We constructed an AAV that includes ~1 kb homologous arms around *Alb* exon 14 to express *Alb-mNeonGreen* instead of *Alb*. Systemic injection of this AAV with AAV-CMV-Cas9 in postnatal day 3 mice resulted in two-photon visualization of the cerebral cortex vasculature within ten days. The expression level of *Alb-mNeonGreen* was high for at least three months. As an alternative approach, we have generated a mouse in which the bright red fluorescent protein mScarlet is knocked in to *Alb* exon 14 by CRISPR/Cas9 genome editing with homologous arms of ~1.5 kb. The heterozygous knock-in infant mice are clearly distinguishable by their skin fluorescence using a commercial genotyping LED/filter set. The presumed few hundred micromolar mScarlet-tagged albumin concentration in the blood plasma is sufficient to visualize the entire depth of the vasculature in the cerebral cortex of the adult heterozygous knock-in mouse by two-photon microscopy. Furthermore, the extracellular space appears to have low-level fluorescent signals in the cerebral cortex parenchyma while the cellular elements do not contain notable fluorescence. These two genome-editing approaches are expected to provide powerful means for micro and macroscopic imaging of vascular dynamics in the mouse brain and other organs.

Disclosures: **M. Vittani:** None. **P.A.G. Knak:** None. **Y. Hiraoka:** None. **A.B. Lee:** None. **X. Wang:** None. **A. Konno:** None. **P. Kusk:** None. **M. Nagao:** None. **A. Asiminas:** None. **T.**

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Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.08/LL22

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: U19NS128613
R01NS078168
R01NS101353
T32NS115667
T32GM108563

Title: The dynamics of Tac1-expressing neuron activity during sleep and wake states

Authors: *F. SALEHI SHAHRBABAKI[1], K. L. TURNER[1], M. HOSSAIN[1], D. GREENAWALT[2], Q. ZHANG[3,4], P. J. DREW[1,3,4,5];
[1]Dept. of Biomed. Engin., [2]Grad. Program in Molecular, Cellular, and Integrative Biosci., [3]Ctr. for Neural Engin., [4]Dept. of Engin. Sci. and Mechanics, [5]Biol. and Neurosurg., The Pennsylvania State Univ., State College, PA

Abstract: Sleep is accompanied by large arterial dilations and constrictions in the cortex, and vascular changes are thought to play an important role in movement of cerebrospinal fluid and clearance of waste. The vascular changes are thought to move cerebrospinal fluid (CSF) via periarterial pumping, potentially removing wastes from the brain. However, which neuronal cell types control vascular changes during sleep has yet to be fully elucidated. Previous work has shown that a subset of vasodilatory neuronal nitric oxide synthase-expressing neurons are activated by the Substance P (SP) peptide, which is released by a subset of parvalbumin (PV) neurons, and local infusion of SP leads to vasodilation via activation of these neurons. Under anesthesia, optogenetic activation of PV neurons leads to a biphasic hemodynamic response under anesthesia, with initial vasoconstriction followed by a prolonged vasodilation that is mediated by SP lasting tens of seconds. We sought to understand under what arousal state conditions SP-releasing neurons were active by expressing GCaMP7 under control of a TAC1 promoter. We used wide-field imaging in head-fixed mice to visualize the activity of the SP-releasing subset of PV neurons and blood volume during sleep and wake states. We found that TAC1-expressing neurons were activated by sensory stimulation, as well as during NREM and REM sleep. Our results suggest these neurons could play a role in neurovascular coupling in the brain.

Disclosures: F. Salehi Shahrbabaki: None. **K.L. Turner:** None. **M. Hossain:** None. **D. Greenawalt:** None. **Q. Zhang:** None. **P.J. Drew:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.09/LL23

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: Cerebrovascular reactivity reflects trajectories of post-acute sequelae of COVID-19

Authors: *Y. MATOS, N. SHAFF, S. NITSCHKE, K. JULIO, S. RYMAN, A. A. VAKHTIN; The Mind Res. Network, Albuquerque, NM

Abstract: Along with the hallmark symptoms of COVID-19, around 35% of patients report neurocognitive issues that persist long-term as part of post-acute sequelae of COVID-19 (neuro-PASC). The SARS-CoV-2 virus has been histologically shown to interact directly with brain vasculature via angiotensin-converting enzyme 2 (ACE2) receptors - which are widely expressed by endothelial and smooth muscle cells. Given that adequate cerebral perfusion depends on the healthy functioning of these cells, it is of interest to establish the *in-vivo* consequences of the virus-vasculature interactions in the brain. We used cerebrovascular reactivity (CVR) functional magnetic resonance imaging (fMRI) to quantify brain vasculature dynamics in 23 former COVID-19 patients who were at least 3 months post-infection. Specifically, vasoreactivity to 5% CO2 gas respiration was assessed in terms of its response Magnitude and Delay in global gray (GM) and white matter (WM). A multiple regression analysis revealed that Time Since COVID-19 (TSC) and Age significantly predicted shorter CVR Delay in the WM ($F_{2, 20} = 3.640$, $p = 0.045$), but not GM ($p = 0.242$). The TSC term specifically was predictive of lower WM CVR Delay ($p = 0.050$). Follow-up analyses examined the relationship between TSC and WM CVR Delay in participants who reported full recoveries (PASC-) and those reporting persistent neurocognitive symptoms (PASC+). The relationship between TSC and WM CVR Delay appeared to be driven by the PASC- group, which showed a pattern that suggested decreases in WM CVR Delay as time post-COVID-19 increased ($p = 0.112$). On the other hand, the PASC+ group showed no effect of TSC on WM CVR Delay ($p = 0.941$). Collectively, these findings suggest that the delay in cerebrovascular response in the white matter may reflect the post-infection timeline of neuro-PASC. Cerebrovascular dysfunction may contribute to neuro-PASC symptomology via neuronal malnourishment due to episodic hypoperfusion secondary to a delayed vascular response, as well as via accumulation of cellular metabolic byproducts due to inadequate cerebrovascular mechanical action necessary for their removal by the glymphatic system. Our findings motivate further examinations of the effects of COVID-19 on brain vasculature and its sequelae on neural and cognitive function via other imaging modalities and larger samples.

Disclosures: Y. Matos: None. **N. Shaff:** None. **S. Nitschke:** None. **K. Julio:** None. **S. Ryman:** None. **A.A. Vakhtin:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.10/LL24

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant 1F31NS132422-01
NIH Grant R01- NS090444
NIH Grant R01-NS117515
NIH Grant R01-NS119410

Title: Unraveling the Intricate Neural Circuitry of Neurovascular Coupling: Exploring How a Subset of Somatostatin Neurons Regulates Changes in Cerebral Blood Flow

Authors: *F. JUAREZ ANAYA, C. F. RUFF, S. E. ROSS, A. L. VAZQUEZ;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Cerebral blood flow (CBF) and neuronal activity exhibit variations in different brain states. Specifically, CBF changes are more significant during high gamma-band activity associated with sensory-evoked responses and during delta-band activity associated with non-rapid eye movement (NREM) sleep. Inhibitory neurons play a crucial role in mediating these brain states and are believed to be involved in the changes observed in CBF. However, it remains unclear which neurons regulate these CBF changes across different brain states. Previous studies have indicated that a particular subpopulation of somatostatin neurons, depolarized by Tac1 (also known as substance P) and expressing both Tachykinin Receptor 1 (Tacr1) and neuronal nitric oxide synthase (nNOS), regulate CBF and exhibit Fos induction during NREM sleep. In this study, we aim to characterize the activity of cortical Tacr1 neurons in various brain states and identify potential sources of Tac1-mediated inputs to Tacr1 neurons. To measure changes in Tacr1 neuron activity across different brain states, we utilized two-photon calcium imaging and pharmacological manipulations in $Tacr1^{CreER}$ mice expressing GCaMP, while simultaneously monitoring EEG and EMG signals. Our findings reveal increased Tacr1 neuron activity during NREM sleep periods and following whisker stimulation. Additionally, the administration of an antihistamine, known to enhance the likelihood of sleep, resulted in increased calcium activity in Tacr1 neurons. Moreover, we employed viral transfection mapping to identify potential cortical sources of Tac1. Our mapping data indicate that in addition to local projections of $Tac1^{+}$ neurons, a population of $Tac1^{+}$ neurons in the perirhinal cortex sends long-range projections to the cortex. Notably, the perirhinal cortex is known to play a critical role in memory consolidation, which predominantly occurs during nREM sleep. In summary, our results highlight the variability of activity in Tacr1 neurons across different brain states and suggest that the perirhinal cortex might be a source of Tac1 during NREM sleep. This study sheds light on the intricate relationship between Tacr1 neurons, Tac1 signaling, and the regulation of cerebral blood flow in different brain states.

Disclosures: F. Juarez Anaya: None. **C.F. Ruff:** None. **S.E. Ross:** None. **A.L. Vazquez:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.11/LL25

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Læge Sofus Carl Emil Friis og Hustru Olga Doris Friis' Legat
Dagmar Marshalls Fond
Hørslev fonden
The Independent Research Fund Denmark (1030-00374B)

Title: Neurovascular coupling during volitional whisking in mice barrel cortex

Authors: A. KROGSGAARD[1], J. A. STELZNER[1], G. VYDMANTAITE[1,2], L. SPERLING[1], ***B. L. LIND**[1];
[1]Univ. of Copenhagen, Copenhagen, Denmark; [2]Inst. of Pharmaceut. Technologies, Lithuanian Univ. of Hlth. Sci., Kaunas, Lithuania

Abstract: Neurovascular coupling (NVC) is the basis of energy delivery to activated brain areas. The majority of NVC studies involve imposed stimulation, but much less is known with regards to how self-initiated activity in awake mice modulates NVC. During volitional whisking, different pathways target the barrel cortex besides the excitatory thalamocortical projections. Several neuromodulators have been shown to elicit a blood flow response. These may thus contribute to the NVC response during volitional whisking, dependent on the accompanying behaviour of the mouse. Here we used two-photon microscopy imaging of layer II/III barrel cortex, through chronically implanted cranial window, to relate changes in cerebral blood flow (CBF) and astrocyte Ca^{2+} to volitional whisking activity. We found that CBF responses increased linearly with the duration of self-initiated whisking (SIW) and were amplified by whiskers touching a piece of sandpaper. Astrocyte activity peaked earlier during SIW than for air puff stimulation and SIW evoked biphasic astrocytic Ca^{2+} responses while airpuff stimulation of whiskers evoked monophasic responses. The early astrocytic Ca^{2+} rise preceded, and the late phase succeeded, the accompanying rise in CBF. Correlation between astrocytic Ca^{2+} and vasodilation was dependent on noradrenaline, reflecting a contribution from brain wide noradrenergic modulation to the NVC response. This study introduces the concept of SIW evoked NVC in awake behaving mice as a tool to study hemodynamic responses related to cognitive tasks and thus under conditions that are relevant for human studies.

Disclosures: A. Krogsgaard: None. **J.A. Stelzner:** None. **G. Vydmantaite:** None. **L. Sperling:** None. **B.L. Lind:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.12/LL26

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: Columbia University Medical Center Target of Opportunity Provost award to the Department of Anesthesiology

Title: Age-dependent cerebral vasodilatory responses to anesthetics: the role of $NG2^+$ pericytes

Authors: *H. ZHOU, V. NEUDECKER, J. F. PEREZ-ZOGHBI, A. M. BRAMBRINK, M. LI, G. YANG;
Anesthesiol., Columbia Univ. Irving Med. Ctr., New York, NY

Abstract: Volatile anesthetics may regulate cerebral blood flow by inducing alterations in vessel diameter. Using *in vivo* two-photon microscopy, optogenetic stimulation, and conditional cell ablation, we examined anesthetic-induced changes in cerebral vessel diameter in young and adult mice and the role of vascular pericytes expressing neural/glial antigen 2 (NG2). We found that commonly used anesthetics in clinics (*e.g.*, sevoflurane and isoflurane) rapidly and significantly dilated cortical arterioles in adult mice, to a lesser extent in juveniles, and no at all in infants. In the adult cortex, anesthetic exposure led to a reduction in cytosolic Ca^{2+} concentrations, resulting in pericyte hyperpolarization. Optogenetic inhibition of $NG2^+$ pericytes increased the diameter of cortical arterioles, whereas activation reversed the anesthetic-induced dilation. Furthermore, local ablation of $NG2^+$ pericytes eliminated the vasodilatory response of arterioles to anesthetics. These results highlight the crucial role of cortical pericytes in anesthetic-induced cerebral vasodilation and suggest that the lower density of $NG2^+$ pericytes may contribute to the deficient vasodilatory response observed in infant mice.

Disclosures: H. Zhou: None. **V. Neudecker:** None. **J.F. Perez-Zoghbi:** None. **A.M. Brambrink:** None. **M. Li:** None. **G. Yang:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.13/LL27

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: NIH Grant
American Heart Association pre-doctoral fellowship

Title: Parvalbumin neuron-mediated modulation of gamma oscillations and cerebral blood flow in awake mice while at rest

Authors: *A. RAKYMZHAN[1], A. VAZQUEZ[2];
[2]Univ. of Pittsburgh, [1]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Low-frequency oscillations in the envelope of local field potential (LFP) signals exhibit significant fluctuations below 0.1 Hz across multiple brain regions. These fluctuations demonstrate cross-coupling coherence with the gamma (γ) frequency range (30-100 Hz), irrespective of cortical distance or behavioral state. Resting wakefulness is characterized by temporally correlated low-frequency fluctuations in cerebral blood flow (CBF) measured by fMRI. Simultaneous neurophysiological and hemodynamic recordings reveal a strong correlation between γ-band power and CBF during stimulation and rest. The neuronal mechanisms behind this correlation, particularly involving Parvalbumin (PV) neurons responsible for γ oscillations, remain unclear. Our study proposes that PV neurons generate both γ rhythms and spontaneous vascular fluctuations, elucidating the correlation between CBF and γ power, which aligns with findings from previous resting-state studies.We used transgenic PV-cre mice (2 females, 4 m.o.) to selectively express hM4Di-DREADD-mCherry or m-Cherry alone (control animal) in PV neurons and Ca^{2+} reporter GCaMP7f in all neurons in S1. Awake imaging was enabled through cranial window implantation. Two-photon microscopy captured GCaMP7f and vessel diameter changes using an injected vascular dye. EEG γ power was measured through bone screws and Laser Doppler flowmetry was employed to measure cortical CBF changes. Resting-state imaging was performed for 30 minutes while the mouse was awake. Subsequently, the mice received an IP injection of one of several DREADD agonists to enhance Gi signalling. Imaging was repeated after 10-30 minutes and compared to the pre-injection measurements. We administered a DREADD agonist, which resulted in a 2.51% reduction in the average GCaMP7f signal (F/F_0) in PV neurons, confirming the desired DREADD action. Additionally, there was a 14.29% decrease in the number of PV Ca^{2+} events observed. As anticipated, the inhibition of PV neurons led to a slight 10.46% reduction in γ-band power. Furthermore, the chemogenetic suppression of PV neuron activity correspondingly caused a 1.65% decrease in the average basal diameter (D/D_0) in arteries, a 0.54% decrease in penetrating arterioles, and a 12.71% reduction in basal CBF. We are currently investigating the effects of different DREADD agonists on control mice to further understand their impact. Our preliminary results suggest that the spontaneous vascular fluctuations are modulated by the ongoing activity of PV neurons, and that the signalling of PV cells may serve as a primary neural contributor to the strong correlation observed between γ oscillations and CBF.

Disclosures: A. Rakymzhan: None. **A. Vazquez:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

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Program #/Poster #: PSTR357.14/LL28

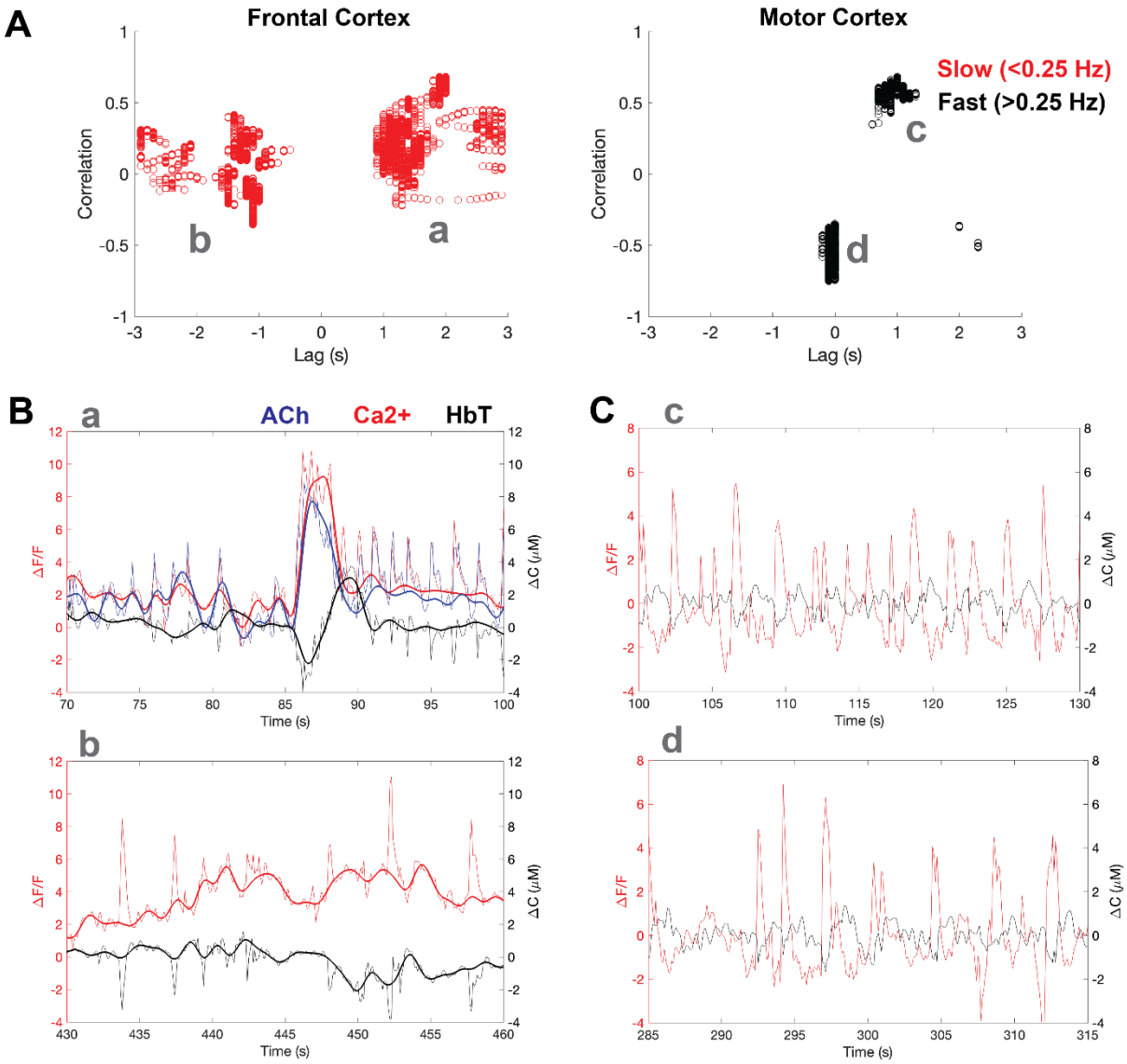
Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: U19NS123717
R01DA050159

Title: Coupling between Acetylcholine and hemodynamics varies across cortical areas

Authors: *B. RAUSCHER[1], N. FOMIN-THUNEMANN[1], P. DORAN[1], S. KURA[1], K. KILIÇ[1], E. MARTIN[1], R. TANG[1], J. X. JIANG[1], S. KNUDSTRUP[2], J. GAVORNIK[2], D. A. BOAS[1], M. THUNEMANN[1], A. DEVOR[1,3];
[1]Biomed. Engin., Boston Univ. Neurophotonics Ctr., Boston, MA; [2]Dept. of Biol., Boston Univ., Boston, MA; [3]Martinos Ctr. for Biomed. Imaging, Massachusetts Gen. Hosp., Boston, MA

Abstract: Ascending cholinergic projections from the basal forebrain (BF) generate internal brain states of arousal coupled with an increase in γ-band brain waves. It has been shown that modulation in the γ-band power entrains the dynamics of arteriolar vasomotion potentially linking cholinergic activity to the "resting state" fMRI signal (Mateo et al.). Therefore, understanding the role of acetylcholine (ACh) in driving hemodynamic fluctuations may open the door for inference of the internal brain state (and, possibly, cholinergic activity) from noninvasive imaging signals in humans.
We used mesoscopic imaging to simultaneously visualize the release of ACh, Ca2+ activity of local cortical neurons, and changes in hemoglobin concentration and oxygenation across the dorsal surface of the cerebral cortex. To measure ACh, we used the fluorescent biosensor GRAB-ACh. The sensor was expressed in transgenic mice expressing red Ca2+ biosensor jRGECO1a. To measure oxy, deoxy, and total hemoglobin, we used 525 nm and 625 nm reflectance LEDs. In addition to resting state (spontaneous) activity, we implemented a sensory discrimination task to engage the mice.
Our results show that during resting state, the ACh and Ca2+ signals were tightly coupled across brain areas. Lag cross correlation analysis on slow and fast frequency bands of the Ca2+ and hemodynamics signals revealed separate clusters over time and across brain regions (Fig. 1A). In the somatomotor cortex, slow increases in ACh/Ca2+ were coupled to increases in total hemoglobin at all times, but fast oscillations showed time-varying levels of coupling (Fig. 1C). In higher cortical areas, slow oscillations showed time-varying levels of coupling generally fitting a bimodal distribution, suggesting changes in brain state (Fig. 1B).
These results demonstrate temporal and regional specificity in neurovascular coupling (NVC) that was not significantly perturbed during engagement. The time-varying levels of NVC suggest a possible inference of brain state from NVC in higher cortical areas and also parcellation of NVC across the cortex.



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Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.15/MM1

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: 23POST1023086
DP2OD02944801
5R01NS115401
R01AG066645

Title: Mechanistic insights into vascular signaling plasticity: remodeling of existing blood flow delivery mechanisms in response to neuronal energy fluctuations

Authors: *N. WEIR[1], L. XIANG[2], A. VIGDERMAN[2], D. P. ISAACS[2], D. C. G. GARCIA[2], H. QADIR[2], M. S. PATTON[2], B. MATHUR[2], F. DABERTRAND[3], T. A. LONGDEN[2];
[1]Univ. Maryland, Baltimore, Baltimore, MD; [2]Dept. of Physiol., Univ. of Maryland Baltimore, Baltimore, MD; [3]Univ. of Colorado Anschutz Med. Campus, Denver, CO

Abstract: Neuronal computation is metabolically costly and demands precise pairing of energy demand and supply via tightly coordinated blood flow increases in response to neuronal activity. The matching of neural activity to local blood flow is referred to as neurovascular coupling (NVC) and is facilitated by a range of mechanisms. NVC mechanisms are considered to be immutable and their plasticity in response to fluctuating neuronal energy needs has not been investigated. We have discovered that neuronal activity induces plasticity in NVC mechanisms to precisely adapt blood flow in accordance with changing local energetic demands, referred to as vascular signaling plasticity (VSP). Using a 7-day environmental enrichment paradigm to elevate neuronal activity and plasticity within the barrel cortex, we observe potentiated K^+-dependent NVC mechanisms, augmenting blood flow elevations in response to manipulations beyond that elicited in naïve mice. Potentiation of capillary-to-arteriole electrical signaling—a bedrock mechanism of brain blood flow control—was observed *in vivo*, manifesting as increased red blood cell flux in response to capillary endothelial cell (cEC) stimulation with K^+ to simulate local neuronal activation. Augmentation of this pathway was also observed *ex vivo* as a significantly decreased EC_{50} for K^+-evoked dilations in an isolated capillary-to-arteriole pressure myography preparation. This cEC sensitization to K^+ resulting from environmental enrichment is mediated by an increase in current density of the strong inward rectifier K+ channel (Kir2.1) that underpins K^+ dependent NVC. These drastic changes in vascular functionality are dependent on protein synthesis, relying on a transcriptional regulator; p300. Using patch clamp electrophysiology, we probed the mechanism of Kir2.1 current augmentation and have established a PKA-p300 signaling axis that is activated during periods of sustained neuronal activity to facilitate VSP. Our data thus recasts the brain vasculature as plastic and capable of fine-tuning blood flow in response to fluctuating energetic demand. VSP represents a novel dimension to brain plasticity that may be vital to physiological process and likely exhibits disruption in brain pathologies with a blood flow-dependent etiology.

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Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.16/MM2

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Title: An Electrical Circuit Model of Blood Flow in the Circle of Willis in the Human

Authors: *B. SHETH[1], D. ANANDAYUVARAJ[2];
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Abstract: Our current goal is to model blood flow in the Circle of Willis (CoW) in humans and leverage the model to pinpoint structural weaknesses in individual arteries of the CoW, both across and within individuals. The CoW is a circulatory anastomosis of several arteries at the bottom (inferior) side of the brain that supplies oxygenated blood to over 80% of the brain. Computational fluid dynamics models of blood flow have investigated structure of arterial segments and junctions, turbulent flow and resulting stagnation. However, these models are not scalable to arterial networks, in part because of their computational complexity, and are thus limited in their clinical applicability. Sidestepping the above critique, we designed a zeroth-order electrical circuit model of the CoW. Individual arteries of the CoW were modeled as resistors, with the internal carotid artery (ICA) and basilar artery modeled as inlets to the CoW, and the remaining arteries serving as outlets leaving to the rest of the brain. Difference in blood pressure along individual arteries was modeled using Poiseuille's Law, with experimental measurements of diameter and length of arteries informed by the literature (e.g. Iqbal et al. 2013, Hillen et al. 1986, and five others). The range of anatomical dimensions reported in human studies was used to constrain randomly generated 4 billion+ models, which also allowed us to model individual variation. Specifically, we modeled three common variations in the CoW, which together account for nearly 90% of the total variation and also for which experimental flow data are available, including a complete CoW, observed in 50% of the population. The flow (current) solution to each individual model was then computed using theorems from circuit analysis based on Kirchhoff's Current Law and Kirchhoff's Voltage Law, and then compared with empirical data on blood flow from the literature (e.g. Zarinkoob et al., 2015). We analyzed further models whose current solutions (flow rates) for all CoW arteries fell within empirical range. Two distinct analyses were performed on the flows in the various arteries of the CoW to pinpoint arteries where the flow exceeded expectations and thus suggest shear stress and likely sites for aneurysm and/or stroke. Of significance, both analyses converged in demonstrating that the artery in the CoW most likely to show excessive blood flows was the middle cerebral artery, which dovetails with the known finding that it is the most common artery involved in stroke. The model is now being extended to incorporate non-stationary blood flows stemming from the innate nature of the human heart, and more complex arterial networks.

Disclosures: B. Sheth: None. **D. Anandayuvaraj:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

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Program #/Poster #: PSTR357.17/MM3

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: 2021ZD0204500

Title: Locus coeruleus-norepinephrine orchestrates global cerebral blood flow via hierarchical modulation of pericyte functions

Authors: *L. SUN[1,2], X. PENG[1], Z. ZHANG[1], Y. GONG[1,2], H. CAO[3], D. HU[3], K. WANG[1,2], J. DU[1,2];
[1]Chinese Acad. of Sciences, Inst. of Neurosci., Shanghai, China; [2]Sch. of Future Technol., Univ. of the Chinese Acad. of Sci., Beijing, China; [3]Inst. of Natural Sci., Shanghai Jiao Tong Univ., Shanghai, China

Abstract: Cerebral blood flow (CBF) is intricately regulated on both local and global scales for both meeting energy demands and maintaining homeostasis of the brain. While much has been known about mechanisms of CBF regulation by local neurochemical and metabolic factors, on the global level it remains unclear whether there's a "headquarter" in the brain that coordinates global CBF across brain regions, as well as the cellular and molecular mechanisms by which such process is realized. Here by brain-wide imaging in larval zebrafish, we investigated the spatiotemporal patterns of global pericyte activities under spontaneous and sensory-evoked conditions. We observed synchronized pericyte events in which pericytes on different levels of the vascular bed show order-specific activity patterns. Physiological pericyte activities are accompanied by vasoconstriction and decreased blood flow. By calcium imaging and targeted cell manipulations, we show that Locus coeruleus-norepinephrinergic (LC-NE) neuronal events are coupled with and orchestrate global pericyte activities in an intensity-dependent manner. Based on morphological evidence and functional perturbations, we further show that LC signal to capillary and precapillary pericytes preferentially through direct NE and indirect radial astrocyte-mediated actions respectively. Furthermore, by mathematical modeling of CBF, we demonstrate that LC-mediated active vasoconstriction state-dependently augments neurovascular coupling (NVC)-induced local blood flow increase. Hereby we unveil two complementary pathways of CBF regulation by LC-NE which tunes vascular states to brain states and enables efficient allocation of limited resource upon metabolic demands.

Disclosures: L. Sun: None. **X. Peng:** None. **Z. Zhang:** None. **Y. Gong:** None. **H. Cao:** None. **D. Hu:** None. **K. Wang:** None. **J. Du:** None.

Poster

PSTR357. Blood Flow: Measurement and Neural Functions

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR357.18/MM4

Topic: F.05. Brain Blood Flow, Metabolism, and Homeostasis

Support: R01AG029523
R01AG047972

Title: Convergent and Divergent Patterns between Age-differential fMRI BOLD, Blood Flow and Oxygen Metabolism During Parametric Task Performance

Authors: *Y. ZHAO[1], D. ABDELKARIM[2], M. TURNER[3], P. LIU[6], B. P. THOMAS[7], J. SPENCE[4], H. LU[8], B. RYPMA[5];
[1]Univ. of Texas At Dallas, Dallas, TX; [2]Univ. of Illinois, Urbana-Champaign, IL; [3]Univ. of Texas at Dallas, Dallas, TX; [5]Sch. of Behavioral and Brain Sci., [4]Univ. of Texas at Dallas, Richardson, TX; [6]Univ. of Maryland, Baltimore, MD; [7]Advanced Imaging Res. Ctr., Univ. of Texas Southwestern Med. Ctr., Dallas, TX; [8]Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Age-related decline in the modulation of neural recruitment with increasing cognitive task demand has been observed in many studies using blood-oxygen-level-dependent (BOLD) signals. We measured age- changes in demand-related neural modulation in physiological factors underlying BOLD signal while participants performed a digit-symbol-verification task (DSVT). 48 younger (Mean Age = 24.0, SD = 4.04, 24 females) and 43 older (Mean Age = 61.6, SD =4.75, 28 females) healthy adults were scanned while performing the DSVT. On each trial, they were presented with a key of multiple digit-symbol pairings simultaneously with a single digit-symbol probe pair for 3000 ms. Participants judged if the probe pair matched one of the key-pairings and responded by pressing a right or left thumb-button. The digit-symbol pairings in the key varied parametrically in set size between 1-, 3-, and 9 pairs. Utilizing a dual-echo fMRI sequence, participants' BOLD signal and cerebral blood flow (CBF) were simultaneously measured. A CO_2 ingestion procedure enabled the calculation of cerebral metabolic rate of oxygen ($CMRO_2$). Spatial patterns of task-positive (Task greater than Baseline) suprathreshold signal maps were relatively convergent across the three metrics of BOLD, CBF, and $CMRO_2$. However, spatial patterns for task-negative effect (Task greater than Baseline) showed considerable divergence: while BOLD and CBF showed significant task-negative effects, no such effects were found in $CMRO_2$. Positive age effects (Old greater than Young) were observed in all three metrics. None of the metrics revealed Young less than Older effects. Conjunction analyses showed that age effects mostly overlapped with task-positive regions. In terms of parametric task modulation, significant disparities were observed between BOLD and other physiological measures. While CBF and $CMRO_2$ showed convergent interaction patterns (linear increasing in younger, inverted quadratic in older) with increasing set size, BOLD often indicated linearly increasing in both groups but without age by set size interaction. The neurovascular coupling (NVC) ratio showed spatial variability for both groups. Significant positive age effects were observed in nearly every task-positive brain region. In terms of parametric modulation, younger exhibited spatially focused NVC modulation patterns in task-related regions, while older exhibited more diffuse patterns. These results suggest that complex neurophysiological phenomena underlie apparently orderly BOLD observations in task effects, age effects, and their modulation with increasing task demand.

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Poster

PSTR358. Molecular Biology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.01/MM5

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant DA039865
NIH Grant DA046346
NIH Grant MH111601
NIH Grant MH106460
NIH Grant MH128763

Title: Comparative rhythmic transcriptome profiling of human and mouse striatal subregions

Authors: *K. A. PETERSEN[1], W. ZONG[2], L. DEPOY[1], M. SCOTT[1], J. BURNS[1], S.-M. KIM[1], K. KETCHESIN[1], G. C. TSENG[2], C. A. MCCLUNG[1];
[1]Psychiatry, [2]Biostatistics, Univ. of Pittsburgh, Pittsburgh, PA

Abstract: The human striatum can be subdivided into the caudate, putamen, and nucleus accumbens (NAc). In mice, this roughly corresponds to the dorsal medial striatum (DMS), dorsal lateral striatum (DLS), and ventral striatum (NAc). Each of these structures have some overlapping and distinct functions related to motor control, cognitive processing, motivation, and reward. Previously, we used a “time-of-death” approach to identify diurnal rhythms in RNA transcripts in the human striatal subregions. Here, we identified molecular rhythms across the three striatal subregions collected from C57BL/6J mice across 6 times of day and compared results to the human striatum. Pathway analysis indicated a large degree of overlap between species in rhythmic transcripts involved in processes like cellular stress, energy metabolism, and translation. Notably, a striking finding in humans was that small nucleolar RNAs (snoRNAs) and long non-coding RNAs (lncRNAs) were among the most highly rhythmic transcripts in the NAc and this was not conserved in mice, suggesting the rhythmicity of RNA processing in this region could be uniquely human. Furthermore, the peak timing of overlapping rhythmic genes was shifted between species, but not consistently in one direction. Taken together, these studies reveal conserved as well as distinct transcriptome rhythms across the human and mouse striatum and are an important step in helping to understand the normal function of diurnal rhythms in humans and model organisms in these regions and how disruption could lead to pathology.

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Poster

PSTR358. Molecular Biology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.02/MM6

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R01MH111601
BBRF NARSAD Independent Investigator Award
NIH Grant 5T32MH016804
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NIH Grant K01MH128763
BBRF Young Investigator Award

Title: Sex Differences in Striatal Gene Expression in Psychosis and Comparison Subject

Authors: *M. PEREZ[1], W. ZONG[2], M. A. HILDEBRAND[3], M. R. SCOTT[3], M. L. SENEY[3], K. M. CAHILL[2], V. G. SHANKAR[3], J. R. GLAUSIER[3], D. A. LEWIS[3], G. C. TSENG[2], K. D. KETCHESIN[3], C. A. MCCLUNG[3];
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Abstract: Introduction: Psychosis is a highly disruptive and often debilitating symptom found in disorders such as schizophrenia and bipolar disorder. Prior studies from our group have found diurnal alterations in gene expression across the human striatum in subjects with psychosis. Furthermore, previous studies have reported that subjects with schizophrenia show sex differences in both symptomology and the transcriptome in cortical regions. Here, we investigated sex differences in the transcriptome within striatal subregions in psychosis and comparison subjects. In a separate set of analyses, we also investigated gene expression patterns across striatal subregions within disease groups.
Methods: RNA-seq was performed on nucleus accumbens (NAc), caudate, and putamen samples from subjects with psychosis (n=36) or unaffected subjects (n=59). For analysis of sex differences (psychosis: n=10/sex; unaffected: n=11/sex), we evaluated sex by disease interaction effects within each brain region using an ANOVA of expression data. In a separate set of analyses, we performed differential expression analyses between striatal regions within disease groups. Ingenuity Pathway Analyses and Metascape were used for pathway and biological process enrichment, respectively.
Results: Significant sex and psychosis effects were observed across striatal regions. Notably, immune/inflammation-related transcripts were significantly enriched in the putamen of unaffected male comparison subjects compared to unaffected female subjects. In female subjects with psychosis (relative to unaffected female subjects), angiogenesis- and immune/inflammation-related transcripts were upregulated across all striatal regions, while mitochondrial-related transcripts were downregulated in the NAc. Between region comparisons in both psychosis and unaffected subjects showed similar patterns of expression, with the NAc being transcriptionally unique. Interestingly, transcriptional gradients in expression across striatal regions were also observed.
Conclusion: We found significant effects of sex and psychosis across the dorsal and ventral striatum, which may provide insight into sex differences in psychosis. We also identified unique expression patterns across striatal subregions, suggesting regional

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Poster

PSTR358. Molecular Biology of Clocks

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Program #/Poster #: PSTR358.03/MM7

Topic:

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NIMH T32MH016804
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NIH DP2GM140924
NIMH K01MH128763
NIDA DA051390

Title: The 12 h transcriptome in human striatal subregions is upregulated in schizophrenia

Authors: *M. R. SCOTT, K. D. KETCHESIN, W. ZONG, M. A. HILDEBRAND, M. L. SENEY, J. R. GLAUSIER, D. A. LEWIS, G. C. TSENG, B. ZHU, C. A. MCCLUNG; Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Biological rhythms allow organisms to anticipate environmental changes across the light/dark cycle and adapt accordingly. These rhythms occur on many scales, and the importance of circadian rhythms in health and disease has become increasingly clear, particularly in psychiatric illnesses. Far less is known about ultradian rhythms, including their prevalence in the human brain and whether they are disrupted in subjects with psychiatric disorders. We performed rhythmicity analyses on RNA-sequencing data from the nucleus accumbens (NAc), caudate, and putament of subjects with no psychiatric diagnosis (NP; n = 59) and subjects with schizophrenia (SZ; n = 28) to determine 12 h rhythms in gene expression. In NP subjects, we observe fewer genes with 12 h rhythms in the NAc and Caudate relative to the Putamen. Across the subregions there was relatively little overlap in genes with 12 h rhythms, and in the timing of these rhythms. In SZ, we observe a strikingly high number of genes with 12 h rhythms in all three subregions, with the largest amount in the Caudate. Unlike in NP, 12 h rhythms in subjects with SZ were consistently associated with mitochondria and protein translation/quality control pathways regardless of striatal subregion. Additionally, the timing of these rhythms were in sync across the three striatal subregions in SZ. A previous study in these same subjects found that there were fewer genes with 24 h rhythms in SZ, suggesting that there may be a shift in the balance of genes with 12 and 24 h rhythms within the striatum in SZ.

Disclosures: **M.R. Scott:** None. **K.D. Ketchesin:** None. **W. Zong:** None. **M.A. Hildebrand:** None. **M.L. Seney:** None. **J.R. Glausier:** None. **D.A. Lewis:** None. **G.C. Tseng:** None. **B. Zhu:** None. **C.A. McClung:** None.

Poster

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Program #/Poster #: PSTR358.04/MM8

Topic: F.07. Biological Rhythms and Sleep

Support: MSU College of Social Science Faculty Initiative Fund
NIH/NINDS R21NS125449

Title: Daily Rhthms and Sleep in Rai1 deficient Nile Grass Rat, A Diurnal Model of Smith-Magenis Syndrome

Authors: *N. E. LUCERA[1,2], J. SHI[2], K. LINNING-DUFFY[2], B. ZHOU[4,5], S. IWASE[4,5], L. YAN[2,3];
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Abstract: The Retinoic-acid induced 1 (RAI1) gene, that encodes a histone-binding protein, is a major gene contributing to a rare neurodevelopmental disorder, Smith-Magenis Syndrome (SMS).
SMS is characterized by a low intellectual quotient, obesity, behavioral problems, and disrupted circadian rhythms in sleep and melatonin secretion. Every reported individual with SMS experiences sleep disturbances including daytime sleepiness, frequent nighttime awakenings, and decreased total sleep time beginning in early childhood. Additionally, most of them display an inverted secretion pattern of melatonin. Multiple Rai1-mutant mouse lines exist, but these mice (C57BL/6) are melatonin-deficient and lack circadian rhythm phenotype. It is unclear whether the lack of circadian rhythm phenotype in Rai1 mice was due to melatonin deficiency, their nocturnal nature, or both. There are striking differences between nocturnal and diurnal species in their circadian rhythms and central responses to light and to melatonin. For instance, light promotes sleep in nocturnal species but wakefulness in diurnal ones. Melatonin is a key modulator of both sleep and neurodevelopment. As the hormone of darkness, melatonin promotes sleep in diurnal species, including humans, but has no such effect in nocturnal species. Therefore, a diurnal model with intact melatonin secretion will help better understand the role of Rai1 in regulating circadian rhythms and sleep, which may better translate to humans. To address the fundamental issues—lack of diurnal animal models and limited knowledge about RAI1 biology—we set out Rai1 gene targeting in the Nile grass rat, Arvicanthis niloticus, a diurnal rodent species. Nile grass rat is a well-established diurnal model used in circadian research. However, gene targeting has remained as a challenge in this species. Leveraged on the recently

available genome sequence and CRISPR-mediated gene editing, we were able to generate several Nile grass rat founders carrying deletions of Rai1. Using offspring produced by these founders crossed with wild-type (WT), we compared daily rhythms and sleep between mutant animals and their WT littermates (Rai1+/- vs. Rai1+/+). In-cage locomotor activity was monitored by a motion sensor mounted on top of each cage. Sleep/wake was analyzed using a PiezoSleep system (Signal Solutions). Although the results still need be confirmed with a bigger sample size, our preliminary data indicate altered daily rhythms in locomotor activities and sleep in Rai1+/- grass rats. These results will guide future studies elucidating the neural mechanisms underlying sleep rhythm disturbance and inverted melatonin rhythm in SMS.

Disclosures: **N.E. Lucera:** None. **J. Shi:** None. **K. Linning-Duffy:** None. **B. Zhou:** None. **S. Iwase:** None. **L. Yan:** None.

Poster

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Topic: F.07. Biological Rhythms and Sleep

Support: MSU College of Social Science Faculty Initiative Fund
NIH Grant NS125449

Title: Crispr-based genome editing of the diurnal nile grass rat Arvicanthis niloticus

Authors: *J. SHI[1], H. XIE[2,3], K. LINNING-DUFFY[1], E. Y. DEMIREVA[2,3], B. ABOLIBDEH[2,3], B. ZHOU[5,6], S. IWASE[5,6], L. YAN[1,4];
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Abstract: The Nile grass rat (*Arvicanthis Niloticus*) is a diurnal rodent species that has been used in the circadian field for over 20 years in studies that uncovered important neural mechanisms regulating circadian rhythms, sleep/arousal, and photic responses in this species and how they differ from nocturnal rodents. The circadian system in nocturnal and diurnal species differs in a more complex manner than a simply flipped daily pattern. The evolutionary divergence likely involves distinct wiring of neural circuits and gene-regulatory networks. Thus, direct studies of diurnal species are necessary to untangle the relationship between circadian dysregulation, sleep disturbance, and health. Despite being a well-established rodent model, grass rats have been genetically intractable until recently, due to the absence of a complete genome sequence or applicable genome editing tools. However, the Genome10K project has recently released the initial build of the Nile grass rat genome, which opens an exciting opportunity for gene editing in this species. We have initiated a project to generate the first genome edited grass rat. Through a series of experiments to understand the fundamental

reproductive biology of this species, we made progress in several critical steps for gene targeting, including egg donor superovulation, embryo culture, and direct embryonic injection of genome editing reagents. Using an improved Genome editing via Oviductal Nucleic Acids Delivery (iGONAD) method that involves delivering CRISPR-Cas9 gene editing components into zygotes *in vivo* via oviduct electroporation, we have successfully generated founder animals carrying large deletions of the Retinoic Acid-Induced 1 (*Rai1*) gene to model the human neurodevelopmental disorder Smith-Magenis Syndrome. Several of these founder animals have produced G1 offspring carrying the edited *Rai1* deletion allele, confirming germline transmission. The method developed in this project provides a strong foundation for future studies of successful development of genetically modified grass rats, which will open the possibility of creating a variety of mutant lines with greater utility of this non-traditional diurnal rodent model for translational research.

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Poster

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Program #/Poster #: PSTR358.06/MM10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant R35GM133440

Title: Alternative polyadenylation sites in the mammalian brain linked to human brain health and disorders are driven by circadian rhythms and sleep homeostasis.

Authors: C. C. FLORES[1], N. PASETTO[1], K. C. SCHUPPE[1], Z. JIANG[2], A. DIMITROV[3], C. J. DAVIS[1], ***J. GERSTNER**[1];
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Abstract: Disruption of sleep and circadian rhythms are a comorbid feature of many pathologies, and can negatively influence various adverse health conditions, such as degenerative disease, metabolic illness, cancer, and neurological disorders. Genetic association studies linking sleep and circadian disruption with disease susceptibility have mainly focused on changes in gene expression from mutations, such as single-nucleotide polymorphisms. An interaction between sleep and/or circadian rhythms with the use of Alternative Polyadenylation (APA) has been largely undescribed, especially in the context of brain disorders. APA is a process that generates various transcript isoforms of the same gene affecting the ultimate expression of its mRNA translation, stability, localization, and subsequent function. Here we used Whole Transcriptome Termini Sequencing (WTTS-seq) and identified unique polyadenylation sites (PASs) expressed in rat brain over time-of-day, immediately following sleep deprivation, and the

subsequent recovery period following sleep loss. From these data, we performed a meta-analytic of sleep- or circadian-associated PASs with a dataset of recently described APA-linked brain disorder susceptibility genes in humans. These data suggest phylogenetic alterations in PAS usage of specific transcripts tether circadian and/or sleep homeostatic molecular mechanisms with human brain health and disorders.

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Poster

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Program #/Poster #: PSTR358.07/MM11

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant NS108713
NIH Grant NS095775

Title: Translational profiling with BAC-TRAP reveals differential gene expression in dopaminergic neurons in the substantia nigra during the day and night

Authors: R. M. COWELL[1], L. J. MCMEEKIN[3], J. R. PAUL[2], A. SWAROOP[2], **A. M. COLAFRANCESCO**[2], M. SIMMONS[2], D. K. CROSSMAN[2], *K. L. GAMBLE[2];
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Abstract: Dopaminergic neurons of the substantia nigra are particularly susceptible to dysfunction and loss with aging and disease. A potential contributor to this vulnerability is the requirement for the maintenance of firing patterns that must be in sync with voluntary motor behavior that varies over time-of-day. Therefore, we sought to test the hypothesis that there are day-night differences in the gene expression encoding ion channels important for regulating neurophysiological patterns of dopaminergic neurons. To quantitatively evaluate gene expression in a cell-selective manner, we used translating ribosome affinity purification (BAC-TRAP) to isolate and sequence mRNA from midbrain dopaminergic neurons. We dissected the substantia nigra and the ventral tegmental area (VTA) from these mice at two times of day: Zeitgeber Time (ZT) 10 (2 hours before lights off) and ZT 22 (2 hours before lights on), corresponding to the late day and late night, respectively. Samples from 10 mice were pooled and RNA isolated using the BAC-TRAP procedure, followed by RNA sequencing. We used a differential gene expression analysis to determine which genes were differentially expressed in the VTA versus nigra. For the substantia nigra, we also identified 239 genes that showed day-night differences in expression ($p < 0.05$ as well as a 1.5-fold change). Interestingly, four genes related to firing patterns and/or

bursting (*Cacna1a*, *Cacna1b*, *Grin2c*, and *Grin2d*) showed significantly higher gene expression during the late day than late night. Fluorescent *in situ* hybridization (RNAScope) revealed significant circadian rhythms in expression of the core circadian genes, *Per1*, *Per2* and *Rora* in dopaminergic neurons of substantia nigra sections from wild-type mice housed in constant darkness. Ongoing experiments are using organotypic cultures from *Slc6a3*-Cre/TdTomato/Per2Luc/+ mice to provide real-time measures of clock gene rhythmicity in living dopaminergic neurons. We will also use RNAScope to validate any rhythmic changes in expression of gene targets identified via BAC-TRAP. Altogether, these experiments have the potential to point to the circadian clock as a novel regulatory mechanism of nigral function that could give critical insight into dopaminergic neuron vulnerability in aging and disease.

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Poster

PSTR358. Molecular Biology of Clocks

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Topic: F.07. Biological Rhythms and Sleep

Title: SIRT6 mediates central circadian clock control via modulating REV-ERBa stability

Authors: *Y.-C. TANG;
Lingang Lab., Shanghai City, China

Abstract: Circadian rhythms couple with metabolic regulations that take place in the brain and peripheral tissues. Notably, many enzymes that can connect metabolic status often cooperate with circadian clock components for the modulation of diurnal oscillations. Sirtuins are prominent examples for such a coordination. Sirtuins are NAD^+-dependent protein deacylase that can respond to the NAD^+/NADH status further involve in many physiological pathways. While SIRT1 have been demonstrated in regulating circadian rhythms in many tissues including in the brain, SIRT6 activity in circadian control was mainly discussed in the liver. Here we found that SIRT6 in the brain also participated in regulating circadian period and amplitude. Ablating SIRT6 via NMS-Cre and Vgat-Cre all led to decreased locomotor activity, shortened circadian period and compromised response to light entrainment. Under normal light-dark cycle, we noted the phase of respiratory exchange ratio rhythm and sleep in SIRT6 knockout mice appeared with advance shift. At the cellular level, devoid of SIRT6 caused increased REV-ERBα protein with elevated acetylation. We further conducted a lysine-residue replacement test on REV-ERBα and confirmed that SIRT6 acted as a key deacetylase to regulate REV-ERBα stability. We found dysregulated REV-ERBα could alter sleep homeostasis via affecting cholinergic signaling. The deteriorated situation was also identified in aged mice, we then applied a SIRT6 agonist to treat aged mice and found the strategy could ameliorate age-associated sleep disorder with lowering

the REV-ERBα level. All told, our findings showed that SIRT6 assisted to maintain the central circadian clock, and approaches to raise SIRT6 activity may play a role in treating circadian dysfunction.

Disclosures: Y. Tang: None.

Poster

PSTR358. Molecular Biology of Clocks

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Program #/Poster #: PSTR358.09/MM13

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01 GM117650

Title: Molecular mechanisms underlying photoperiodic plasticity in the suprachiasmatic nucleus

Authors: *O. H. COX[1], M. A. GIANNONI-GUZMÁN[2], S. KIM[5], M. COTTAM[3], J.-P. CARTAILLER[3], D. G. MCMAHON[4];
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Abstract: Photoperiod is an important environmental factor affecting circadian rhythms produced by the brain's master clock, the suprachiasmatic nucleus (SCN). Photoperiod can be encoded by the SCN perinatally through alteration of the period and the waveform of individual neurons and these changes can persist into adulthood. The mechanisms by which photoperiod induces plasticity in the SCN are largely undetermined.
To assay the effects of encoding of seasonal photoperiods on SCN gene expression, we exposed a mouse strain with intact melatonin signaling (C3Hf+/+) to distinct seasonal photoperiods. Breeder pairs were placed under short (LD 8:16), equinox (LD 12:12), or long (LD 16:8) photoperiods. Litters were born and maintained under these conditions. At postnatal day 50, mice were placed into constant darkness for a min. of 36h. After this interval, whole SCN dissections were conducted at 6 distinct timepoints in 4h intervals over 2 circadian cycles. mRNA was extracted from each SCN sample for RNAseq.
RNAseq data was analyzed using a suite of quantitative analysis tools to find differential rhythmic amplitude, mean expression, and peak phase in rhythmic transcripts between photoperiods. Non-rhythmic transcripts were analyzed for differential expression. Additionally, we employed a descriptive method of rhythmic analysis that does not impose a perfect sine wave on the data, but rather uses spline-fitting for less biased approach. It was therefore possible to generate high-resolution representations of gene expression across the day to validate the results of the quantitative analyses, including circadian-relevant hits. Clock gene temporal profiles in our 12:12 data matched previous datasets. Candidate gene hits are currently being validated using q-RTPCR.

We have tested a candidate gene hit *ex vivo* in optogenetically-entrained SCN slice culture through aav-mediated shRNA knock-down. SCN slices are taken from a mouse strain expressing luciferase under the Period 2 gene promoter (Per2::Luc+/+) crossed with our C3Hf+/+ line. Slices are then transduced with pAAV1-Syn-ChrimsonR-tdT and pAAV9-GFP-U6- Gene shRNA. Together, this allows 1.) a bioluminescent readout of endogenous circadian rhythm of the slice, 2.) knock-down of our candidate gene, and 3.) the ability to stimulate the slice with optogenetics. The SCN slices are entrained to seasonal photoperiods *ex vivo* by optogenetic stimulation using a 2-pulse skeleton photoperiod design.

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Poster

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Topic: F.07. Biological Rhythms and Sleep

Support: NRF-2019R1A5A2026045
NRF-2020R1A2C2007158

Title: The Identification of the Peptidyl-prolyl cis/trans isomerase as an essential regulator of the circadian clock in Drosophila

Authors: *S. KANG[1,2], H. T. TRAN[3], E. KIM[1,2];
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Abstract: The circadian clock system allows living organisms to exhibit about 24-hour rhythmic behavior and physiology in anticipation of the Earth's daily rhythmic variations. The molecular and cellular mechanism of circadian clock, which is highly conserved in all eucaryotes, is primarily based on the transcriptional translational feedback loop (TTFL) involving core clock genes. Additionally, clock proteins undergo post-translational modifications (PTMs) including phosphorylation, O-GlcNAcylation, and ubiquitination, which lead to time-dependent alterations in their molecular properties such as stability, activities, and subcellular localization. Peptidyl-prolyl cis/trans isomerases (PPIases) are enzymes that facilitate cis/trans conformational isomerization of proline. These conformational changes act as molecular switches allowing proteins to modulate their function by transitioning between cellular locations, interacting with other molecules, or altering their activity state. In search for PPIases that potentially play a crucial role in circadian clock function, we performed genome-wide in vivo RNAi screen. Among the 23 PPIases examined, we observed that downregulation of a cyclophilin family

member in clock cells resulted in a significant lengthening of free-running period by approximately 4.4hr (28.1hr) compared to control flies (23.7hr). This PPIase was expressed ubiquitously in the fly's brain including clock cells. In this PPIase knockdown (KD) flies, the levels of core clock protein PER were significantly reduced in clock neurons. Furthermore, the levels of *per* pre-mRNA and mRNA levels were also reduced. The expression of *per* transgene specifically in the clock cells restored the lengthened period, which indicate reduction of PER was responsible for long period of this PPIase KD flies. Interestingly, KD of this PPIase in per^0 flies expressing the *tim*-Gal4 driven UAS-*per* transgene resulted in increased levels of PER levels during the early hours of the day. These results suggest that this PPIase controls *per* transcription during the early phase of PER oscillation and regulates PER degradation during the later phase of PER oscillation. In summary, our study identifies a PPIase as an essential regulator supporting the high amplitude of PER molecular rhythm in the circadian clock of *Drosophila*.

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Poster

PSTR358. Molecular Biology of Clocks

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Topic: F.07. Biological Rhythms and Sleep

Support: Research Grants Council of HKSAR Project No. CityU 11101019 and 11102220 to J.Y.K.

Title: Microglial circadian clocks regulate neural cell recruitment

Authors: *Q. LU[1], J. KIM[2];
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Abstract: Circadian clocks are endogenous oscillators built on a transcriptional-translational negative feedback loop and are intrinsic to nearly all cell types, including microglia. Microglia are phagocytes in the central nervous system and are critical in maintaining immune responses and homeostasis. However, how circadian clocks regulate microglia functions is still unclear. Here, we report that the rhythmic expression pattern of the circadian transcription factor Bmal1 is modified with a phase shift in microglia by activation with lipopolysaccharide in the mouse brain. In addition, rhythmically expressed *Iba1* and *Itgam*, activated microglia markers, lose their rhythmicity in activated microglia, suggesting relationships between circadian clocks and microglia state and functions. By local injection of microglia, we find that resting microglia recruit oligodendrocyte progenitor cells (OPCs), while this ability is lost after activation. Similar to activated microglia, Bmal1-deleted microglia also do not recruit OPCs. This shows that altered microglial clocks via reduced Bmal1 are involved in regulating OPC recruitments. Thus,

this study suggests the potential roles of microglial clocks in cell recruitment and communication.

Disclosures: Q. Lu: None. **J. Kim:** None.

Poster

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Program #/Poster #: PSTR358.12/MM16

Topic: F.07. Biological Rhythms and Sleep

Title: The effects of a STAT3 inhibitor on the C6 in vitro model for circadian rhythms in glioma cells

Authors: *C. W. CHANDLER, Jr, M. GEUSZ;
Biol. Sci., Bowling Green State Univ., Bowling Green, OH

Abstract: Glioma cells have intrinsic circadian clocks that likely impact properties of malignant tumors. How tumor cells respond to circadian rhythms in the activity of the immune system is not understood. Whether these daily cycles that challenge cancer cell survival synchronize with the circadian clocks in cancer cells is also unknown. The C6 rat astrocytoma cell line is a useful model for understanding circadian clock control of cancer progression, particularly the epithelial-mesenchymal transition (EMT) that is linked to tumor invasiveness and metastasis. The core circadian clock protein BMAL1 acts through transcription factor STAT3 to promote EMT. Furthermore, the daily activity cycle of the inflammatory cytokine interleukin 6 acts through STAT3 and reaches a peak during the sleep phase in healthy individuals. Cancer cells have elevated STAT3 activity that appears to regulate the poorly differentiated state of cancer stem cells. The phytochemical curcumin acts through STAT3 to target cancer cells and suppress de-differentiation into cancer stem cells, resulting in potentially more treatable tumor cells. The circadian clock in C6 cells determines when during the 24-hour cycle curcumin's anticancer effects are most effective. Because curcumin affects multiple molecular signaling pathways it is difficult to evaluate its molecular actions on the circadian clock. We sought a more selective STAT3 inhibitor and began testing HO-3867, a curcumin analog and known STAT3 inhibitor. We found that it acts much like curcumin to inhibit and kill C6 cells within 24 hours after application, although with a higher EC_{50}, around 40 μM. Its absorbance is compatible with cell viability stain PrestoBlue in multi-well, cell culture plate assays. HO-3867 may provide a way to manipulate the circadian timing mechanism of cancer cells to understand the role of STAT3 at different phases of the circadian cycle.

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Poster

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Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.13/MM17

Topic: F.07. Biological Rhythms and Sleep

Title: Circadian rhythm of HIF-1 alpha in the brain of crayfish Procambarus clarkii.

Authors: *E. G. ESCAMILLA-CHIMAL[1], C. I. LÓPEZ-BECERRIL[1], C. O. LARA-FIGUEROA[2], C. R. JUÁREZ-TAPIA[3];
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Abstract: Cells possess homeostatic mechanisms that enable them to regulate their functions in the presence of stressors, such as low oxygen concentrations. Among these mechanisms is the hypoxia-induced factor (HIF-1), which compromises two protein subunits: HIF-1α and HIF-1β. The binding of these subunits promotes the transcription of erythropoietin genes, glucose transporters, glycolytic enzymes, and clock-controlled genes. The aim of this study was to characterize the expression of HIF-1α protein over 24-hour period in the crayfish brain, which is considered a putative pacemaker in this species, to determine if it exhibits a rhythm. We used 36 adults *Procambarus clarkii* crayfish that were acclimatized to a Light-Dark cycle (LD 12h:12h). Some animals were sacrificed at 6 Zeitgeber times (ZT: 0, 4, 8, 12, 16 and 20 h), while others remained in constant darkness (DD) for an additional 72 hours and were subsequently sacrificed at 6 Circadian times (CT: 0, 4, 8, 12, 16 and 20 h). The brain tissue was dissected and homogenized for Western blot processing using an anti-HIF-1α primary antibody. The experimental design was approved by the Ethics and Scientific Responsibility Committee of the Faculty of Sciences (T_2019_02_014). The results of relative HIF-1α concentrations under LD conditions showed no significant daily variations (Kruskal Wallis, $P>0.05$); however, COSINOR analysis showed a bimodal rhythm ($P<0.05$) with acrophases at 04:00 and 16: 00h. In the DD condition, significant differences were observed throughout the day among the sampled hours, as determined by ANOVA ($P>0.05$). COSINOR analysis revealed a circadian rhythm ($P<0.05$) with an acrophase at 07:57 hours. Based on the obtained results, it was determined that the relative concentration of HIF-1α exhibited a circadian rhythm in the crayfish brain, suggesting that HIF-1α is a master regulator in the brain of *P. clarkii*, being involved in circadian system´s feedback loop in these animals, in addition to its role in homeostatic mechanisms in response to stressful situations.

Disclosures: E.G. Escamilla-Chimal: None. **C.I. López-Becerril:** None. **C.O. Lara-Figueroa:** None. **C.R. Juárez-Tapia:** None.

Poster

PSTR358. Molecular Biology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.14/MM18

Topic: F.07. Biological Rhythms and Sleep

Support: CHDI Foundation, Inc.

Title: Mapping brain gene coexpression in daytime transcriptomes unveils diurnal molecular networks and deciphers perturbation gene signatures

Authors: N. WANG[1], P. LANGFELDER[1], M. STRICOS[2], L. RAMANATHAN[2], J. RICHMAN[1], R. VACA[1], M. PLASCENCIA[2], *X. GU[2], S. ZHANG[2], K. TAMAI[1], L. ZHANG[3], F. GAO[1], K. OUK[4], X. LU[1], L. IVANOV[5], T. VOGT[6], Q. LU[3], J. MORTON[4], C. COLDWELL[1], J. AARONSON[6], J. ROSINSKI[6], S. HORVATH[7], X. YANG[1];
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Abstract: Brain tissue transcriptomes may be organized into gene coexpression networks, but their underlying biological drivers remain incompletely understood. Here, we undertook a large-scale transcriptomic study using 508 wild-type mouse striatal tissue samples dissected exclusively in the afternoons to define 38 highly reproducible gene coexpression modules using weighted gene co-expression network analysis (WGCNA). We found that 13 and 11 modules are enriched in cell-type and molecular complex markers, respectively. Importantly, 18 modules are highly enriched in daily rhythmically expressed genes that peak or trough with distinct temporal kinetics, revealing the underlying biology of striatal diurnal gene networks. Moreover, the diurnal coexpression networks are a dominant feature of daytime transcriptomes in the mouse cortex. We next employed the striatal coexpression modules to decipher the striatal transcriptomic signatures from Huntington's disease models and heterozygous null mice for 52 genes, uncovering novel functions for Prkcq and Kdm4b in oligodendrocyte differentiation and bipolar disorder-associated Trank1 in regulating anxiety-like behaviors and nocturnal locomotion. Additionally, we provide online searchable maps that can help visualize and search the gene coexpression networks in wild-type mouse striatum and cortex, which serves as a powerful tool to portrait perturbation and disease gene signatures.

Disclosures: N. Wang: None. **P. Langfelder:** None. **M. Stricos:** None. **L. Ramanathan:** None. **J. Richman:** None. **R. Vaca:** None. **M. Plascencia:** None. **X. Gu:** None. **S. Zhang:** None. **K. Tamai:** None. **L. Zhang:** None. **F. Gao:** None. **K. Ouk:** None. **X. Lu:** None. **L. ivanov:** None. **T. Vogt:** None. **Q. Lu:** None. **J. Morton:** None. **C. Coldwell:** None. **J. Aaronson:** None. **J. Rosinski:** None. **S. Horvath:** None. **X. Yang:** None.

Poster

PSTR358. Molecular Biology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.15/MM19

Topic: F.07. Biological Rhythms and Sleep

Support: NSF Faculty Early Career Development (CAREER) Award
Barnard Summer Research Institute (SRI) Funds
Hermione STEM Fund

Title: Cycle controls axonal development in drosophila clock neurons

Authors: *G. C. BIONDI, G. MCCORMICK, M. FERNANDEZ;
Neurosci. & Behavior, Barnard Col., NYC, NY

Abstract: *Cycle* controls axonal development in *Drosophila* clock neurons
Grace Biondi, Gina McCormick and Maria P. Fernandez
Circadian rhythms are regulated by an endogenous molecular clock, which controls various aspects of an animal's physiology and behavior. This internal clock is based on a transcriptional feedback loop, which in the case of adult *Drosophila*, functions in ~ 150 brain neurons and several peripheral tissues. Heterodimers of CLOCK-CYCLE (CLK-CYC) activate the transcription of *period* (per) and *timeless* (tim). Subsequently, PER-TIM complexes provide feedback to repress CLK-CYC transcription, and the degradation of PER-TIM complexes releases CLK-CYC. Null mutations in both CLK and CYC genes result in abnormal expression of the key circadian synchronizing factor PDF. Although CYC and CLK are expressed days before the functional clock is established, their roles in the development of clock neurons are still unknown.
Here, we show that the silencing of *cyc* expression in clock neurons leads to early defasciculation, hypo-fasciculation, and excessive growth of axonal projections. This effect is due to a developmental effect of *cyc*, as its silencing specifically during early development leads to phenotypes in adults similar to those seen with constitutive silencing. In contrast, adult-specific silencing leads to mild phenotypes, mostly in projection length. The fasciculation phenotypes can also be observed in larval brains. Interestingly, clock genes that are known to be directly downstream of CLK-CYC do not exhibit these phenotypes. Finally, silencing of *cyc* and *clk* lead to distinct phenotypes in adult brains, indicating a potential role for CYC that is independent of its heterodimerization with CLK. Our research reveals a non-circadian role of the clock gene *cycle*, shedding light on the additional functions of circadian clock genes in the development and functioning of the nervous system.

Disclosures: G.C. Biondi: None. **G. McCormick:** None. **M. Fernandez:** None.

Poster

PSTR358. Molecular Biology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR358.16/MM20

Topic: F.07. Biological Rhythms and Sleep

Support: VA I01 BX004626
NIH R01 NS073899

Title: Role of FKBP51 in age-related disruptions of the circadian clock

Authors: *N. GEBRU[1,3], L. VERDINA[1,3], J. GUERGUES[2], J. WOHLFAHRT[2], S. M. STEVENS[2], D. GULICK[1,3], L. BLAIR[1,3,4];
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Abstract: Sleep complaints and depression are commonly reported in the elderly population. Interestingly, though not surprisingly, the circadian rhythm is altered with aging, which leads to earlier wake times. The FK506-binding protein 51 (FKBP51) is a negative regulator of the glucocorticoid receptor (GR) that is upregulated with age. Mice lacking the gene that encodes FKBP51, *Fkbp5*, (*Fkbp5*$^{-/-}$) show improved resiliency to stressful and sleep deprived conditions. Moreover, *Fkbp5*$^{-/-}$ mice indicate lower peak corticosterone levels compared to wild-type mice; consistent with the reduced HPA activity. We **hypothesized** that FKBP51 ablation will prevent age-associated circadian desynchrony. To test this, we used aged *Fkbp5*$^{-/-}$ mice and control littermates housed in individual circadian phenotyping cages. Wheel running activity was measured as a proxy of circadian rhythmicity during 12-hour light/dark (establish basal rhythm), 24-hour darkness (measure internal clock rhythm) and 7-hour phase advance periods. At the end of the study, blood serum was collected to assess corticosterone and brain tissues were processed to assess protein changes in distinct brain regions contributing to circadian rhythmicity by immunohistochemistry, proteomics, and western blot. A significant decrease in circadian period was also observed in wild-type males during stress exposure, a change well tolerated by the *Fkbp5*$^{-/-}$. Female *Fkbp5*$^{-/-}$ mice are more susceptible to stress induced rhythm fragmentation. Aged *Fkbp5*$^{-/-}$ showed a sex specific increase in *Per1* protein levels in the hypothalamus with no major changes in the other clock genes assessed so far. As expected, basal corticosterone levels were significantly decreased all *Fkbp5*$^{-/-}$ mice. Proteomic analysis was performed in the hippocampus and amygdala and the analysis is ongoing. Preliminary results suggest that FKBP51 regulates ubiquitination through altered levels of discrete E3 ligases. Additional analyses and comparison to young animals is in progress. In conclusion, FKBP51 alters circadian rhythm in response to stress. These alterations are portrayed in sex dependent changes in clock proteins in the hypothalamus and amygdala suggesting a possible role of FKBP51 as a mediator between circadian rhythm and stress response.

Disclosures: N. Gebru: None. **L. Verdina:** None. **J. Guergues:** None. **J. Wohlfahrt:** None. **S.M. Stevens:** None. **D. Gulick:** None. **L. Blair:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.01/MM21

Topic: F.07. Biological Rhythms and Sleep

Title: Contribution of circadian rhythms to sensory neuron activity

Authors: *A. BRÉCIER[1], N. GHASEMLOU[2];
[1]Queen's Univ., Kingston, ON, Canada; [2]Queen's Univ., Queens Univ., Kingston, ON, Canada

Abstract: Recent studies have unravelled a daily rhythm of thermal and mechanical sensitivity in humans and mice, suggesting a circadian control of nociception. However, the mechanisms underlying this phenomenon remain unclear. At the molecular level, circadian rhythms operate in each mammalian cell owing to core clock genes. The anti-correlated expression of *Bmal1*, the master clock gene, and *Nr1d1*, one of its principal repressors, participate in the molecular clock establishment and maintenance that further regulates the rhythmic expression of approximately 40% of the genome. While nociceptive information is primarily transduced by the sensory neurons of the dorsal root ganglia (DRG), a link between the activity of DRG neurons and the circadian regulation of nociception has never been established. We propose that circadian rhythms control the excitability of DRG sensory neurons. RT-qPCR analysis revealed abnormal expression over time of the two main clock genes *Bmal1* and *Nr1d1* in the non-treated cultured DRG neurons compared to DRG tissues, suggesting a disruption of the circadian clock *in vitro.* In contrast, dexamethasone-treated cultures successfully expressed *Bmal1* and *Nr1d1* in an anti-correlated manner. Interestingly, the excitability of dexamethasone-synchronized neurons remains identical 12h and 24h post-treatment, while recordings from whole-mount DRGs revealed a decreased excitability of sensory neurons at ZT14 compared to ZT2. Our study first revealed that cultured DRG neurons present an altered molecular clock. Secondly, despite the molecular clock restoration in neuronal cultures with dexamethasone, the circadian fluctuation of sensory neuron activity is absent. Overall, we suggest that *in vitro* experiments are not a good model for studying circadian rhythm in DRG sensory neurons. More importantly, our study uncovered a daily fluctuation in the excitability level of sensory neurons *ex vivo* in healthy mice.

Disclosures: A. Brécier: None. **N. Ghasemlou:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.02/MM22

Topic: F.07. Biological Rhythms and Sleep

Support: NIH

Title: Sleep deprivation disturbs circadian rhythmicity of gut microbiome, immune system, SCFAs and the correlation among them

Authors: *W. SHAN[1], M. GUO[3], W. ZANG[1], Z. ZUO[2];
[1]Univ. of Virginia, Charlottesville, VA; [2]Univ. of Virginia, Univ. of Virginia, Charlottesvle, VA; [3]Sun Yat-sen Univ. Mem. Hosp., GuangZhou, China

Abstract: Sleep deprivation disturbs circadian rhythmicity of gut microbiome, immune system,SCFAs and the correlation among them. Weiran Shan[1], Mingyan Guo[1,2], Wenzhe Zang[1], Zhiyi Zuo[11]: University of Virginia. [2]: Sun Yat-sen University Memorial Hospital**Background:**Circadian rhythms coordinate our mental and physiological systems throughout the body. Ourphysiological, metabolic, biochemical functions and immune system all display circadianoscillations. Recent studies have shown that the intestinal microbiome have diurnal oscillationsand they are synchronized by host's circadian clock and feeding rhythms. Sleep physiologicalstate is linked to the immune system. Partial sleep deprivation alters gut microbiota composition.Despite the close relationship among sleep, cytokine activity and gut microbiome, the overallinteractions and functions remain unclear. Our aim is to determine the variation of circadianoscillation of gut microbiota and cytokine activity caused by sleep deprivation and theinteraction between sleep, immune system, and gut microbiome.**Methods:**CD1 male mice, 6 - 8-week-old, were randomly assigned into two groups. First group is controlwithout sleep deprivation. Second group is subjected to platform sleep deprivation for 24 h.Water and food are ad libitum. The sleep deprivation started at 8:00 am, ended next day 8:00 am.Blood plasma and ileum feces were harvested immediately next day at 8:00, 12:00 and 16:00,20:00, 24:00, 4:00 from both groups (n = 6 for each time point). Plasma interleukin (IL)-6, IL-1β, IL-17 and corticosterone were measured by ELISA. Short chain fatty acid (SCFA) in theplasma were measured by HPLC-UV method. Microbiome composition from ileum feces wereevaluated by 16s rDNA sequencing.**Results:**Sleep deprivation significantly decreased the richness of microbiome, changed the compositionof ileum microbiome compared to control, and disturbed the diurnal rhythmicity of gutmicrobiome *Bacteroidales* and *Clostridiales* (order level) in CD1 mice. The normal diurnalrhythmicity of IL-6 and IL-17 was abolished by sleep deprivation. Sleep deprivation alsodisturbed the normal diurnal rhythmicity of IL-1β and corticosterone to establish newrhythmicity. IL-17 was positively correlated with several bacteria. Corticosterone was negativelyrelated with some bacteria.**Conclusions:**Our results suggest that sleep is a key modulator of immune system and gut microbiota. Acutesleep deprivation changes the composition of intestinal microbiome and disrupts the circadianrhythmicity of IL-6, IL-1β, IL-17 and corticosterone. These changes may have significantfunctional consequences.

Disclosures: W. Shan: None. **M. Guo:** None. **W. Zang:** None. **Z. Zuo:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.03/MM23

Topic: F.07. Biological Rhythms and Sleep

Support: NCI Grant K99CA273424
NCI Grant R21CA276027

Title: Light at night and daily rhythms in blood-brain barrier permeability

Authors: W. H. WALKER II[1], ***C. KISAMORE**[2], B. D. ELLIOTT[1], A. C. DEVRIES[3], R. J. NELSON[3];
[1]Neurosci., [3]Med., [2]West Virginia Univ., Morgantown, WV

Abstract: Physiology and behavior are optimally regulated in most organisms via circadian rhythms. These ~24-h rhythms are controlled by a primary molecular clock within the suprachiasmatic nucleus of the hypothalamus and are reset daily to precisely 24 h by exposure to the light-dark cycle. Circadian clocks are ubiquitously located throughout peripheral tissues, where they maintain proper synchronization with the environmental light-dark cycle of resident biological processes via humoral and neural signals from the SCN. The widespread adoption of electric lighting has dramatically affected the circadian organization of physiology and behavior. Although initially assumed to be innocuous, exposure to artificial light at night (ALAN) is associated with several disorders, including increased incidence of cancer, metabolic disorders, and mood disorders. Recent studies have demonstrated circadian regulation of blood-brain barrier (BBB) permeability. However, the effect of ALAN on BBB permeability remains unspecified. We hypothesized that ALAN disrupts rhythms in BBB permeability and predicted that mice exposed to LAN will demonstrate altered daily rhythms and increased permeability of the BBB. To properly measure alterations in permeability across the day, we first optimized methods for the extraction and measurement of rhodamine 123 (Rh123), a marker of efflux at the BBB, and sodium fluorescein (NaFl), a marker of paracellular permeability. Adult male and female mice received an intraperitoneal injection of rhodamine 123 (25 mg/kg) or sodium fluorescein (25 mg/kg). Tissue collection occurred 45, 90, or 120 min, or 30, 60, or 90 min after perfusion, respectively. Rh123 concentrations peaked within the brain 120 minutes and NaFl 30 minutes following injections. Next, adult (>8 weeks) male and female Balb/C mice were housed in either LD (150 lux day; 0 lux dark nights) or ALAN (150 lux day; 5 lux of ALAN) for four weeks. After four weeks in LD or ALAN, mice received an intraperitoneal injection of rhodamine 123 or sodium fluorescein, at one of four time points, ZT0 (lights on), ZT6, ZT12 (lights off), or ZT18. Tissue collection occurred 120 min after Rh123 injections and 30 min following NaFl injections. Similar to our previous studies, we predict that mice housed in LD will demonstrate a daily rhythm in BBB permeability to Rh123, with peak permeability occurring during the active phase. Additionally, mice housed in ALAN will not display daily rhythms in Rh123 or sodium fluorescein. Future studies will examine the effect of ALAN on BBB permeability to chemotherapeutics.

Disclosures: W.H. Walker II: None. **C. Kisamore:** None. **B.D. Elliott:** None. **A.C. DeVries:** None. **R.J. Nelson:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.04/MM24

Topic: F.07. Biological Rhythms and Sleep

Support: Canadian Institutes of Health Research

Title: Circadian clock genes Bmal1 and Per2 in the nucleus accumbens are negative regulators of alcohol drinking in male and female mice

Authors: *S. AMIR, J. HERRERA, C. GOLDFARB, M. BUTTON, P. DOHERTY-HAIGH, N. QUIETSHAT, K. SCHOETTNER;
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Abstract: Voluntary alcohol consumption is influenced by a variety of environmental and genetic factors, including circadian clock genes. We have shown previously that selective ablation of the clock genes *Bmal1* or *Per2* from neurons of the striatum augments alcohol intake in male mice. In females, deletion of *Bmal1* repressed alcohol intake whereas deletion of Per2 had no effect on alcohol consumption. To study the contribution of specific striatal subregions to the observed drinking behavior, alcohol intake and preference was investigated in male and female mice with a conditional deletion of *Bmal1* or *Per2* from cells in the nucleus accumbens (NAc). Mood- and anxiety-related behaviors were assessed prior to alcohol drinking to exclude potential confounding effects of the animal's behavioral state on alcohol consumption. Alcohol consumption and preference were increased in male and female mice with a conditional knockout of *Bmal1*, whereas the same effect was only found in males with a deletion of *Per2*. Because affective behaviors were only mildly influenced by the conditional gene knockouts, the observed alcohol-drinking phenotypes can be directly associated with the NAc-specific clock gene deletion. The results thus suggest an inhibitory role of *Bmal1* and *Per2* in the NAc on alcohol consumption in male mice. In females, the inhibitory effect of *Bmal1* is strictly localized to the NAc, because striatal-wide deletion of *Bmal1* caused a suppression of alcohol consumption. This sex-dependent stimulatory effect of *Bmal1* on alcohol drinking is probably mediated through other striatal subregions such as the dorsal striatum.

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Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.05/MM25

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant AG062716
NIH Grant AG078758

Title: The ontogeny of circadian rhythms in the mouse neuroimmune system

Authors: *K. BELL, R. CHEN, L. K. FONKEN;
Col. of Pharm., Univ. of Texas at Austin, Austin, TX

Abstract: Inflammation in the developing brain has been closely associated with the progression of neurodevelopmental disorders (NDDs). Inflammatory responses are under control of the circadian system, which governs the ~24-hour biological rhythms driven by the suprachiasmatic nucleus (SCN) of the hypothalamus. However, the development of circadian rhythms in the brain and neuroimmune system is not fully established. Here, we reveal the emergence of circadian, immune, and neuroimmune gene oscillation in discrete brain structures and the brain's resident immune cell (i.e., microglia) throughout postnatal development. Male and female C57BL/6 mice were bred and housed in a standard light-dark cycle [12:12 light (150 lux) / dark (0 lux)]. For gene expression analysis, whole brains were flash frozen on postnatal days (PND) 1, 4, 10, 24 and 60, and hippocampus, prefrontal cortex, and SCN were micropunched. All regions analyzed presented 24-hr oscillations in the mRNA expressions of clock genes *Per1* and *Reverbα* by PND4. Oscillations in the mRNA expression of microglia marker *Iba1* and phagocytic marker *CD68* appeared by PND24. For additional neuroimmune analysis, microglia were isolated from whole brains on PND10, 24, and 60. There were no time-of-day differences detected in phagocytic activity of fluorescently labeled beads in isolated microglia at either PND 10 or 24. Ongoing work is analyzing time-of-day differences in phagocytic activity at a later age. Future work will characterize the timing of circadian gene oscillations in microglia as well as in other components of the neuroimmune system such as the meninges and choroid plexus. Understanding the emergence of circadian and immune systems may help our understanding of critical windows and thus potential intervention for the development of NDDs.

Disclosures: K. Bell: None. **R. Chen:** None. **L.K. Fonken:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.06/NN1

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI 19K06360

Title: The suprachiasmatic nucleus is required for light-induced behavioral rhythms in mice lacking circadian rhythms

Authors: *T. J. NAKAMURA[1], S. MIYAZAKI[1], K. WATANABE[1], N. N. TAKASU[2], W. NAKAMURA[2];
[1]Meiji Univ., Kawasaki, Japan; [2]Nagasaki Univ., Nagasaki, Japan

Abstract: The circadian rhythm exists in almost all organisms on the Earth, controlling behavioral and physiological functions to align with the 24-hour cycle associated with the planet's rotation. *Period* (*Per*) genes are the major clock genes responsible for generating the circadian clock. Three homologs of *Per* genes, *Per1*, *Per2*, and *Per3*, have been identified in mammals. The locomotor activity rhythm of mice lacking all homologs (*Per1/2/3* KO mice) entrains with a 12-hour light /12-hour dark cycle but does not exhibit circadian rhythm under constant darkness. However, light pulses in constant darkness can induce the expression of short-period (15-20 hours) locomotor activity rhythms. The mechanisms underlying the generation of the light-induced behavioral rhythm remain unknown. In the present study, we investigated whether the suprachiasmatic nucleus (SCN) is involved in the light-induced rhythms observed in *Per1/2/3* KO mice. We conducted experiments to lesion the SCN and measure the neuronal firing activity rhythms *in vivo* and *in vitro*. In the experiment where the SCN was unilaterally or bilaterally lesioned in *Per1/2/3* KO mice, the bilateral lesioned group under constant darkness did not show the light-induced rhythm. In contrast, the unilateral lesioned group exhibited a light-induced rhythm similar to the sham-operated group. *In vivo* multi-unit neuronal firing activity rhythm recordings, neuronal firing activity rhythms in the SCN were observed under light-dark conditions but disappeared under constant darkness. However, after a 6-hour light pulse, a rhythm of approximately 19.5 hours, similar to the locomotor activity rhythm, was observed. *In vitro* experiments, the SCN was dissected from newborn *Per1/2/3* KO mice, and dispersed cultures were performed using multi-electrode dishes to evaluate the presence of neuronal firing activity in each SCN neuron. As a result, circadian rhythms in the spontaneous firing frequency of some neurons were detected, even in the absence of stimulation. Additionally, to explore the physiological significance of the approximately 19.5-hour rhythm, female *Per1/2/3* KO mice were housed under a light-dark cycle with a period of 19.5 hours, which resulted in an increased number of individuals showing a stable estrous cycle of 4 to 5 days, similar to wild-type mice. These results suggest that the SCN, as the central circadian clock, is essential for the expression of light-induced rhythms in *Per1/2/3* KO mice, and SCN neurons have the ability to generate short-period circadian rhythms even in the absence of major clock genes.

Disclosures: T.J. Nakamura: None. **S. Miyazaki:** None. **K. Watanabe:** None. **N.N. Takasu:** None. **W. Nakamura:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.07/NN2

Topic: F.07. Biological Rhythms and Sleep

Support: 1RO1NS 122589-01

Title: Neuropeptidergic modulation of the hypothalamic subparaventricular neurons in mouse brain slices

Authors: *F. RAFFIN[1], R. DE LUCA[1], A. TRUCCO[2], A. N. CASTAGNO[2], P. SPAIARDI[2], F. TALPO[2], P. M. FULLER[3], G. R. BIELLA[2], E. ARRIGONI[1];
[1]Beth Israel Deaconess Med. Ctr., Boston, MA; [2]Dept. of Biol. and Biotech., Univ. of Pavia, Pavia, Italy; [3]Dept. of Neurolog. Surgery, Univ. of California, Davis, CA

Abstract: The suprachiasmatic nucleus (SCN) is the master circadian clock in mammals. It sends a major output to the subparaventricular zone (SPZ) through which it regulates homeostatic functions including endocrine rhythms, sleep-wake cycle and circadian changes in body temperature and metabolism. The SPZ is a GABAergic nucleus located just dorsal of the SCN and it is an important relay for the circadian timing system. In this study we investigated the efferent output of the SCN to the SPZ neurons. All the studies were conducted in brain slices from mice. We first tested by ex vivo electrophysiology the synaptic connectivity of the SCNNMS and SCNVIP neurons with the SPZ neurons. We expressed channelrhodopsin2 (ChR2) in the SCN neuromedin-S (NMS) neurons and in the SCN vasoactive intestinal polypeptide (VIP) neurons. We recorded SPZ neurons in brain slices while photostimulating the SCNNMS or SCNVIP inputs. We found that photostimulation of the SCNNMS input resulted in opto-evoked inhibitory post synaptic current (oIPSC) in 60% of the recorded SPZ neurons. These oIPSCs were abolished by bicuculline indicating a GABAA signaling, and they were maintained in the presence of TTX indicating monosynaptic connectivity. Photostimulation of the SCNVIP input evoked oIPSCs in 66% of SPZ recorded neurons. These oIPSCs were also blocked by bicuculline. We then conducted ex vivo calcium imaging recordings. We expressed GCaMP7f in SPZ Vgat neurons and recorded changes in intracellular calcium in response to the application of VIP, AVP (vasopressin) and NMS in brain slices. We found that the VIP activated neurons in both the dorsal (dSPZ) and the ventral SPZ (vSPZ) likely through VPAC2 receptors as indicated by the responses to the VPAC2 specific agonist BAY-55-9837. The effects of BAY-55-9837 were maintained in TTX indicating a direct activation through VPAC2. We also found that 52% of the vSPZ neurons and 66% of the dSPZ directly respond to AVP with a significant change in intracellular calcium levels. Lastly, we tested the NMS, focusing on the vSPZ region. We found that NMS directly activated vSPZ neurons (recordings in TTX). Our results indicate a dual response of the activation of the NMS and VIP inputs in the SPZ. The release of GABA inhibits the SPZ postsynaptic neurons whereas the peptides NMS, AVP and VIP excite the SPZ neurons. The net effect of the co-release of GABA and the NMS or VIP is unclear but as in the nocturnal animal the SCN and the SPZ show an antiphase activity, it is likely that the GABA signaling is the more prominent effect.

Disclosures: F. Raffin: None. **R. De Luca:** None. **A. Trucco:** None. **A.N. Castagno:** None. **P. Spaiardi:** None. **F. Talpo:** None. **P.M. Fuller:** None. **G.R. Biella:** None. **E. Arrigoni:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.08/NN3

Topic: F.07. Biological Rhythms and Sleep

Support: Texas A&M University Department of Biology Startup Funds

Title: The molecular clock in the dorsomedial hypothalamus is necessary for robust circadian behavioral output

Authors: *N. E. ARMITAGE[1], J. JONES[2];
[1]Texas A&M Univ., College Station, TX; [2]Biol., Texas A&M Chapter, College Station, TX

Abstract: The dorsomedial hypothalamus (DMH) encodes circadian information from the central biological pacemaker, the suprachiasmatic nucleus (SCN), to regulate rhythmic behaviors including locomotion, sleep, and feeding. Because lesioning the DMH leads to arrhythmic locomotor activity, intact DMH neurons are required to maintain rhythmic outputs. However, the role of the molecular clock within the DMH and how it regulates rhythmic behavior are not fully understood. To test the hypothesis that the molecular clock in the DMH is required for circadian rhythms in behavior, we used CRISPR/Cas9-mediated genome editing to selectively ablate the core clock gene Bmal1 in genetically-defined DMH neurons in male and female mice. We ablated Bmal1 in the SCN as a positive control and injected AAV encoding sgRNA targeting Lacz into the DMH as a negative control. After virus injection and recovery, we housed mice under a 12:12 light/dark cycle followed by constant darkness (DD) to analyze free-running locomotor activity. We found that mice with SCN-specific Bmal1 ablation had arrhythmic wheel-running activity, consistent with previous reports using targeted Bmal1 ablation in the SCN. Surprisingly, we discovered that locomotor activity remained rhythmic in mice with DMH-specific Bmal1 ablation. However, these mice exhibited an increased number of bouts during the subjective day and decreased rhythm amplitudes compared to the negative controls. This retention of rhythmicity but irregular activity pattern in DD suggests that when DMH neurons are intact but have a broken molecular clock, they are unable to fully encode SCN input. We predict that these neurons are incapable of propagating a coherent rhythm forward to other brain circuits to generate high-amplitude daily rhythms in locomotor activity.

Disclosures: N.E. Armitage: None. **J. Jones:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.09/NN4

Topic: F.07. Biological Rhythms and Sleep

Support: NINDS R21NS120003
Washington University Siteman Cancer Center

Title: Sensitivity to the chemotherapy agent Temozolomide depends on circadian time of treatment in murine models of glioblastoma

Authors: *M. F. GONZALEZ[1], A. R. DAMATO[2], L. L. TREBUCQ[4], S. P. CARDENAS-GARCIA[3], T. SIMON[3], E. D. HERZOG[5];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Washington Univ. in St. Louis, Saint Louis, MO; [3]Biol., Washington Univ. in St. Louis, St. Louis, MO; [4]Univ. Nacional de Quilmes, Bernal, Argentina; [5]Biol., Washington Univ., St. Louis, MO

Abstract: Glioblastoma (GBM) is the primary and most aggressive brain tumor in adults. The current standard of care consists of maximal surgical resection, followed by radiation and chemotherapy with Temozolomide (TMZ). Despite extensive research and clinical trials, average survival post-treatment remains at 15 months. Thus, all opportunities to optimize current treatments and improve patient outcomes should be considered. Previous work has shown that murine and human models of GBM have cell-intrinsic circadian rhythms in the expression of the core clock genes *Bmal1* and *Per2*. In a retrospective clinical study, our lab found that taking morning TMZ increased patient overall survival by 6 months compared to evening. Because expression of the DNA repair enzyme, O6-Methylguanine-DNA Methyltransferase (MGMT), is circadian and has been associated with GBM outcomes, we tested whether TMZ efficacy depends on time-of-day and MGMT activity. We transduced two models of GBM (i.e., LN229 and GL261) with a luciferase reporter of *Per2*, *Bmal1* or *Ef1-α* transcription (hereafter, GBM-P2L, GBM-B1L, or GBM-*Ef1-α*, respectively). We found that GBM-P2L and GBM-B1L cells have anti-phase daily rhythms in bioluminescence, while GBM-*Ef1-α* cells luminesce constitutively. We next treated GBM cells at different circadian phases with varying concentrations of TMZ. We found that TMZ dose-dependently induced greater cell death when delivered at the peak of *Bmal1 in vitro*. Sensitivity to TMZ increased and the daily rhythm in sensitivity was abrogated when inhibiting MGMT activity with O6-Benzylguanine. We next implanted these cells into the basal ganglia of male and female mice and tracked tumor size daily using *in vivo* bioluminescence imaging. Once tumor growth was established, we administered TMZ by oral gavage at either the peak (ZT4, morning) or trough (ZT11, evening) of *Bmal1* for 5 consecutive days. We found elevated sensitivity to morning TMZ administration compared to evening as measured by decreased tumor size and increased body weight. These results suggest that chemotherapy with TMZ can be dramatically enhanced by delivering at the daily maximum of tumor *Bmal1* expression and minimum of MGMT activity. This work may inform personalized circadian medicine (e.g., timed TMZ) to improve individual patient outcomes.

Disclosures: M.F. Gonzalez: None. **A.R. Damato:** None. **L.L. Trebucq:** None. **S.P. Cardenas-Garcia:** None. **T. Simon:** None. **E.D. Herzog:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.10/NN5

Topic: F.07. Biological Rhythms and Sleep

Support: R01 NS078410

Title: Sex differences in murine myoblast circadian gene expression

Authors: *M. R. RALSTON[1], M. DOVER[1], M. MADANI[1], C. GHIANI[2], K. LAMIA[3], K. PAUL[1,2];
[1]Integrative Biol. and Physiol., [2]Psychiatry, UCLA, Los Angeles, CA; [3]Mol. Med., Scripps Res. Inst., La Jolla, CA

Abstract: The mammalian circadian rhythm is controlled by an intricate molecular feedback loop of circadian transcription factors. The cyclic expression and interaction of these circadian genes are driven by the core of the mammalian circadian clock, the suprachiasmatic nucleus (SCN). Clock genes are not limited to the SCN and have been identified in several peripheral tissues, including skeletal muscle. Muscle overexpression of Brain and Muscle ARNT Like 1 (BMAL1) gene has recently been shown to regulate sleep homeostasis and mitigate sleep loss; however, the expression of BMAL1 and other circadian genes in the cellular precursor to muscle, myoblasts, have not yet been examined. In this study, we lay the foundation for a potential molecular mechanism through which circadian factors in peripheral tissues act directly on sleep regulatory processes in the brain. We selected the genes BMAL1, PER1, PER2, BDNF, PGC-1α;, CRY1, and CRY2, which are commonly associated with regulating circadian rhythms and neuronal functions via skeletal muscle expression. In a time-course cellular assay of 4-week-old mouse myoblast explants, RT-qPCR data reveals significant sex differences in the expression of all circadian genes, and they may be oscillatory in nature. In all genes examined, the expression of circadian genes was significantly higher in the myoblasts of male mice compared to their female littermates. The observed higher profile of circadian gene expression in male skeletal muscle indicates that females may require an enhanced therapeutic intervention for treating sleep disturbances. Further characterization of these sex differences may open avenues for developing targeted therapeutics for sleep homeostasis disruption that weigh sex as a biological variable. Further investigation of these mechanisms would require a larger sample size, procedure timing optimization, and potential age-related effects.

Disclosures: M.R. Ralston: None. **M. Dover:** None. **M. Madani:** None. **C. Ghiani:** None. **K. Lamia:** None. **K. Paul:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.11/NN6

Topic: F.07. Biological Rhythms and Sleep

Title: Assessing pharmacological and non- pharmacological interventions on motor deficient Bmal1 habenula knockout mice

Authors: *C. GOLDFARB[1], N. BAHARAV[1], H. JIANG[1], K. SCHÖTTNER[2], S. AMIR[2];
[2]Concordia Univ., [1]Concordia Univ., Montreal, QC, Canada

Abstract: Circadian clocks coordinate cellular functions like metabolism and signaling according tothe time of day through an interconnected loop of clock genes and protein expression - enabling systemic homeostasis and proper functioning of the biological system. Previous research has indicated the importance of one such signalling pathway, the nigrostriatal dopamine pathway, on clock functioning in the striatum (DS). The striatum being an essential hub in the brain and alterations to this region have been associated with various disorders and diseases - addiction, schizophrenia, and Parkinson's. While daily rhythms of dopamine synthesis and release in the nigrostriatal pathway have been found in previous work, the underlying pacemaker mechanism is still being investigated. One region of interest is the habenula, an SCN independent oscillator that projects to both the VTA and substantia nigra (SN). Using male and female *Bmal1* floxed mice, we injected AAV-2/9-CAGCre-eGFP virus into the habenula to selectively knockout *Bmal1*. We found a significant impact on motor coordination in both male and female knockout mice as well as changes in gene expression in the SN and DS. Causal to this could be alterations in dopamine levels in the DS over the 24-h day, as indicated by liquid chromatography coupled mass spectrometry. We investigated if pharmacological and non-pharmacological interventions could improve dopaminergic rhythms in the DS through daily timed injections of a D2 agonist quinpirole or through voluntary running activity. Results indicate changes in performance on motor tasks in treatment mice compared to controls, suggesting that circadian interventions are critical to the motor functioning.

Disclosures: C. Goldfarb: None. **N. Baharav:** None. **H. Jiang:** None. **K. Schöttner:** None. **S. Amir:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.12/NN7

Topic: F.07. Biological Rhythms and Sleep

Support: Texas A&M University Department of Biology Startup Funds

Title: Machine learning classification of estradiol-dependent circadian behaviors in mice

Authors: *L. PERRY, J. JONES;
Biol., Texas A&M Univ., College Station, TX

Abstract: Organisms have evolved circadian rhythms in a diverse set of behaviors to anticipate daily opportunities and challenges such as mating and predation. However, the ethological investigation of behavioral rhythms in mice has been traditionally limited to studying behaviors that are easily monitored and measured. While manual human labeling of recorded videos can expand this set of studied behaviors, this can be inaccurate, variable across labelers, and, particularly for multi-day circadian analysis, extremely time-consuming. Here, we tested the hypothesis that the sex hormone estradiol affects circadian rhythms of eight home-cage

behaviors: eating, drinking, rearing, climbing, nesting, digging, grooming, and resting. We used infrared-capable, high-resolution video cameras and a novel continuous video processing pipeline to stream and record videos from wild-type male, ovariectomized female, and ovariectomized, estradiol capsule-implanted female mice housed in a 12:12 light:dark cycle followed by constant darkness over eight days. Using a custom machine learning classification model to perform real-time behavioral analysis, we identified several behavioral rhythms (including digging, rearing, and grooming) that differed significantly depending on the presence or absence of estradiol. Future experiments using our real-time behavioral classification tool will allow us to investigate how hormones, genotypes, and specific neuronal circuits influence multiple circadian behaviors at unprecedented temporal resolution.

Disclosures: L. Perry: None. **J. Jones:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.13/NN8

Topic: F.07. Biological Rhythms and Sleep

Support: NIDA Grant R01DA039865

Title: Circadian Rhythms in Neural Mechanisms Underlying Motivated Behaviors

Authors: *T. A. STOWE[1], L. M. DEPOY[1], Y. H. HUANG[2], C. A. MCCLUNG[3];
[2]Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Psychiatry, Univ. of Pittsburgh Med. Sch., Pittsburgh, PA

Abstract: Circadian rhythms are near 24-hour oscillations in physiology and behaviors and have a significant impact on psychiatric disorders, such as substance use disorders (SUDs). Notably, drug-taking patterns can vary throughout the day, indicating that certain times may render individuals more susceptible to drug use. Thus, it is crucial to explore the neural mechanisms that underlie the relationship between circadian rhythms and SUDs. Cholinergic interneurons (CINs) play a key role in motivated behaviors. However, the existence and relevance of rhythms in CIN activity remain largely unexplored. Previous research found that CIN modulation of dopamine release was higher midway through the light cycle in comparison to midway through the dark cycle, which would suggest higher CIN activity in the light cycle. To expand on these data, we utilized slice electrophysiology to determine whether there are rhythms in CIN activity. Our preliminary data suggest that CIN activity is higher during the beginning of the dark cycle in comparison to the beginning of the light cycle, particularly in male mice. Given the essential role CINs play in motivated behaviors, the rhythmic activity in CINs may influence behaviors like drug-taking. In the future, we aim to investigate whether chronic drug exposure alters these rhythms in CINs and if eliminating these rhythms affects drug-taking behaviors. Overall, these

novel findings bring us closer to characterizing the role of circadian rhythms in the neural mechanisms that drive motivated behaviors associated with SUDs.

Disclosures: T.A. Stowe: None. **L.M. Depoy:** None. **Y.H. Huang:** None. **C.A. McClung:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.14/NN9

Topic: F.07. Biological Rhythms and Sleep

Title: Electroencephalography analysis in BMAL1-deficient mouse and macaque models revealed a dysregulated high-frequency neural activity feature

Authors: *H.-C. CHANG;
Lingang Lab., Shanghai city, China

Abstract: Sleep disturbance led by BMAL1-deficiency has been recognized both in rodent and non-human primate models. Yet it remined unclear how their diurnal brain oscillations were affected upon BMAL1 ablation, and what caused the discrepancy in the quantity of sleep between the two species. Here we investigated diurnal electroencephalographs (EEG) of BMAL1-deficient mice and cynomolgus monkeys at young adult age, and found a shared defect of dysregulated high frequency oscillations in both models based on relative entropy measurement. By determining the divergence between wild-type and BMAL1-deficient monkeys, we found beta and gamma oscillations were significantly disturbed in a day versus night manner, while in rodents the beta band difference was less evident. We further studied the contrast and noted that beta oscillation dysregulation was highly associated with psychiatric behaviors including the occurrence of confusion and delusion in BMAL1-deficient monkeys. As such psychiatric phenotypes were difficult to uncover in rodent models, our results offered a unique method to study the correlation between circadian clock dysregulation and psychiatric disorders, at the same time established a way to examine EEG features associated with BMAL1-deficiency.

Disclosures: H. Chang: None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.15/NN10

Topic: F.07. Biological Rhythms and Sleep

Support: Texas A&M Department of Biology Start-up Funds

Title: Circadian Modulation of in vivo Basal Dopamine Release in the Mesolimbic Pathway

Authors: *J. N. COOK[1], J. JONES[2];
[1]Biol., Texas A&M Univ. Neurosci. Inst. For Neurosci., College Station, TX; [2]Biol., Texas A&M Chapter, College Station, TX

Abstract: The mesolimbic pathway drives motivated behaviors such as reward pursuit or aversion via dopaminergic axons from the ventral tegmental area (VTA) that synapse onto the nucleus accumbens (NAc). Circadian modulation of basal (non-evoked) dopamine remains poorly understood, including its impact on reward-related behavior. While some studies have identified daily variations in dopamine production and reward behaviors such as food- or drug-seeking, it is unclear how neuron activity in the VTA generates these rhythmic outputs. To test the hypothesis that dopamine release is regulated by the circadian activity of VTA neurons within this circuit, we injected male and female mice with adeno-associated viruses encoding either a genetically-encoded dopamine sensor (DA3m) into the NAc or a genetically-encoded calcium indicator (GCaMP8m) into the VTA. We then used in vivo fiber photometry to measure dopamine release in the NAc and calcium activity in the VTA of freely behaving mice. We continuously recorded mice over 5 days in a 12:12 light:dark cycle followed by 5 days in constant darkness. Surprisingly, we found that in vivo VTA calcium activity was arrhythmic. However, we observed circadian rhythms in basal dopamine release in the NAc that peaked during the subjective day, consistent with prior reports. These results suggest that circadian information is transmitted to the NAc via the rhythmic release of dopamine. Ongoing experiments aim to identify daily changes in evoked dopamine release both ex vivo and in vivo and to determine if genetically-defined subpopulations of VTA neurons exhibit circadian activity in vivo. Defining the temporal dynamics of basal and evoked dopamine release will ultimately lead to a better understanding of the circadian outputs of the mesolimbic pathway that regulate daily rhythms in motivated behavior.

Disclosures: J.N. Cook: None. **J. Jones:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.16/NN12

Topic: F.07. Biological Rhythms and Sleep

Support: NIH-NINDS R00 NS105942

Title: Sexually dimorphic roles for Drosophila circadian clock neuropeptides in regulating rest-activity rhythms

Authors: *S. CRESPO-FLORES, M. FETCHKO, A. BARBER;
Rutgers Univ. - New Brunswick, Piscataway, NJ

Abstract: Circadian clocks in the brain act as master pacemakers that synchronize molecular oscillators across an organism to allow anticipation of daily environmental changes. Across species, the circadian system consists of a central oscillator that is entrained by environmental inputs and controls behavioral and physiological outputs. In *Drosophila*, the brain clock network consists of ~150 neurons that express the core molecular clock components and is entrainable by light and temperature. Despite our advancements in uncovering the mechanisms of the molecular clock, and how daily environmental cues entrain the brain clock, we don't understand how information exits the clock network to drive rhythmic behavior. Recent single-cell RNAseq of the fly clock network uncovered novel neuropeptides expression, which may act as output signals from the clock to other brain regions or may serve roles in intra-clock circuit communication. To evaluate the roles of these neuropeptide in modulating circadian rest activity behavior I conducted a CRISPR deletion screen of eight candidate neuropeptides in the whole clock circuit and characterized changes in circadian locomotor behavior in male and female flies. We identified several hits that increase or decrease rhythm strength when knocked out in the whole clock network. Unexpectedly, we also observed sexually dimorphic effects of neuropeptide knockout in the clock network. CRISPR deletion of Allatostatin A (AstA) or CNMamide (CNMa) increases rhythm strength in female, but not male, flies, with no effects on period length or daily activity amounts. Neuropeptide F (NPF) deletion has a more severe phenotype with female-specific increases in rhythm strength and male-specific decreases in rhythm strength accompanied by reduced daily activity, however clock-specific NPF deletion also reduced survival. Further restriction of CNMa and NPF deletion to DN1 neurons continues to increase female rhythm strength, suggesting that expression in this population has a key role. These findings suggest novel sexually dimorphic roles for neuropeptides in mediating circadian locomotor behavior. Future work will identify downstream signaling targets inside and outside the clock network in both sexes to expand our understanding of sexually dimorphic neuropeptide regulation of behavior.

Disclosures: S. Crespo-Flores: None. **M. Fetchko:** None. **A. Barber:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.17/NN13

Topic: F.07. Biological Rhythms and Sleep

Support: MOST111- 2320-B-182-008
CMRPD1M0162

Title: Protein kinase C modulates state-dependent Ca^{2+} response to glutamate in the rat suprachiasmatic nucleus

Authors: *P.-C. CHENG, R.-C. HUANG;
Physiol., Chang Gung Univ., Taoyuan, Taiwan

Abstract: Glutamate evokes intracellular Ca^{2+} signaling mechanisms to mediate photic entrainment of the central clock in the suprachiasmatic nucleus (SCN). We recently showed that in the rat SCN cells glutamate produced two different Ca^{2+} responses: a reproducible, tonic Ca^{2+} increase and a variable Ca^{2+} decrease. In this study, we used ratiometric Ca^{2+} imaging technique to investigate the underlying mechanisms. For cells exhibiting a Ca^{2+} decrease response to the first 20-s exposure to glutamate (100 μM), repetitive applications of glutamate every 5 min evoked Ca^{2+} decreases for the first one to few applications, followed by variable, slow Ca^{2+} increases for the next few applications, and then reproducibly rapid, tonic Ca^{2+} increases for the last applications. Similarly, 2-min application of glutamate evoked a Ca^{2+} response with initial Ca^{2+} decrease followed by sigmoidal Ca^{2+} increase to reach a plateau. The results indicated a glutamate-induced change in the state-dependent Ca^{2+} response to glutamate. In sharp contrast to the totally different Ca^{2+} responses to glutamate, the Ca^{2+} increase responses to AMPA or NMDA were the same between the glutamate inhibition state and the glutamate excitation state. The result indicated no change in the responsiveness of ionotropic glutamate receptors (iGluRs) to their specific agonists, suggesting that the glutamate-evoked Ca^{2+} decrease response at the glutamate inhibition state most likely involved the activation of metabotropic glutamate receptors (mGluRs) to inhibit the activation of iGluRs. Indeed, the type II mGluRs antagonist LY 341495, but not the type I mGluRs antagonist AIDA, reversibly converted the Ca^{2+} decrease to a Ca^{2+} increase response to glutamate, suggesting that glutamate activation of type II mGluRs inhibited glutamate activation of iGluRs. On the other hand, AIDA, but not LY 341495, could reversibly convert the Ca^{2+} increase to a Ca^{2+} decrease response to glutamate, suggesting the involvement of type I mGluRs in promoting glutamate activation of iGluRs. Importantly, calphostin C inhibition of protein kinase C (PKC) mimicked the AIDA effect, whereas phorbol 12-myristate 13-acetate activation of PKC mimicked the LY 341495 effect. Taken together, our results indicated a complicated regulation of iGluRs by mGluRs to mediate the glutamate-induced change in the state-dependent Ca^{2+} response to glutamate. The glutamate inhibition state appeared to be mediated by the activation of type II mGluRs to inhibit glutamate activation of iGluRs, whereas the glutamate excitation state appeared to be mediated by the activation of type I mGluRs, along with PKC activation, to promote glutamate activation of iGluRs.

Disclosures: P. Cheng: None. **R. Huang:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.18/NN14

Topic: F.07. Biological Rhythms and Sleep

Support: NIH 0P50HD103557
Taiwan Ministry of Education #1100090595

Title: Role of inflammation in mediating the effects of dim light at night in the Cntnap2 KO mouse model of autism.

Authors: H.-B. WANG[1], C. A. GHIANI[2], ***C. COLWELL**[3];
[1]Dept. of Psychiatry and Biobehavioral Sci., [2]Pathology, UCLA, Los Angeles, CA; [3]Psychiatry, Univ. of California - Los Angeles, Los Angeles, CA

Abstract: Our prior work suggests that exposure to dim light at night (DLAN) causes disruptions to the sleep/wake cycles as well as increased autism-like behaviors in the *Contactin Associated Protein-like 2* knockout (*Cntnap2* KO) mice. In this study, we sought to assess the contribution of inflammation to these DLaN driven behavioral changes. The wild-type (WT) and *Cntnap2* KO mice were held under control light/dark (LD) cycles or DLAN for 2 weeks and, after behavioral assays, the plasma and the prefrontal cortex (PFC) and basolateral amygdala (BLA) were sampled in the middle of the night. Under the DLAN, many of the plasma inflammatory markers were elevated in both genotypes and correlated with the behavioral deficits observed in these same mice. In addition, we found that the levels of IL-6 in the PFC were significantly increased in both genotypes. Furthermore, the mutant mice exposed to DLAN exhibited an increased number of microglia cells with altered complexity. We further tested the role of inflammation in mediating the effects of DLAN by treating the mice with the non-steroidal anti-inflammatory drug carprofen. This treatment effectively reduced the inflammatory signature and reduced the impacts of DLAN on social impairments and repetitive grooming. Together, our data suggest that exposure to DLAN increases inflammation and blocking the inflammatory pathways appears protective.

Disclosures: H. Wang: None. **C.A. Ghiani:** None. **C. Colwell:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.19/NN15

Topic: F.07. Biological Rhythms and Sleep

Support: NINDS R01 NS121161

Title: Sodium leak conductance (NaLCN) is required for circadian rhythms in gene expression and sleep-wake

Authors: *S. P. CARDENAS GARCIA[1], E. D. HERZOG[2];
[1]Biol., Washington Univ. in St. Louis, St. Louis, MO; [2]Washington Univ., St. Louis, MO

Abstract: Daily rhythms arise through an intracellular, transcription-translation negative feedback loop. In this near 24- hour cycle, clock proteins including PERIOD2 (PER2) actively suppress their own transcription. In mammals, the suprachiasmatic nucleus (SCN) acts as the central circadian pacemaker coordinating daily rhythms in other tissues. SCN neurons must

synchronize their activity with each other and to the local environment to drive synchronous physiological and behavioral cycles. The "Bicycle Model" proposes that daytime increases in intrinsic currents generated by sodium (Na+) channel and nighttime potassium (K+) channel opening underlie daily rhythms in firing circadian pacemaker neurons in flies and mammals. Although membrane depolarization can alter clock gene transcription, it remains uncertain whether membrane potential or specific ionic conductances play a role in generating daily patterns in gene expression, neuronal firing, and sleep-wake cycles. We examined the role of NaLCN (sodium leak channel) in regulating the daily rhythms of clock gene expression and behavior. With viral-mediated targeting in adult mice, we deleted Nalcn in neurons of the bilateral SCN. We found that, with the loss of Nalcn expression in SCN neurons, the amplitude of daily rhythms in locomotion was reduced in the light cycle and abolished in constant darkness. The daily onsets and offsets of locomotion also shifted to produce shorter daily activity in a light cycle. Furthermore, blocking Na+ leak conductances with N-methyl-d-glucamine (NMDG+) dose-dependently reduced the amplitude and shifted the phase of PER2 expression in SCN slices in vitro. These preliminary results indicate that Na+ leak conductances in SCN neurons are required for the generation of daily patterns in gene expression and behavior. Supported by NINDS R01 NS121161.

Disclosures: S.P. Cardenas Garcia: None. **E.D. Herzog:** None.

Poster

PSTR359. Physiology of Clocks

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.20/NN16

Topic: F.07. Biological Rhythms and Sleep

Support: Academy of Finland
Finnish Medical Society

Title: Effects of painful nerve injury on sleep architecture and circadian rhythmicity in mice

Authors: W. DAI, ***V. PALADA**;
Dept. of Physiol. and SleepWell Res. Programme, Univ. of Helsinki, Helsinki, Finland

Abstract: The relationship between sleep and pain is bidirectional since insufficient sleep can precede and exacerbate pain while pain can disturb the quality of sleep. In current study, we wanted to assess the effects of spared nerve injury (SNI) in a mouse model of neuropathic pain (NP) on sleep architecture using telemetric electroencephalography (EEG) and to access the effects of the SNI on circadian rhythmicity in mice. EEG was recorded from 7 SNI and 4 control sham C57BL/6JRj mice (both males and females). HD-X02 implants (DSI, Harvard Bioscience) simultaneously recorded the EEG, EMG, temperature and locomotor activity. Recordings were done for 48h at the baseline before the injury and at 7, 14 and 21 days after the SNI. Mechanical and thermal hyperalgesia were assessed using Von Frey filament test, dynamic test, hot and cold

plate testing. Sleep scoring was performed by Spike 2 and CircaCompare was used to compare the temperature and activity differences in mesor, amplitude and phase between the SNI and controls. As a result, EEG revealed significant reduction in total duration of REM sleep in both male and female mice upon SNI and the effects were the most severe at 21 days after the injury. Wakefulness and NREM sleep were not affected. Additionally, SNI disrupted the temperature and activity cycles in mice. These findings might be highly clinically relevant to better understand the mechanisms behind the comorbidity between sleep and pain and for developing novel pain treatments by taking into account the circadian effects of painful nerve injury.

Disclosures: W. Dai: None. **V. Palada:** None.

Poster

PSTR359. Physiology of Clocks

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR359.21/NN17

Topic: D.07. Visual Sensory-Motor Processing

Support: NIH F30MH129056

Title: Circadian changes in cerebral and systemic physiology and behavior in rhesus macaques

Authors: *D. ISSAR, E. C. CRANE, E. E. SKOG, J. M. KAINERSTORFER, M. A. SMITH; Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Our overall engagement with the environment, often termed arousal level, changes with both external stimuli as well as natural rhythms, such as the circadian cycle. Typically, in humans and many diurnal mammals, behavioral performance fluctuates over the circadian cycle, peaking during the middle of the day and waning at dawn and dusk, with accompanying fluctuations in systemic physiology including heart rate. Despite these familiar trends, time of day is not always controlled or reported as a variable in behavioral experiments. Furthermore, we do not have a strong understanding of how the circadian cycle affects our brain activity to give rise to these behavioral differences. Our goal was to quantify circadian changes across multiple behavioral and physiologic variables in rhesus macaque monkeys to understand how time of day may influence arousal as indexed by numerous common measures. We recorded heart rate, respiration rate, pupil diameter, and cerebral blood volume changes over the course of minutes to hours from four subjects while they performed a behavioral task (approximately 60 sessions). The task was either an active fixation or working memory task, in which a response was measured with an eye movement or joystick movement to a cued location. In a single session, subjects worked for several hours, and on each day we initiated the sessions at different times to assess circadian effects. We recorded heart rate and cerebral blood volume changes using a near-infrared spectroscopy sensor placed over the scalp, respiration rate using a nasal thermal sensor, and pupil diameter using an infrared eye tracking system. We observed circadian trends across subjects and task types in behavior and physiology. Compared to early in the morning or late at

night, heart rate, respiration rate, and pupil diameter peaked midday. Reaction time and accuracy also showed similar trends. Additionally, in both physiology and behavior there were subject-specific trends over the hours-long sessions that were consistent across sessions and time of day. Understanding both circadian and subject-specific within-session trends in arousal-linked behavior and physiology is important for studies that use these measures as correlates of cognitive states.

Disclosures: D. Issar: None. **E.C. Crane:** None. **E.E. Skog:** None. **J.M. Kainerstorfer:** None. **M.A. Smith:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.01/NN18

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH 1R01HL137103-01A1

Title: Novel role for prefrontal cortex angiotensin type 2 receptor expressing interneurons in learned fear

Authors: *H. C. SMITH, Z. YU, L. IYER, A. KISNER, A. M. POLTER, P. J. MARVAR; George Washington Univ., Washington, DC

Abstract: Evidence suggests that the renin-angiotensin system (RAS) is a potential therapeutic target for PTSD. Brain angiotensin type 2 receptors (AT2Rs) are expressed across corticolimbic circuits such as the medial prefrontal cortex (mPFC), though their role and mechanism are largely unknown. Given the importance of the mPFC in fear-related behavior, we sought to examine the role of AT2R-expressing mPFC neurons in fear learning. To first characterize AT2R neurons in the mPFC, AT2R-Cre/td-Tomato and AT2R-eGFP BAC reporter male and female mice were used for immunohistochemistry (IHC) and whole-cell patch-clamp recording. To characterize AT2R-cre/td-Tomato+ cells, brain sections were stained with glutamatergic marker Tbr1 or with one of 5 interneuron markers and colocalization was quantified. To assess fear-related behaviors in AT2R-flox mice, we selectively deleted AT2R from mPFC neurons using an AAV-Cre or GFP virus (n=9-12). Following viral integration (3 weeks), mice underwent Pavlovian auditory fear conditioning and anxiety-like and locomotor behavior testing. IHC analysis revealed that AT2R-cre/td-Tomato+ cells in the mPFC primarily consist of interneurons (*14.0% Tbr1+, 67.6% interneuron+)* and are highly expressed in the prelimbic (*180.9 cells/mm*^{2}) and infralimbic (*219.6 cells/mm*^{2}) cortices. Slice electrophysiology revealed that ex-vivo activation of mPFC-AT2R-eGFP+ neurons decreased the distribution of frequency (*D=0.19, p<0.01*) and amplitude (*D=0.13, p<0.01*) of spontaneous excitatory postsynaptic currents (sEPSC). Following Pavlovian fear conditioning and extinction learning, selective mPFC-AT2R deletion impaired extinction learning across testing days in female (day 1, $p<0.01$; day 2,

p=0.02) but not male (day 1, p=0.6; day 2, p=0.6) mice. Generalized anxiety and locomotion measures, which include percent time in open arm (M: GFP 14.4%, Cre 13.6%, p=0.8; F: GFP 14.9%, Cre 21.7%, p=0.2) and total distance traveled, (M: GFP 44.1m, Cre 56.0m, p=0.4; F: GFP 42.9m, Cre 42.4m, p=0.9) were unaltered by mPFC-AT2R deletion. These results provide support for mPFC-AT2R+ neurons as a newly identified interneuron subgroup that may influence the extinction of learned fear in a sex-dependent manner.

Disclosures: H.C. Smith: None. **Z. Yu:** None. **L. Iyer:** None. **A. Kisner:** None. **A.M. Polter:** None. **P.J. Marvar:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.02/NN19

Topic: G.01. Fear and Aversive Learning and Memory

Title: Sex Differences in Frontal Cortical Extinction Circuits

Authors: *K. GRAHAM[1], L. KENT-WEBBER[1], G. K. O'BRIEN[1], E. BLOSS[2];
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Abstract: The brain uses the results of past experiences to produce adaptive behaviors in the present. A clear example of this process is associative fear learning, where a strong memory is formed rapidly between specific cues that are paired with an aversive footshock. Although the generation of defensive behaviors when these cues are later encountered could be seen as adaptive, these behavioral responses become maladaptive once cue-response contingencies change. The process of abandoning fear responses when no longer useful is colloquially called fear extinction, and impairments in extinction is a hallmark of multiple psychiatric disorders including post-traumatic stress disorder and generalized anxiety disorder. These disorders show a prominent sex difference in prevalence, with women several-fold more vulnerable than men, yet the underlying neurobiology driving this sex difference remains unknown. Here, we test the hypothesis that sex differences in extinction result from differential processing in the infralimbic (IL) cortex. Specifically, we posit that two IL projection pathways mediate fear extinction: neurons targeting the basal lateral amygdala (BLA) and neurons targeting the nucleus reuniens of the thalamus (RE). Within these pathways, we show differential expression of two related yet functionally distinct immediate early gene transcription factors (cFos and FosB) across the sexes following extinction learning. Consistent with extinction learning as a plasticity-related processes, we show neurons in these projection pathways show sexual divergent plasticity of spine densities and morphology following extinction learning. Interestingly, genetic deletion of FosB specifically from IL neurons facilitates fear extinction in both sexes, suggesting a sex-independent transcriptional pathway that drives fear extinction. Together, these findings suggest that IL-to-BLA and IL-to-RE projections undergo plasticity during extinction learning and that

their differential expression of Fos proteins may underlie the sex differences seen in fear extinction.

Disclosures: K. Graham: None. **L. Kent-Webber:** None. **G.K. O'Brien:** None. **E. Bloss:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.03/NN20

Topic: G.01. Fear and Aversive Learning and Memory

Support: National Institutes of Health grant 2T32NS041228-21
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Brain and Behavior Research Foundation NARSAD Young Investigator Grant
Connecticut Mental Health Center
National Center for PTSD

Title: Prefrontal effects of norepinephrine prediction errors

Authors: *A. BASU[1], J.-H. YANG[2], A. YU[2], S. GLAESER-KHAN[3], J. FENG[5], Y. LI[5], T. YOKOYAMA[6], M. SAKAMOTO[6], A. P. KAYE[4];
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Abstract: Individuals must learn to predict levels of threat in environments containing uncertainty to respond with appropriate defensive behaviors. Other neuromodulators have been shown to represent components of predictive learning models such as prediction error during both appetitive and aversive learning. However, sensitive investigations of the role of NE in threat computation have been limited by the lack of high-resolution measurements and manipulations of NE and downstream effectors in aversive learning. To determine the role of norepinephrine in aversive learning, we expressed the fluorescent NE sensor GRAB-NE2h in the mouse medial prefrontal cortex (mPFC) and measured its fluorescence with fiber photometry during classical aversive conditioning. We found that predictive-cue evoked NE is consistent with an aversive prediction error. However, several features of cue evoked NE, such as NE evoked by the cue offset, could only be explained by a reinforcement learning model that incorporates temporal uncertainty. In order to understand the dynamics of molecular signaling downstream of NE, we employed a cAMP sensor to elucidate prefrontal cortical second

messenger dynamics. cAMP represents both associative and unconditioned stimuli related to threat in the PFC. Ongoing studies utilize optogenetic manipulations of NE in the context of two-photon imaging to identify the consequences of PFC-NE temporal dynamics for threat prediction and responsivity. Determination of the effects of threat NE on second messengers and downstream behavior will inform the chemical substrates of learned fear behavior, and how neuromodulators and second messengers contribute to the computation of threat.

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Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.04/OO1

Topic: G.01. Fear and Aversive Learning and Memory

Support: NRF-2012R1A3A1050385

Title: Cellular Correlates of Remote Cued Fear Memory in the Infralimbic Cortex

Authors: *Y. SUNG[1], ***Y. SUNG**[2], D. HAN[1], E.-R. LEE[1], B.-K. KAANG[1];
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Abstract: Extinguishing outdated fearful memory is crucial for proper adaptation. Previous studies have shown that the infralimbic cortex (IL) in mice regulates the extinction of fear memory. We showed that the IL neurons display elevated c-fos activity after one-day auditory fear extinction, compared to three-day extinction or memory recall. However, whether fear memory trace resides in the IL is still elusive. To address this question, we captured remote fear memory recall-induced neuronal ensemble in IL using Arc-CreERT2 mice. The reactivation ratio of the ensemble, as measured by c-fos expression, correlated with the freezing level to the conditioned stimulus (CS). CRISPR/Cas9-mediated knockout (KO) of N-Methyl-D-Aspartate Receptors (NMDARs) in the recall-induced IL ensemble diminished the c-fos reactivation ratio after extinction recall. These results suggest that fear memory recall-induced IL ensemble might underlie remote cued fear memory in NMDAR-dependent manner.

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Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.05/OO2

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIMH R01 42066

Title: The contribution of Infralimbic-Basal Forebrain communication to fear extinction

Authors: *C. FERNANDES HENRIQUES[1], Y. GUETTA[2], M. SCLAR[3], Y. MIURA[4], L. YUSUFOVA[3], A. K. FRIEDMAN[5], E. LIKHTIK[3];
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Abstract: Activation of projections from the infralimbic region (IL) of the medial prefrontal cortex to the basolateral amygdala (BLA) are needed to suppress fear responses after extinction. However, how the IL organizes extinction is still poorly understood. Given the importance of the basal forebrain (BF) in attention and learning, and its connectivity to IL and BLA, we investigated whether IL-BF communication contributes to fear extinction and to IL-BLA physiology. Retrograde neuroanatomical tracers were used to study IL projections to the BLA and BF, and combined with functional immunohistochemistry to assess the activity of IL-BF versus IL-BLA projectors during fear and extinction recall. Anatomical data confirmed IL-BLA projections arising in LII-III and showed IL-BF projections from LII-III and LIV-V. Immunohistochemical analyses showed that during extinction recall the IL-BLA projectors are more active, whereas activity of deep layer IL-BF projectors decreases. We then studied excitability of IL-BF neurons using *in vitro* patch clamp recordings and found that IL-BF projectors are more excitable during extinction learning than extinction recall. To better understand the patterns of communication of these three regions during extinction learning and recall in this circuit, we used multisite local field potential recordings in the IL, BF, and BLA during extinction learning and recall. These experiments showed that during extinction learning and recall, cue-evoked IL and BF theta power (4-8Hz) started higher and progressively decreased to pre-tone levels with training, with similar changes but slower dynamics in the BLA. Additionally, percent freezing in the first two trials of extinction recall correlated with evoked theta power only in IL and BF. We are currently investigating how optogenetic manipulation of the IL-BF projection affects extinction learning and recall. Taken together, these data open the possibility that IL activation of the BF during extinction may be involved in the expression of a fear memory, possibly through BF's cholinergic neurons.

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Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.06/OO3

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIH Grant R01 DA052108

Title: Sex differences in oscillatory signaling dynamics during affect processing in the infralimbic cortex and nucleus accumbens shell

Authors: *J. E. DOUTON, R. M. CARELLI;
Psychology and Neurosci., The Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Many psychiatric disorders are associated with dysfunctional hedonic processing that can lead to maladaptive behaviors. Specifically, the development of negative affect is prevalent in diseases such as depression and addiction. Corticolimbic circuits have been shown to play a vital role in hedonic processing. For example, projections from the infralimbic cortex (IL) to the nucleus accumbens shell (NAcSh) have been linked to learned negative affect as 20 Hz optogenetic stimulation of this circuit reduces condition taste aversion (CTA) in male rats. However, the same stimulation did not produce an effect in female rats. Hence, the goal of this study was to characterize the neurobiological processes underlying these sex differences. Here, we used taste reactivity to assess affective state and Lithium Chloride (LiCl) CTA to study the development of learned negative affect. We simultaneously recorded local field potential (LFP) activity in the IL and NAcSh in response to 30 intraoral infusions (3.5 sec infusion, VT30 sec schedule) of commonly rewarding (0.15% saccharin) and aversive (1mM quinine) tastants and following induction of CTA (LiCl, 127 mg/kg ip) in male (n=9) and female (n=6) Sprague-Dawley rats. We found that rewarding saccharin elicited distinct oscillatory activity within each brain region across sex. In addition, we observed that CTA development increased IL resting-state power, and this increase correlated with the strength of the learned aversion. Moreover, although no behavioral differences were observed between males and females, CTA reduced beta power and IL-NAcSh coherence in response to the LiCl-paired saccharin in male rats. On the contrary, CTA only increased gamma power in the NAcSh of female rats in response to paired saccharin. When analyzing phase-amplitude coupling (PAC), generalized effects were observed, as CTA reduced theta-low gamma PAC following the saccharin infusion in both sexes. Finally, while the infusion of quinine produced similar effects in oscillatory power across sex, the NAcSh of female rats showed a differential PAC response to this tastant. Collectively, our data reveals clear sex-specific differences in the processing of normal hedonic stimuli in the IL and NAcSh and that CTA development differentially affects oscillatory activity within these brain regions in male and female rats. Elucidating such sex-specific differences in oscillatory signaling dynamics is important to help develop targeted treatment strategies for negative affect in humans such as non-invasive brain stimulation.

Disclosures: J.E. Douton: None. **R.M. Carelli:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.07/OO4

Topic: G.01. Fear and Aversive Learning and Memory

Support: R01 DA052108
T32 DA007244

Title: Effects of optical stimulation of infralimbic cortex to nucleus accumbens shell pathway at beta (20 Hz) and gamma (80 Hz) frequencies on conditioned taste aversion in female rats

Authors: E. GRABLIN[1], J. DOUTON[2], S. MULLER[3], *R. CARELLI[4];
[1]Univ. of North Carolina At Chapel H Behavioral Neurosci. Program - Psychology Dept., Chapel Hill, NC; [2]The Univ. of North Carolina, Chapel Hill, NC; [3]The Univ. of North Carolina, Chapel Hill, Chapel Hill, NC; [4]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Disrupted hedonic processing has been implicated in many psychiatric illnesses such as substance use disorders (SUDs). This disruption can lead to negative affect which is prevalent during drug withdrawal and often leads to relapse. Thus, understanding the neural pathways that underlie hedonic processing is vital to develop effective treatments for SUDs. A common method used to study affect in rats is taste reactivity (TR). TR involves analyzing positive (appetitive) and negative (aversive) orofacial responses when a taste stimulus is intraorally infused, allowing insight into the rat's current affective state. Additionally, conditioned taste aversion (CTA) can be used to study the development of learned negative affect and involves pairing naturally rewarding stimuli (e.g., saccharin) with a malaise-producing agent such as lithium chloride (LiCl). This pairing causes the once-rewarding stimuli to become aversive. The infralimbic cortex (IL) to nucleus accumbens shell (NAcSh) pathway has been implicated in hedonic processing, specifically in regulating negative affect, as 20 Hz optogenetic stimulation of this circuit effectively reduced learned aversion in male, but not female rats. The goal of this study was to compare the effects of 20 Hz beta versus high gamma 80 Hz optogenetic frequency on reducing learned negative affect in female rats. Female, Sprague-Dawley rats received injections of ChR2 (AAV-CaMKIIa-hChR2(H134R)-mCherry) or control virus (AAV-CaMKIIa-mCherry) bilaterally into the IL. Eight weeks later, rats underwent a second surgery where they were outfitted with intraoral (IO) cannulas and optical fibers bilaterally positioned into the rostral NAc shell. After recovery, rats received 30 IO infusions (3 seconds/infusion) of 0.15% saccharin followed by an IP injection of 0.3 M LiCl to induce CTA. On test day (2 days later), rats received 30 IO saccharin infusions with simultaneous optical stimulation at 20 Hz (n=7 mcherry; n=5 ChR2) or 80 Hz (n =6 mcherry; n=5 ChR2). After 5 days of extinction, rats received 30 IO infusions of quinine paired with optical stimulation to study the effects on innate aversion. We found that 20 Hz stimulation was ineffective in rescuing appetitive responses or attenuating negative responses in female rats, consistent with prior work. However, preliminary data indicate that stimulation at 80 Hz tended to reduce the number of aversive responses during learned but not innate aversion in females indicating that higher frequency (high gamma) stimulation may be more effective at reducing learned negative affect in female rats.

Disclosures: E. Grablin: None. **J. Douton:** None. **S. Muller:** None. **R. Carelli:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.08/OO5

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIDA R01 DA052108
NIDA T32 DA007244

Title: Oscillatory signaling dynamics in the prelimbic cortex and the nucleus accumbens core following conditioned taste aversion in male and female rats

Authors: *P. RODRIGUEZ-ECHEMENDIA, J. E. DOUTON, R. M. CARELLI;
Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Affective state is integral to informing appropriate behavior in situations that elicit pleasure or lead to aversion. When affective processing becomes dysfunctional, several psychiatric illnesses including depression and substance use disorders can occur. Conditioned taste aversion (CTA) is a preclinical behavioral model used to study shifts in affective processing; here taste reactivity (TR) is used to track the affective properties of appetitive and aversive tastants before and after the induction of a CTA. The infralimbic cortex (IL) and its projections to the nucleus accumbens (NAc) shell play a key role in affective processing and electrophysiological (local field potential, LFP) recording methods show that oscillatory signaling dynamics in the IL and NAc shell track hedonic shifts following CTA in male, but not female rats. Because the prelimbic cortex (PrL) has been shown to be linked to the NAc core and the NAc core has been implicated in tastant learning, the goal of the present study was to determine if this particular circuit also plays a role in learned negative affect. Adult, male and female Sprague Dawley rats (n=16, 8M, 8F) were surgically prepared for LFP recording in the PrL and NAc core and intraoral cannula; 1 week later rats received 30 intraoral (IO) 0.15% saccharin infusions (3.5s/inf, VT30s schedule) immediately followed by an IP injection of LiCl (127 mg/kg) on the naïve day. After recovery, rats were tested for CTA with 30 IO saccharin infusions. After four extinction days, rats were given 30 IO bitter quinine infusions to examine neural responses to an innate aversive tastant. LFP data in the PrL-NAc core was recorded during a baseline 15-min period before each session and during all IO infusions, and TR during IO infusions was recorded on video. Behavioral results indicate that CTA elicited a hedonic shift from appetitive to aversive TR, regardless of sex ($p<.0001$). However, preliminary analysis of LFP power shows a reduction of power in delta, theta, and beta frequency bands following CTA in the NAc core only in males. Moreover, preliminary analysis of LFP functional connectivity suggests that the PrL-NAc core circuit does not track any aspect of CTA in both males and females. Ongoing studies include time-frequency analyses to assess how CTA affected neural oscillations in response to the tastants in the PrL and NAc core and phase-amplitude coupling to determine interactions between oscillations of different frequency bands within the same brain regions. Collectively, these data will provide an understanding of real-time oscillatory signaling dynamics in the PrL and NAc core following learned negative affect in both male and female rats.

Disclosures: **P. Rodriguez-Echemendia:** None. **J.E. Douton:** None. **R.M. Carelli:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.09/OO6

Topic: G.01. Fear and Aversive Learning and Memory

Support: DoD/ARO
NIH/NIMH R01MH106617
Brain and Behavior Research Foundation

Title: Distinct Patterns of Neuronal Communities in Prelimbic Cortex Associated with Fear Discrimination Learning

Authors: *K. SHULER, J. PASTORE, V. PAPALEXAKIS, E. KORZUS;
Univ. of California, Riverside, Riverside, CA

Abstract: Fear discrimination learning (FDL), the ability to discriminate between safe and threatening stimuli, is an adaptive safety learning process that is important for animal survival. Disruptions to this form of safety learning can result in maladaptive overgeneralized fear towards neutral stimuli, as commonly observed in post-traumatic stress disorder pathology in humans. Previous research has found that the medial prefrontal cortex (mPFC) in rodents may exert modulatory control of fear expression via connections with the amygdala during fear discrimination learning, but the precise roles of the prelimbic (PL) versus infralimbic (IL) subregions in the mPFC during FDL as well as the underlying neural mechanisms remain unclear. The current project utilizes *in vivo* calcium imaging in PL during a differential fear conditioning (DFC) paradigm to assess network dynamics during FDL. Additionally, we compared a control (PL-Control) and a mutant group (PL-CBPΔHAT) with disrupted long-term memory consolidation in PL in order to identify if the ability of PL to form long-term memories, which is needed for successful FDL across multiple days, will affect performance in the DFC task. While the PL-Control group was able to successfully discriminate, the PL-CBPΔHAT group was unable to effectively discriminate between safe and threatening contextual stimuli, thus implicating a role of long-term memory consolidation in PL in FDL. Since PL-CBPΔHAT behavioral performance was expected to be disrupted, we also expected that disruption to translate to the PL network activity. To analyze PL network dynamics during DFC, we generated a neuronal network activity graph of each mouse that represented the functional relationships between neurons across time and probed the underlying structure of these relationships using community analysis. Community analysis identified subpopulations of neurons in the PL network that were frequently co-active across DFC. Three neuronal communities that were relevant to stimulus- and trial-specific aspects of DFC were identified consistently across PL-Control mice. The PL-CBPΔHAT network also reliably displayed three communities across mice with similar trial-associated communities as the PL-Control network, but differences in

stimulus-associated communities. The PL-CBPΔHAT neuronal communities did not differentiate between more ambiguous conditioned stimuli and unambiguous non-conditioned neutral stimuli as effectively as PL-Control. This distinction in network operations may reflect the observed behavioral differences and explain how the PL network may be functioning to support successful FDL.

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Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

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Program #/Poster #: PSTR360.10/OO7

Topic: G.01. Fear and Aversive Learning and Memory

Support: DOD ARO
NIH 5R01MH106617
NARSAD/BBRF

Title: Prefrontal neural dynamics and fear modulation

Authors: *J. STEINHAUSER, J. PASTORE, V. PAPALEXAKIS, E. KORZUS;
Univ. of California, Riverside, Riverside, CA

Abstract: Understanding fear and where it is generated, manipulated, and interpreted is paramount when trying to develop our knowledge of anxiety and stressor type disorders and how they develop. Regions within the fear circuit, specifically amygdala, hippocampus, and prefrontal cortex, have all been heavily investigated at the synaptic and global level; however, further research is needed to elucidate the network- and population-level activity leading to modulation of fear behavior. Previous research has implicated the ventromedial prefrontal cortex (vmPFC) in modulating fear behavior via inputs received by hippocampus and amygdala that are interpreted and sent back to amygdala for fear behavior output. Researchers used a variety of methods, including lesioning and histological studies, to conclude that the prelimbic region (PL) of vmPFC is critical for fear expression and the infralimbic region (IL) of vmPFC is necessary for fear inhibition. Our study uses calcium imaging as a method to record the network level activity of PL to investigate specifically, how populations of cells in this region modulate fear behavior. Mice underwent fear conditioning and differential fear conditioning paradigms to first acquire fear, then to learn to discriminate between similar, yet novel contexts. Tensor decomposition, an advanced, unsupervised data mining technique used to identify latent factors within a multidimensional array, was used to discover different neuronal populations within PL that differentially regulate the output of fear behavior to amygdala. Tensor decomposition identified distinctive populations of co-active neurons engaged during different phases of fear discrimination learning. One of the identified neuronal populations was highly engaged before fear conditioning. In addition, the tensor decomposition identified two populations of neurons

that showed activity correlating well with behavioral performance and were highly engaged after fear conditioning, during distinct phases of the differential fear conditioning paradigms. These results support the idea that neuronal populations within PL are differentially active based on the uncertainty of the contextual experience and may play a role in contributing a more nuanced response of fear to diverse contextual situations. Future studies are necessary to investigate how different neuronal populations within prefrontal cortex are recruited to navigate situations of ambiguity.

Disclosures: **J. Steinhauser:** None. **J. Pastore:** None. **V. Papalexakis:** None. **E. Korzus:** None.

Poster

PSTR360. Neural Circuits of Fear and Aversive Processing: Prefrontal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR360.11/OO8

Topic: G.01. Fear and Aversive Learning and Memory

Support: MOST 110-2326-B-007-001-MY3 (Taiwan) to CHC.

Title: The role of infralimbic cortex to nucleus accumbens pathway and chronic restraint stress in switching defensive strategies in rats

Authors: *H.-Y. KUAN, C.-H. CHANG;
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Abstract: Animals adopt different defensive behaviors when encountering stress events. Passive defensive behavior, such as freezing, requires the animals to stay still to avoid drawing attention from the threats. Active defensive behavior, such as fight or flight, requires the individuals to actively respond to challenging situations. Previous studies have shown that the infralimbic region (IL) of the medial prefrontal cortex (mPFC) and the nucleus accumbens (NAc) are essential to regulate active avoidance behaviors, and tracing studies revealed that the IL has projections to the nucleus accumbens core (NAcc). However, it remains unclear whether this pathway is involved in the regulation of active avoidance behaviors. In the present study, we first used functional disconnection approach to determine whether the information exchange between the IL and the NAcc is necessary to sustain cued lever-press active avoidance to foot shock in rats. Next, we examined whether chronic restraint stress led to a shift from active to passive defensive strategies, which is commonly reported in patients of anxiety-related disorders. Our results suggested that for stress-free rats, disconnection of the IL to NAcc pathway decreased active avoidance responses, but increased freezing to cues (i.e., white noise), the number of feces left in chambers during the session, and the number of punishment (i.e., foot shocks) the animal received. For the animals that underwent 14 days of daily 2-hr restraint stress, they were slower in gaining body weight and showed an increase in behavioral despair in forced swim test. However, there was no difference compared to stress-free controls in anxiety-related behavior in

the elevated plus maze or anhedonia in sucrose preference test. Chronic restraint stress did not affect active avoidance behavior, freezing time to cues, the number of feces left, and the number of shock received. These findings provided an insight into the IL-NAcc circuit that advanced our understanding of its function in shifting the defensive behaviors in animals. However, we did not find supporting evidence that chronic restraint stress would lead to the switch of strategies from active to passive coping.

Disclosures: H. Kuan: None. **C. Chang:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.01/OO9

Topic: G.01. Fear and Aversive Learning and Memory

Title: Study of Mozart's Sonata K448 influence on the contextual memory loss of fear in old female mice

Authors: *M. M. SILVA[1], R. M. E. VILELA[1], G. S. AZEVEDO[1], Â. C. M. PAIVA[1], L. V. MIRANDA[1], A. DI GESU[1], C. M. F. TRZESNIAK[1], L. M. VITORINO[1], C. R. SARTORI[2], R. S. DE FARIA[1];
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Abstract: Memory is the ability to store and recall information acquired from experiences. The memory of fear consists of a neutral impulse that are associated with an aversive impulse, resulting in fear responses. The neural structures involved include amygdala and hippocampus. Memory extinction is important to prevent unwanted behavior. Exposure to Mozart's Sonata K.448 has been associated with memory benefits in adult mice, however, there is no information about the effects of this sonata in the memory of old animals, especially in the loss of the memory of fear. Thus, it was studied the possible influence of this Sonata in the contextual loss of fear memory in old female mice. The study was performed with 30 female mice of the C57BL/6J lineage from the vivarium of the University "*Faculdade de Medicina de Itajubá (FMIT)*", Minas Gerais/Brazil. The use of animals for this study was approved by Ethics Commission in the Use of Animals (CEUA AISI) registered under No. 10/10/2018. The animals were divided in three groups: G1-Mozart (*n*=11), G2-Ambience (*n*=11) e G3-Control (*n*=08). Only group G1 was exposed, from the intrauterine period to the Mozart Sonata K.448 from 7am to 9pm, the groups G2 and G3 were exposed to ambient sound. From the 361st to 365th day, after birth, the animals were accustomed. In the day 366th it was performed aversive memory training with groups G1 and G2. From day 396th, the memory extinction test was carried out, lasting five days. On day 421st happened the memory recall test. Significant results were considered $p<0.050$. In the memory extinction test, there was a main effect of the days ($p=0.013$). Follow-up contrasts between days 1 vs. 2 ($p=0.082$) and 4 vs. 5 ($p=0.077$) were not

significant. No main effect was observed between the three groups (p=0.800), but there was a significant interaction between days and groups (p=0.008). The follow-up contrasts between the groups on days 1 and 2 (p=0.052), 2 and 3 (p=0.023), 3 and 4 (p=0.022), and 4 and 5 (p=0.020) were significant, indicating an alternating pattern of freezing behavior over time. In the memory recall test, the ambience group presented more freezing than the control group (p=0.018). Thus, from our results, we conclude that exposure to Mozart's K.448 sonata in old female mice has no positive effect on the extinction of contextual fear memory. However, future studies with larger samples and different species are opportune to better clarify this music in the extinction of the memory of fear.

Disclosures: **M.M. Silva:** None. **R.M.E. Vilela:** None. **G.S. Azevedo:** None. **Â.C.M. Paiva:** None. **L.V. Miranda:** None. **A. Di Gesu:** None. **C.M.F. Trzesniak:** None. **L.M. Vitorino:** None. **C.R. Sartori:** None. **R.S. De Faria:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.02/OO10

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program
NIDA Grant P30 DA048736
NIMH Grants MH107435 and MH119817

Title: Loss of endocannabinoid CB1 receptors in prefrontal-amygdala neurons impairs fear extinction

Authors: *E. VAN LEER[1], *E. VAN LEER[2], O. GUNDUZ CINAR[2], L. CASTILLO[2], M. XIA[2], E. T. BROCKWAY[2], G. POLLACK[2], O. BUKALO[2], A. W. LIMOGES[2], S. OREIZI-ESFAHANI[2], F. YASMIN[3], V. KONDEV[4], R. BALDI[3], J. HARVEY-WHITE[2], R. CINAR[2], G. KUNOS[2], L. S. ZWEIFEL[5], S. PATEL[3], A. HOLMES[2];
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Abstract: Previous work shows that endocannabinoids (eCBs) and the ventromedial prefrontal cortex (vmPFC)→basolateral amygdala (BLA) pathway are involved in fear extinction. Optogenetic activation of this circuit causes elevated levels of eCBs in BLA, while pharmacologically blocking the cannabinoid receptor type 1 (CB1R) impairs extinction. The current study aims to elucidate the connection between eCBs and the vmPFC→BLA pathway by selectively virally expressing a CRISPR-Cas9 engineered *Cnr1* (CB1R) loss of function mutation in vmPFC→BLA neurons. qPCR and BaseScope *in situ* hybridization confirmed reduced *Cnr1* in mice expressing the mutation, and slice electrophysiological recordings

demonstrated impaired CB1R-mediated inhibition of glutamate release at vmPFC→BLA synapses. Behaviorally, mice underwent fear conditioning followed by extinction training and retrieval testing, and were also tested for anxiety-like behavior and food consumption. As compared to non-mutant controls, vmPFC→BLA *Cnr1* mutated mice had higher levels of freezing during extinction retrieval, suggesting an impairment in extinction memory formation or expression. *Cnr1* mutant mice also ate less food when fasted (but not when sated) and showed higher levels of anxiety-like behavior in the light-dark exploration test. These findings show that eCB-CB1R signaling regulates vmPFC→BLA neuron function to modulate fear extinction, with implications for targeting eCBs extinction-deficient neuropsychiatric disorders.

Disclosures: E. Van Leer: None. **E. Van Leer:** None. **O. Gunduz Cinar:** None. **L. Castillo:** None. **M. Xia:** None. **E.T. Brockway:** None. **G. Pollack:** None. **O. Bukalo:** None. **A.W. Limoges:** None. **S. Oreizi-Esfahani:** None. **F. Yasmin:** None. **V. Kondev:** None. **R. Baldi:** None. **J. Harvey-White:** None. **R. Cinar:** None. **G. Kunos:** None. **L.S. Zweifel:** None. **S. Patel:** None. **A. Holmes:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.03/OO11

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program

Title: Effects of endocannabinoid activity at basolateral amygdala astrocytes in fear and extinction

Authors: *H. BHAGWAT[1], S. ZIMMERMAN[2], V. OFFENBERG[2], A. MENDEZ[2], C. WEINHOLTZ[2], T. CAMPBELL[2], M. YDE[2], O. GUNDUZ-CINAR[2], O. BUKALO[2], A. HOLMES[2];
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Abstract: There is growing evidence that astrocytes contribute to learning and memory. Recent studies also show that astrocytes respond to cannabinoids and endocannabinoids (eCB) through the activation of type-1-cannabinoid receptors (CB1R), which release intracellular calcium and stimulate glutamate-mediated synaptic transmission and plasticity. However, the role of eCB-CB1R activity at astrocytes in the fear-mediating basolateral amygdala (BLA) in learning, expression and extinction fear behavior remains unclear. Here we combined pharmacological and genetic approaches to manipulate CB1R on BLA astrocytes and, in parallel, used *in vivo* fiber photometry to measure changes in BLA astrocytic calcium dynamics during fear conditioning and extinction in mice. We found that systemic injection of CB1R agonist, WIN55,212-2, increased the amplitude of BLA astrocytic Ca^{2+} events; an effect prevented by co-

administration of the CB1R antagonist, rimonabant. Next, using gene mutant and viral strategies to examine the effect of brain-wide and BLA-specific deletion of astrocyte-CB1R, we found that loss of astrocyte-CB1R impaired fear retrieval (reduced levels of cue-related freezing) during early extinction training. This behavioral effect was associated with reduced cue-related Ca^{2+} activity in BLA astrocytes. Notably, in these same mice cue-related freezing and Ca^{2+} activity was not diminished on a post-extinction fear renewal test, demonstrating that loss of astrocyte-CB1R interfered with the retrieval/expression of the fear memory, but did not abolish it. These data show that eCB-CB1R activity at BLA astrocytes supports fear memory retrieval and related astrocytic Ca^{2+} signaling.

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Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.04/OO12

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program

Title: Role of basolateral amygdala astrocyte calcium signaling in fear

Authors: *O. BUKALO, **S. ZIMMERMAN**, V. OFFENBERG, A. MENDEZ, C. WEINHOLTZ, T. CAMPBELL, M. YDE, A. HOLMES;
Natl. Inst. of Health, Natl. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: The ability to retrieve associations between environmental stimuli and previously encountered threat represents a fundamental form of associative memory that is crucial to survival. Recent studies suggest astrocytes support fear memory by modulating memory-encoding neural circuits in cortical and limbic regions. However, the precise mechanisms by which this occurs remain unclear. Here, we monitored and manipulated astrocyte activity in the basolateral amygdala (BLA), a brain region critical to the formation, retrieval, and extinction of a cued fear memory. First, using *in vivo* fiber photometry Ca^{2+} recordings, we found that BLA astrocyte Ca^{2+} activity dynamically tracks shifts in fear state that are evident as mice retrieve, extinguish and renew fear. Next, we found that selective viral expression of a plasma membrane Ca^{2+} extruder, hPMCA2w/b, in BLA astrocytes decreased fear during fear retrieval, extinction training and extinction retrieval. Chemogenetic manipulation of BLA astrocytes, via selective viral expression of hM3D(Gq)- or hM4D(Gi)-coupled DREADDs, and systemic injection of clozapine n-oxide (CNO) bidirectionally altered fear retrieval and produced opposite effects on astrocyte Ca^{2+} activity. Together, these data show that BLA astrocyte Ca^{2+} activity is both tightly coupled and required to maintain fear.

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Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.05/OO13

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program
NIDA grant P30 DA048736
NIMH grants MH107435 and MH119817

Title: Endocannabinoid activity at prefrontal-amygdala neurons dynamically track fear extinction

Authors: *O. GUNDUZ CINAR[1], L. I. CASTILLO[1], M. XIA[1], E. VAN LEER[1], E. T. BROCKWAY[1], G. A. POLLACK[1], F. YASMIN[2], O. BUKALO[1], A. LIMOGES[1], S. OREIZI-ESFAHANI[1], V. KONDEV[3], R. BALDI[2], A. DONG[4], J. HARVEY-WHITE[1], R. CINAR[1], G. KUNOS[1], Y. LI[4], L. S. ZWEIFEL[5], S. PATEL[2], A. HOLMES[1];
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Abstract: Extinction of fear memories is impaired in anxiety and stressor-related disorders. The endocannabinoid (eCB) system plays a key role in fear extinction, as evidenced by preclinical and clinical studies. Basolateral amygdala (BLA)-projecting medial prefrontal cortex (mPFC) neurons have also been implicated in fear extinction. Here we investigated whether eCBs modulate extinction through effects on these neurons. To do so, we virally-expressed a genetically encoded eCB sensor ($GRAB_{eCB2.0}$) at mPFC axon terminals and recorded changes in sensor activity, via *in vivo* fiber photometry, as mice underwent fear extinction testing. By measuring changes in $GRAB_{eCB2.0}$ activity in response to systemically injected eCB-acting drugs, we first pharmacologically validated the efficacy and eCB-selectivity of the biosensor at these neurons. Next, male and female mice were fear conditioned by pairing a white noise cue (conditioned stimulus, CS) with a mild electric foot shock (unconditioned stimulus, US) and, the following day, underwent extinction training via multiple non-reinforced presentation of the CS in a different context. During extinction training, we found increased $GRAB_{eCB2.0}$ activity in the period following CS presentation, and most prominently so early in training when the US is expected but does not occur. These findings reveal the temporal dynamics of eCB activity at BLA-projecting mPFC neurons as animals form a fear extinction memory and provide novel insight into the neural mechanisms underlying the potential therapeutic effects in anxiety and stressor-related disorders.

Disclosures: **O. Gunduz Cinar:** None. **L.I. Castillo:** None. **M. Xia:** None. **E. Van Leer:** None. **E.T. Brockway:** None. **G.A. Pollack:** None. **F. Yasmin:** None. **O. Bukalo:** None. **A. Limoges:** None. **S. Oreizi-Esfahani:** None. **V. Kondev:** None. **R. Baldi:** None. **A. Dong:** None. **J. Harvey-White:** None. **R. Cinar:** None. **G. Kunos:** None. **Y. Li:** None. **L.S. Zweifel:** None. **S. Patel:** None. **A. Holmes:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.06/OO14

Topic: G.01. Fear and Aversive Learning and Memory

Title: The effect of classical music on memory extinction

Authors: *A. DI GESU[1], L. V. MIRANDA[1], M. M. SILVA[1], R. M. E. VILELA[1], C. M. F. TRZESNIAK[1], L. M. VITORINO[1], C. R. SARTORI[2], R. S. FARIA[1];
[1]Dept. of Human Physiol., Faculdade de Medicina de Itajubá - FMIT, Itajubá, Brazil; [2]Dept. of Functional Biol., Campinas State Univ. - UNICAMP, Campinas, Brazil

Abstract: Memory is defined as the aptitude to store and recall information previously assimilated through experiences. These new information associates to those already present in the individual repertoire, thus depending on how frequently they're evoked, they can either be preserved or extinguished.The ability to reframe thoughts and behaviors no longer congruent to the present moment constitutes the extinction process. In other words, it is not a subtraction from the experience but rather a redefinition. It is known that Mozart's K448 Sonata benefits the brain, however, its influence on memory extinction remains poorly understood. Therefore, this project aims to analyze the influence of Mozart's K448 Sonata on contextual memory extinction on male mice. The study was conducted with 22 male C57BL/6J mice, divided into three groups: G1 Mozart (n=07), G2 Environment (n=08) and G3 Control (n=07). Throughout the project, only the first group was exposed to the sonata from 9pm to 7am, since intrauterine life. Groups 2 and 3, on the other hand, were exposed to ambient sound only. From the 50th to the 54th day, animals underwent Habituation phase, aimed at controlling interference from the environment novelties in their behaviors. On the 55th day, the Aversive Training was conducted with groups 1 and 2. Then, on the 82nd day, the Extinction Test was initiated with all three groups for five consecutive days. Subsequently, all of them underwent the Recall Test on the 107th day. The procedures were recorded on video for analysis. The repeated measures ANOVA test was used for analyzing the Extinction Test, and One-Way ANOVA was used for the Recall Test. The values of $p<0,05$ were considered significant. During Aversive Training, there was no significant difference between both groups (Mozart and Environment) freezing time ($p=0,123$). Follow-up contrasts between days of the Extinction Test were significant, showing an overall decrease in freezing behavior over time ($p=0,006$). Regarding the different groups, there was also variation in the observed behavior throughout the Extinction Test ($p=0,006$), but there was no interaction between days and groups ($p=0,074$). Finally, there was no difference between groups in the

Recall Test (p=0,057). From the mentioned results, we concluded male mice exposed to Mozart's K448 Sonata showed increased freezing time, suggesting an impact of the music on memory formation. However, they exhibited a linear process of extinction of this memory. This progressive decrease in freezing behavior was not observed in the Environment and Control groups.

Disclosures: A. Di Gesu: None. **L.V. Miranda:** None. **M.M. Silva:** None. **R.M.E. Vilela:** None. **C.M.F. Trzesniak:** None. **L.M. Vitorino:** None. **C.R. Sartori:** None. **R.S. Faria:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.07/OO15

Topic: G.01. Fear and Aversive Learning and Memory

Support: NIAAA Intramural Research Program

Title: Role of basal amygdala neurons in fear expression and extinction

Authors: *J. GOLDSCHLAGER, S. ZIMMERMAN, V. OFFENBERG, O. BUKALO, A. HOLMES;
Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD

Abstract: Fear and fear extinction can be experimentally assayed in this way across species and is of relevance to anxiety and trauma-related disorders. In a cued typical Pavlovian fear conditioning procedure, a tone conditioned stimulus (CS) is paired with a co-terminating footshock unconditioned stimulus (US) and then is subsequently extinguished by repeated presentation of the CS without coincident US. The amygdala has been identified as a key substrate for fear and extinction, but a complete understanding of the precise role of BA neurons in these processes is currently lacking. To address this issue in the current study, we used *in vivo* fiber photometry to obtain fear and extinction-related correlates of BA neuronal Ca^{2+} activity. We then performed multiple complementary targeted manipulations (chemogenetic, optogenetic, toxin) to determine the causal contribution of BA neurons to fear and extinction. Our findings reveal that BA neurons exhibit correlates of fear and extinction and demonstrate that these responses are necessary for cued fear retrieval and for extinction memory formation.

Disclosures: J. Goldschlager: None. **S. Zimmerman:** None. **V. Offenberg:** None. **O. Bukalo:** None. **A. Holmes:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.08/OO16

Topic: G.03. Motivation

Support: R01-MH117791

Title: Ventral pallidum neurons are necessary to generalize and express fear-related responding in a minimal threat setting

Authors: *E. L. RUSSELL[1], M. A. MCDANNALD[2];
[1]Psychology & Neurosci., [2]Boston Col., Chestnut Hill, MA

Abstract: Fear-related behavior is beneficial when specific to events signaling harm (discrimination), but becomes problematic when displayed in response to neutral events (generalization). Here, we developed minimal threat learning procedures to distinguish discrimination from generalization. We then examined roles for the ventral pallidum - a region anatomically poised to modulate amygdalar threat function - in the generalization and expression of fear-related responding. Experiment 1 established behavioral procedures that would distinguish discrimination from generalization. Long Evans rats (n = 47, 23 females) were mildly food-deprived, trained to nose poke for food pellets, then assigned to one of three probability conditions. For each condition, a threat cue probabilistically predicted foot shock on 10% (n=15), 20% (n=16), or 30% (n=16) of trials. A neutral cue never predicted foot shock. All rats acquired discrimination over the 10 sessions. Rats in the 10% probability condition did not generalize responding to the neutral cue, whereas generalization was evident in the 20% and 30% conditions. During an extinction test, the 10% threat cue supported less responding than the 20% and 30% threat cues. Experiment 2 examined a role for the ventral pallidum in discrimination, generalization and expression of threat cue responding. A dual viral approach (AAV-eSYN-EGFP-T2A-iCre + AAV-flex-taCasp3-TEVp) was used to delete ventral pallidum neurons (Casp3VP, n = 11, 5 female), while neurons were left intact in the Control group (n = 12, 6 female). Control and Casp3VP rats were then assigned to either the 10% or 30% probability conditions. Control rats in the 30% condition, but not the 10% condition, generalized responding. The 30% threat cue supported greater fear-related behavior during extinction than the 10% threat cue. Neither Casp3VP rats in the 10% nor the 30% generalized responding and both groups showed reduced fear-related behavior to the threat cue in extinction. Experiment 3 used the dual viral approach to delete nucleus accumbens neurons projecting directly to the ventral pallidum (Casp3NAc→VP). Control and Casp3NAc→VP rats were assigned to the 30% probability condition (n = 24). Pathway deletion had no impact on generalization or expression of fear-related responding to the 30% threat cue. The results reveal the ventral pallidum is necessary to generalize and express fear-related behavior in a minimal threat setting; however, this function does not depend on nucleus accumbens input.

Disclosures: E.L. Russell: None. **M.A. McDannald:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.09/OO17

Topic: G.03. Motivation

Support: R01-MH117791

Title: Ventral pallidum-defined pathways modulate fear-related behavior during threat discrimination

Authors: *M. MOADDAB, S. QIAN, J. B. BOYCE, N. T. GORDON, A. M. DUBOIS, A. C. FITZPATRICK, M. A. MCDANNALD;
Boston Col., Chestnut Hill, MA

Abstract: The ventral pallidum (VP) is a critical node in the mesolimbic system, contributing to reward-related behavior. Previously, we reported the VP as a neural source of a dynamic, relative threat signal. The VP receives direct projections from the nucleus accumbens (NAc) and paraventricular nucleus of the thalamus (PVT), both important to organizing components of fear behavior. However, it is unclear if the VP, or its inputs from the NAc and PVT, modulate responding or expression of fear-related behavior. Here, we examined the role of the NAc and PVT input pathways to the VP in multi-cue fear discrimination. Male and female Long Evans rats received bilateral infusions of a retrogradely transported adeno-associated virus (AAV) vector encoding Cre recombinase (AAV/retro-eSYN-EGFP-T2A-iCre-WPRE, 0.3 µl per side) in the VP. Controls (n = 16) were injected with an AAV containing mCherry (rAAV5/Ef1a-DIO-mcherry, 0.3 µl per side). Cre-positive cells in the VP, NAc, or PVT were selectively deleted via cre-dependent viral caspase (rAAV5-Flex-taCasp3-TEVp). Cre-caspase was injected into the VP (n = 16, 1.0 µl per side), NAc (n = 16, 1.0 µl per side), or anterior/posterior PVT (n = 16, 0.75 µl per site). Following recovery, rats were food deprived to 85% of their body weight and trained to nose poke to receive food pellets. Rats underwent 16 sessions of Pavlovian fear discrimination before moving on to one extinction session. In fear discrimination sessions, rats were presented with three 10-s auditory cues, each associated with a unique probability of foot shock; danger (p = 1.00), uncertainty (p = 0.25), and safety (p = 0.00). The schedule for rewarded nose poking was completely independent of auditory cue presentation and foot shock. Fear was measured using suppression of rewarded nose poking. Caspase-mediated deletion of local VP neurons and NAc or PVT inputs to the VP accelerated the recovery nose poking after foot shock introduction. Caspase deletion of all three areas had no impact on direct responding to the three cues. Caspase and control rats showed high fear to danger, intermediate fear to uncertainty, and low fear to safety. However, cue-specific effects of caspase deletion emerged during the extinction. We will present the complete histological analysis of the caspase deletion and complete behavioral analyses of fear discrimination and extinction.

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Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.10/OO18

Topic: G.03. Motivation

Support: NIMH R01 MH112505

Title: Axonal collateralization of principal basolateral amygdala neurons

Authors: *I. KIM[1,2], I. HUERTA-OCAMPO[1], O. URENA[1], R. YAMAMOTO[3], D. PARE[1]; [1]Ctr. for Mol. and Behavioral Neurosci., [2]Behavioral and Neural Sci. Grad. Program, Rutgers Univ., Newark, NJ; [3]Kanazawa Med. Univ., Kanazawa Med. Univ., Ishikawa, Japan

Abstract: The basolateral complex of the amygdala (BLA) projects to a diverse array of cortical and subcortical sites through which it influences multiple functions. Although some BLA neurons have branching axons to multiples sites, some BLA targets appear to be innervated by BLA neurons with non-branching axons. At present, the collateralization patterns of BLA neurons remain poorly understood, largely because of technical limitations associated with retrograde tracing methods and the sheer number of possible combinations of target areas. Yet, the extent to which BLA neurons contribute axon collaterals to multiple sites is key for understanding how coding and projection site are related. For instance, it is possible that the BLA contributes multiple output streams, some of which recruit many targets that act in concert because they receive collateralizing BLA axons, while others are engaged independently because the BLA axons contacting them do not collateralize. To shed light on this question, we adopted an intersectional recombinase-based viral tracing strategy that allows visualization of the axon collaterals contributed by different projection-defined BLA subpopulations. We found that BLA neurons projecting to the medial prefrontal cortex (mPFC), dorsal striatum, anterior insula and subiculum (but not the ventromedial hypothalamus) send massive collateral projections to a common array of subcortical structures including nucleus accumbens, the olfactory tubercle, nucleus of the lateral olfactory tract (NLOT), interstitial nucleus of the posterior limb of the anterior commissure, and bed nucleus of the stria terminalis. Ongoing experiments involving the injection of distinct retrograde fluorescent tracers in these structures are so far consistent with the results of the viral tracing. For instance, some of the BLA cells projecting to the mPFC or striatum also project to NLOT. Finally, using optogenetic techniques and whole-cell recordings in vitro, we ascertained that the collateralizing axons are not passing fibers but that they form glutamatergic synapses. Overall, the collateralization patterns we observed indicate that while some BLA targets receive inputs from BLA neurons with non-branching axons, others are innervated by BLA neurons with axons collateralizing to a hitherto underestimated array of targets. Therefore, some BLA functions may depend on the coordinated recruitment of different subsets of BLA targets.

Disclosures: I. Kim: None. **I. Huerta-Ocampo:** None. **O. Urena:** None. **R. Yamamoto:** None. **D. Pare:** None.

Poster

PSTR361. Neural Mechanisms of Fear and Fear Extinction

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR361.11/Web Only

Topic: G.01. Fear and Aversive Learning and Memory

Support: SERB, GOI; CRG/2020/004971
Rashtriya Uchchatar Shiksha Abhiyano, GOI
UGC, GOI; Senior research fellowship

Title: Gut-microbiota accelerates extinction memory formation and prevents relapse of fear memory: A possible role for microbiota in activity-dependent gene expression in the amygdala

Authors: *G. JADHAV, A. SAKHARKAR;
Dept. of Biotech., Savitribai Phule Pune Univ., Pune, India

Abstract: Fear is fundamental for the survival of the host and its processing by the amygdala plays a critical role in its learning and memory consolidation. Studies into fear memory consolidation and extinction are critical for understanding the neural basis of post-traumatic stress disorder (PTSD). Extinction memory formation is the new learning after fear conditioning and therapeutic implications are being made for strengthening these memory networks in order to prevent relapse. Gut microbiota is an intricate part of the host's internal milieu and influences gene expression in the brain responsible for the development of specific phenotypes. Hence, it has been reported that multi-strain probiotic enrichment post-fear conditioning accelerates extinction learning and prevents relapse (Cui et. al., 2021 *Neuropharmacology* 193 (2021): 108613). In the current study, we probe the potential of psychobiotic intervention in modulating synaptic plasticity in the amygdala to accelerate extinction learning. Fear conditioning (tone-shock pairing) was employed in adult male Wistar rats (n=8) followed by either Placebo (0.9% saline) or *Lactobacillus rhamnosus* GG (LGG; ATCC 53103; 1ml of 10^8 CFU/ml) intervention for 10 days. Extinction training was carried out until the percentage freezing was closer to that of the unconditioned controls. Long-term memory test was conducted 7 days post last extinction training for checking relapse of fear memory or retention of new learning, i.e., extinction memory. While extinction training of 12 days was required for the formation of new memory in fear-conditioned rats, LGG treatment accelerated this extinction to achieve in 6 days. Moreover, LGG treatment prevented relapse of fear, which was evident in placebo-treated fear-conditioned rats. Relapse of fear memory is challenging in the treatment of fear-related disorders. Therefore, LGG supplementation may be suggested as an auxiliary method for exposure-based treatment of fear-induced neuropsychiatric conditions. Further, brains were collected at different time points in order to discern the differences in molecular substrates operative at different stages of memory formation and consolidation. Experience-based neuroepigenetic changes modulate activity-dependent gene expression required for behavioral manifestations. The results suggest the implications of LGG in epigenetic regulation of brain-derived neurotrophic factor (*Bdnf*) gene

expression in the amygdala necessary for synaptic plasticity involved in fear processing and memory formation.

Disclosures: G. Jadhav: None. **A. Sakharkar:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.01/OO19

Topic: G.03. Motivation

Support: R01HD095966

Title: Interaction between serotoninergic and dopaminergic signaling in a developmental model of SSRI exposure

Authors: E. DA CUNHA MENEZES[1], L. RACHMANY[1], F. FRANCISCA DE ABREU[1], *C. M. TEIXEIRA[2];
[1]Nathan Kline Inst., Orangeburg, NY; [2]Child and Adolescent Psychiatry, New York Univ., NEW YORK, NY

Abstract: Serotonin is known to regulate multiple systems, from food satiety to mood regulation. Multiple environmental factors affect serotonin levels during development including exposure to Selective Serotonin Reuptake Inhibitors (**SSRIs**) antidepressants. In the US, it is estimated that between 5 and 10% of pregnant mothers take antidepressants during pregnancy with unknown effects on fetal development. It has been shown that altered levels of serotonin during development can lead to behavioral deficits in adult mice such as anhedonia and anxiety-like behavior. However, the mechanisms by which serotonin levels during development affect adult behavior are largely unknown. We hypothesize that early-life exposure to high serotonin levels, induced by SSRI exposure, causes alterations in the adult dopaminergic system and long-lasting dopamine-dependent behavioral changes. To manipulate developmental serotonin levels, we administered the SSRI fluoxetine from postnatal day (**P**)2 to P11 in mice. This period roughly correlated with the third trimester of pregnancy in humans in respect to serotonergic development. We found that early-life SSRI exposure leads to deficits in exploration and deficits in glutamatergic co-transmission between serotonergic and dopaminergic neurons. Interestingly, early-life SSRI exposure (**PN-FLX**) and VGlut-3 knock-out in serotonergic cells led to similar phenotypes in the open-field, elevated plus-maze and light-dark box. Furthermore, using photometry and microdialysis, in live behaving animals, we found a hypoactivation of the dopaminergic system in PN-FLX mice. Importantly, these mice showed reduced motivation in the progressive ratio in an operant task. To test whether we could rescue these deficits in adulthood, by acting in the dopaminergic system, we compared their response to an SSRI (fluoxetine) or a Dopamine-Norepinephrine Reuptake Inhibitors (**DNRI**; bupropion). We found that PN-FLX induced reduction in motivation could be reversed by adult administration of

bupropion but not by fluoxetine. Furthermore, the PN-FLX impaired performance in the novelty suppressed feeding test, a recognized measure of anxiety-like behavior, was successfully ameliorated by administering bupropion in PN-FLX adult mice, while the administration of FLX did not yield similar results. In conclusion, we found that early-life fluoxetine exposure leads to deficits in serotonergic-dopaminergic signaling, deficits in dopaminergic response to rewarding stimulus, deficits in motivation and increased anxiety. There deficits could be ameliorated by enhancement of dopaminergic signaling in the adult.

Disclosures: E. Da Cunha Menezes: None. **L. Rachmany:** None. **F. Francisca de Abreu:** None. **C.M. Teixeira:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.02/OO20

Topic: G.03. Motivation

Support: NIH U19 Grant NS107616
NIH DeNDriTeS Grant 5R25NS107178-03

Title: Neuromodulation of maternal motivated behaviors in mice experiencing pup separation stress

Authors: *H. A. ISSA[1], J. L. SHAN[1], S. VALTCHEVA[2], V. J. IVAN[1], R. C. FROEMKE[1]; [1]Neurosci., New York Univ., New York, NY; [2]Univ. of Cologne, Cologne, Germany

Abstract: Maternal stress has a profound impact on the development of postpartum psychiatric disorders and the maternal-infant bond. For example, prior studies in humans and rodents have reported a higher prevalence of postpartum depression and depressive-like behaviors among mothers who have experienced relatively high levels of emotional stress (Qobadi et al. 2016; Seki et al. 2021). One hallmark of postpartum stress/depression is a reduction in dopamine signaling in response to infant cues, but the underlying causes of this dysregulation remain unknown. Our lab recently showed that oxytocin release from the paraventricular hypothalamus (PVH) to the ventral tegmental area (VTA) occurs during pup interactions, signaling infant sensory cues, and is critical for maternal pup retrieval -- and presumably for stimulation of dopamine release from VTA (Valtcheva, Issa et al. Nature 2023). Thus, we hypothesized that a breakdown in oxytocinergic modulation of VTA dopamine neurons may contribute to maternal stress/depression phenotypes. We induced maternal separation stress in mice by removing pups from their dam's homecage for 4-5 hours. Dams were faster to retrieve pups during the pup retrieval assay after the separation protocol (n=3). Whole brain cFos mapping revealed a significant increase in activity of brain areas known to support maternal care, including the PVH ($p=0.007$), VTA ($p=0.035$) and nucleus accumbens (NAc, $p=0.005$), for dams that underwent the separation protocol (n=4) compared to controls (n=4). We next performed fiber photometry

using the dopamine GRAB sensor in the NAc shell during pup retrieval in dams before and after pup separation. Pup retrieval events coincided with increased dopamine signaling before, but not after, maternal stress was induced (n=3, p=0.006), with normal retrieval-associated dopamine signaling recovering only after a period of reunion with pups. We then performed whole-cell recordings in VTA brain slices, measuring firing rates from identified dopamine neurons before and during/after bath application of oxytocin. We observed that firing rates of VTA dopamine neurons increase with oxytocin administration in brain slices from control animals. Surprisingly, oxytocin had no effect on dopamine neuron firing in slices from post-separation dams (n=3 cells/condition). Our findings support prior work indicating that maternal reward circuits are dysregulated by stress, and point to disruptions in oxytocin's modulation of VTA dopamine neurons as a potential cause. These results also highlight a possible role for stress-related neuromodulators to support critical maternal behaviors in the absence of normal dopamine signaling.

Disclosures: H.A. Issa: None. **J.L. Shan:** None. **S. Valtcheva:** None. **V.J. Ivan:** None. **R.C. Froemke:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.03/OO21

Topic: G.03. Motivation

Title: Social reward and mesolimbic dopamine release

Authors: *T. ERICKSON, H. E. FRANKS, M. J. AZZAWI, D. B. LESTER;
Psychology, Univ. of Memphis, Memphis, TN

Abstract: Deficits in social behavior, such as a loss of motivation and social avoidance are key symptoms in several psychiatric disorders. The mesolimbic dopamine system consists of dopamine cell bodies in the ventral tegmental area (VTA) that project to limbic regions, most notably the nucleus accumbens (NAc). This system plays a critical role in mediating reward-seeking and motivation, most researched in relation to drug reward. Emerging evidence suggests that different circuits facilitate drug versus social reward, with drug reward depending on phasic dopamine release and social reward on tonic dopamine release in the NAc. The current study examined the relationship between aspects of NAc dopamine release and social reward preference in C57Bl/6J mice. Social conditioned place preference (sCPP) was used to assess preference/aversion to chambers associated with social interaction. The sCPP trials consisted of an initial chamber preference test on Day 1, 8 days of conditioning (distinct chambers in which the experimental mouse was placed with a conspecific or alone), and another chamber preference test on Day 10. The degree of social preference/aversion was determined by subtracting the time spent in the social-paired chamber on Day 1 from Day 10, with more time spend in the social-paired chamber on Day 10 compared to Day 1 indicating increased preference for social

interaction. Following sCPP, we used in vivo fixed potential amperometry to measure VTA stimulation-evoked dopamine release in the NAc of anesthetized mice. To challenge this reward system during dopamine recordings, mice were administered an injection of cocaine (10 mg/kg, ip). No significant relationship was observed between baseline (pre-cocaine) phasic dopamine release and social preference; however, there was a sex-dependent, significant relationship between post-cocaine phasic dopamine release and social preference. In males, as the preference for social interaction increased, the dopaminergic response to cocaine decreased. A negative relationship between social preference and the dopaminergic response to cocaine indicates potential differences in the neural processing of social versus drug reward, processing that may vary by sex. Research is ongoing to examine the relationship between tonic dopamine release and social preference.

Disclosures: **T. Erickson:** None. **H.E. Franks:** None. **M.J. Azzawi:** None. **D.B. Lester:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.04/OO22

Topic: G.03. Motivation

Support: NARSAD YIG 25300
NIH R21 MH 129809

Title: Sex differences in habenula-induced inhibition of midbrain dopamine neurons in the rat

Authors: D. BELL, ***P. L. BROWN**;
Dept. of Psychiatry, Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Clinically relevant sex differences have been noted in affective, behavioral, cognitive, and neurological health disorders. Midbrain dopamine neurons have been implicated in several of these same disorders leading to investigations of sex as a biological variable acting on these brain nuclei. The lateral habenula (LHb) exerts significant inhibitory control over dopamine neuronal firing, yet little is known about sex differences in this circuit. Here we compared the ability of the LHb to inhibit dopamine firing by sex. Dopamine neurons from the lateral ventral tegmental area and substantia nigra pars compacta were recorded following single pulse electrical stimulation of the LHb in anesthetized rats. LHb-induced inhibition was significantly less robust in female rats as measured by mean duration amongst inhibited cells and by mean firing rate amongst all recorded cells. In addition, dopamine neurons recorded from male rats were more likely to express a rebound excitation that was largely absent in female rats. Since LHb-induced inhibition of dopamine neurons occurs via the rostromedial tegmental nucleus (RMTg), immunostaining with the neuron-specific marker NeuN was compared between sexes. However, no sex difference in neuronal density of the RMTg was found. There is evidence that estrogen may affect LHb neuronal firing rates and pattern, specifically that estrogen

downregulates the expression of calcium channels responsible for burst firing. An immunostain for ER-alpha demonstrated significantly higher staining in female rats than male rats. Subsequently, in vivo recordings of LHb neurons were performed. Baseline firing rates did not differ by sex. There was a tendency for a higher prevalence of burst firing LHb neurons in male rats, and the coefficient of variation was higher in LHb neurons in male rats, which is indicative of irregular/burst firing. The qualitative and quantitative sex difference in LHb-induced inhibition of dopamine firing, along with the higher ER-alpha expression in the LHb of female rats and greater prevalence of LHb irregular/burst firing in male rats leads us to posit that the sex difference in dopamine inhibition seen here is driven by estrogen altering the firing properties of LHb neurons. In short, we hypothesize that, in the presence of estrogen, activation of LHb neurons is less likely to result in a firing burst, which in turn results in reduced inhibitory input to dopamine neurons. We are currently testing this hypothesis, which may have implications for understanding the etiology of several mental health disorders including depression, schizophrenia, and addiction.

Disclosures: D. Bell: None. **P.L. Brown:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.05/OO23

Topic: G.03. Motivation

Title: Oxytocin administration alters dopamine autoreceptor functioning

Authors: P. A. NALAN, S. B. BERRETTA, R. L. PACE, M. L. LOVE, ***D. B. LESTER**; Univ. of Memphis, Memphis, TN

Abstract: Oxytocin is being researched as a new treatment option for substance use disorder. Oxytocin likely alters the rewarding properties of drugs by altering their effects on mesolimbic dopamine release. Our lab has previously shown that mice pretreated with oxytocin display decreased dopamine release in the nucleus accumbens (NAc) and a reduced dopaminergic response to a psychostimulant. In the NAc, oxytocin receptors form a complex with D2 dopamine receptors, which often serve as autoreceptors. The current study aimed to examine whether oxytocin mediates dopamine release in the NAc by altering dopamine autoreceptor functioning. Stimulation-evoked dopamine release was measured using in vivo fixed potential amperometry in anesthetized C57Bl/6J male and female mice. Stimulation parameters specifically designed to assess dopamine autoreceptor functioning were applied during dopamine recordings following either chronic administration of oxytocin (1.0 mg/kg i.p. daily for 7 days) or a local intra-NAc infusion of oxytocin (0.6 µg in 0.5 µl volume). Chronic pretreatment of oxytocin significantly increased dopamine autoreceptor functioning; however, no differences in autoreceptor functioning were observed following the intra-NAc oxytocin infusion. These results suggest that oxytocin can alter the functioning of dopamine autoreceptors in the NAc, but that

this mechanistic shift in feedback-mediated dopamine release is either delayed or depends on repeated doses of oxytocin. The findings from this study provide further insight on the neural mechanisms of oxytocin and its role in mediating reward-related circuitry.

Disclosures: P.A. Nalan: None. **S.B. Berretta:** None. **R.L. Pace:** None. **M.L. Love:** None. **D.B. Lester:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.06/OO24

Topic: G.03. Motivation

Title: Chronic unpredictable stress affects reward-related behavior and dopamine release in C57BL/6J and DBA2/J mice.

Authors: *C. B. HARTLESS, M. L. EDWARDS, D. B. LESTER, M. N. COOK; Psychology, Univ. of Memphis, Memphis, TN

Abstract: Exposure to chronic stress is associated with substance use disorders and depressive-like behaviors. Anhedonia is a primary feature of depression that involves altered reward related processes (i.e., a reduction in motivation and ability to experience pleasure). The mesolimbic dopamine (DA) pathway is instrumental in reward related processing. Chronic stress has been associated with anhedonia; however, the mechanisms are not well understood. In the current study, C57BL/6J (B6) and DBA2/J (D2) mice, which differ in stress responsivity, emotionality, and reward-related behaviors, were exposed to a chronic unpredictable stress (CUS) paradigm for 4 weeks (PND42-70). Following CUS, one group of animals was assigned to the sucrose preference test (SPT) to determine the extent to which our CUS paradigm produces anhedonia. Mice had 24-hour home cage access to water and a 2% sucrose solution for 14 days. Overall, females consumed more sucrose than males. B6 mice had greater total fluid intake, higher levels of sucrose consumption, and a greater preference for sucrose than D2 mice, whose preference increased over time. CUS decreased sucrose consumption and preference in B6 mice, while increasing sucrose consumption in D2 mice. In a separate group of animals, *in-vivo* fixed potential amperometry was used to examine the effects of CUS on neurotransmission related to reward processing and motivation (i.e., DA release, the synaptic half-life of DA, DA autoreceptor function, DA neuronal reserves, and the DAergic response to 10 mg/kg (i.p.) of the dopamine reuptake inhibitor nomifensine). For these measures, the ventral tegmental area (VTA) was electrically stimulated, and DA release was measured in the nucleus accumbens (Nacc). D2 mice displayed a higher DA release compared to B6 mice. In B6 mice, CUS decreased DA autoreceptor functioning and increased the dopaminergic response to nomifensine. In D2 mice, a trend suggested CUS decreased the synaptic half-life of DA, an indication of more efficient dopamine transporters (DATs). In summary, CUS led to tighter regulation of dopamine release in D2 mice through increased DAT functioning, while in B6 mice, CUS resulted in reduced

control by impairing the functioning of DA autoreceptors. Chronic stress induced alterations in the regulation of dopamine release may contribute to anhedonia. These findings provide insight into how stress affects reward-related circuitry and associated behaviors.

Disclosures: C.B. Hartless: None. **M.L. Edwards:** None. **D.B. Lester:** None. **M.N. Cook:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.07/OO25

Topic: G.03. Motivation

Support: DA042111
DA048931
5T32MH065215- 18

Title: Food restriction influences action control strategies and dopamine release in the dorsal striatum of mice

Authors: *M. CHEVEE[1], C. J. KIM[1], S. D. EMERSON[1], J. TAT[1], J. N. CROW[1], H.-J. YOON[1], E. S. CALIPARI[2];
[1]Vanderbilt Univ., Nashville, TN; [2]Vanderbilt Univ., Vanderbilt Univ. Sch. of Med., Nashville, TN

Abstract: Diverse factors influence how we control our actions. For example, reinforcement schedules bias control strategies via distinct action-outcome contingencies. However, non-learning related factors have equally important influences on behavior and must be studied in combination with reinforcement principles to understand how animals learn and control their actions.One such factor is food deprivation - a condition often experimentally induced to enhance task engagement and learning rate in operant training.
The goal of this study is to determine the neural mechanisms that underlie the influence of food restriction on action control strategies.
1) We first showed that stricter food restriction during training in male and female mice results in behaviors that are more sensitive to extinction.
2) *in vivo* fiber photometry recordings using the dopamine sensor dLight revealed that food restriction modulated dopamine release in DMS, but not DLS. Surprisingly, only the response to sucrose consumption was increased, whereas the responses to randomly delivered external stimuli were not affected by food restriction.
3) We found using slice voltammetry - where the somatic compartment is removed - that food restriction increased dopamine release and slowed the clearance of dopamine in DMS but not in DLS.
4) We found that expression of a phosphorylated version of DAT (T53) - which is known to alter

its uptake function - was reduced specifically in DMS under food restriction conditions.
Thus, our study shows that food restriction fundamentally affects behavioral control strategy and the balance of dopamine release in dorsal striatum. Specifically, our data suggest that food restriction reduces the efficiency of dopamine re-uptake by DAT via phosphorylation of the T53 residue specifically in DMS. This leads to an accumulating of larger dopamine levels and an extended timing of dopamine release in DMS compared to DLS, thus promoting DMS-dependent goal-directed control of actions.
We are currently performing several experiments to test predictions that stem from these observations and to determine whether DAT phosphorylation, enhanced dopamine release and behavioral changes are causally linked.

Disclosures: **M. Chevee:** None. **C.J. Kim:** None. **S.D. Emerson:** None. **J. Tat:** None. **J.N. Crow:** None. **H. Yoon:** None. **E.S. Calipari:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.08/PP1

Topic: G.03. Motivation

Support: NSF GRFP DGE 2140743

Title: Dopamine desensitization devalues repeated behaviors

Authors: *L. E. MINER, A. K. GAUTHAM, M. A. CRICKMORE;
F.M. Kirby Neurobio. Ctr., Boston Children's Hosp., Boston, MA

Abstract: Even the most essential rewarding behaviors seem to lose their urgency after repeated engagement. In this study we sought to identify the circuit and molecular mechanisms that underlie the devaluation of repeated behaviors using the mating behavior of male *Drosophila* as a model. Dopamine sets the persistence of mating when confronted with challenges. We find that this persistence decreases after repeated copulations and that this behavioral devaluation is a direct consequence of dopamine released during prior matings. We ascribe this effect to the use-dependent desensitization of the D2 dopamine receptor on ~8 Copulation Decision Neurons (CDNs) that mediate the decision to end matings. Desensitization requires β-arrestin signaling, tying classical GPCR desensitization mechanisms to the control of natural behavior. Beyond providing a new mechanism of motivational control, the parallels to drug-induced desensitization argue that the widespread devaluation behavior in addiction arises from a corruption of the natural dopaminergic dynamics that regulate our willingness to work for rewards.

Disclosures: **L.E. Miner:** None. **A.K. Gautham:** None. **M.A. Crickmore:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.09/PP2

Topic: G.03. Motivation

Support: JST FOREST Program JPMJFR2040 (MO)
JSPS KAKENHI JP23H01056 (MO)
JSPS KAKENHI JP22K18645 (MO)
JSPS KAKENHI JP21H00961 (SI)
JSPS KAKENHI JP20H03547 (MO)
JSPS KAKENHI JP20K20862 (MO)

Title: A dopamine error signaling to overcome lack of expected reward and reinforce behavioral switching toward future reward pursuit

Authors: *M. OGAWA, R. TSUKASA, S. ISHINO;
Kyoto Univ., Kyoto-shi, Japan

Abstract: Rewards are often uncertain and not easily obtained. In order to obtain a particular uncertain reward, animals need to continue the pursuit of reward, even after the expected reward is not obtained. The neural mechanisms underlying adaptive reward pursuit to overcome the lack of expected reward are poorly understood. Using a behavioral task in which rats were required to continue to pursue a probabilistic reward, we have found that following the responses of the well-known dopamine (DA) neurons in lateral ventral tegmental area (VTA) that signal reward prediction error (RPE) (type 1 DA neurons), a subset of DA neurons, which we called type 2 DA neurons, exhibited increased responses to unexpected reward omission and decreased responses to unexpected reward (Ishino et al., 2023). However, the exact function of the type 2 DA signaling is still unclear. Here we trained head-restrained rats to push a spout-lever to trigger a presentation of an auditory cue and then pull the lever to potentially obtain a liquid reward from the tip of the lever. Rats were required to perform the lever manipulation four times to eventually obtain the reward. Using a genetically encoded DA sensor $GRAB_{DA}$, we measured DA levels in a part of the nucleus accumbens (NAc), i.e., anterior dorsal part of NAc (dNAc), which receives both inputs from the type 1 and type 2 DA neurons (Ishino et al., 2023). At the same time, we recorded DA levels in ventrolateral part of NAc (vlNAc), the region primarily receiving inputs from type 1 DA neurons. Throughout the learning of the task, the DA levels in vlNAc were highest when the rats received the reward and lowest when they did not receive reward after the third lever manipulation, consistent with typical RPE signaling. The DA levels in dNAc at the beginning of the learning were similar to the DA levels in vlNAc, consistent with much stronger inputs from type 1 DA neurons than type 2 DA neurons. However, as the learning proceeded, the DA level in dNAc became highest at the time of no-reward after the first manipulation and lowest at the time of the reward receipt. These results suggest that DA levels in dNAc signal prediction errors about the amount of effort remaining to eventually obtain reward and reinforce behavioral switching toward future reward pursuit to overcome the lack of expected reward.

Disclosures: **M. Ogawa:** None. **R. Tsukasa:** None. **S. Ishino:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.10/PP3

Topic: G.03. Motivation

Support: P50DA044121
ASAP-020600
R01-NS119690
R01-MH110556

Title: A quantitative projection map of molecularly defined midbrain DA populations

Authors: ***O. A. MORENO-RAMOS**[1], D. D. RAJ[1], A. VIGOTSKY[2], M. NISSAN[1], E. P. PHELAN[1], J.-F. POULIN[4], A. V. APKARIAN[5], D. A. DOMBECK[3], R. AWATRAMANI[1]; [1]Neurol., [2]Northwestern Univ., Northwestern Univ., Chicago, IL; [3]Northwestern Univ., Northwestern Univ., Evanston, IL; [4]McGill Univ., McGill Univ., Montreal, QC, Canada; [5]Northwestern Univ. Feinberg Sch. of Med., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Midbrain dopamine (mDA) neurons display a plethora of functions, including movement, reward learning, and motivation. The diverse functions of mDA neurons can be explained partially by their intrinsic heterogeneity shown recently using single-cell RNA sequencing. We sought to examine whether molecular heterogeneity correlates with anatomical projection differences. Recently, using markers derived from such single-cell data, our lab has shown a topographic projection mapping of molecularly distinct midbrain DA neurons from the Substantia Nigra pars compacta (SNc) and the Ventral Tegmental Area (VTA), using different intersectional genetic labeling strategies. We have revealed distinct genetically defined DAergic projection patterns to the caudate putamen, nucleus accumbens, amygdala, and medial prefrontal cortex. Moreover, functional studies on mDA projections in the striatum usually use anatomic references to identify specific roles. Sometimes, independent studies show conflicting roles in the same anatomical region, which we posit arises from overlapping subtype projections in the exact anatomical location. Therefore, our new study seeks to provide a systematic quantitative anatomic characterization of genetically defined mDA subtypes in the striatum from SNc and VTA. To do so, we 1. Utilized intersectional and subtractional (complementary) reporter viruses injected in SNc or VTA to determine the exclusivity of projections 2. Used new driver mouse lines based on newer single-cell taxonomic schemes, allowing greater access to specific subpopulations of DA neurons, and 3. Developed an axonal density quantification method to parcellate the striatum according to the accessed mDA projections. Our work suggests that the striatum displays highly regionalized stereotypic mDA projection domains. This projectome will

serve as a guiding resource to understand better circuitry, functional roles of mDA neurons, and manipulation of specific mDA subtypes.

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Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.11/PP4

Topic: G.03. Motivation

Title: Pain is so close to pleasure: the same dopamine neurons can mediate approach and avoidance in Drosophila

Authors: *L. GUYTON[1], C. ROHRSEN[1], A. KUMPF[1], K. SEMIZ[1], F. AYDIN[1], B. DEBIVORT[3], B. BREMBS[1], *L. GUYTON[2];
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Abstract: In mammals, dopamine is considered a central neuromodulator involved in all kinds of rewarding experiences (‘common currency’ hypothesis). In insects, the role of dopaminergic neurons in aversive stimuli was discovered before dopaminergic neurons were found to also be involved in processing appetitive stimuli. Here, we screened about 50 transgenic Drosophila lines, representing different subpopulations of dopaminergic neurons for their ability to sustain approach or avoidance behavior, when activated optogenetically in four different operant self-stimulation paradigms. None of the lines we screened sustained consistent behavioral valence in all experiments. Individual lines sustained approach in one experiment and avoidance in another, while some lines mediated varying behaviors of the flies over time. One line mediated strong avoidance early in the experiment and weak approach in later stages. The evidence presented here appears to contradict a ‘common currency’ dopamine function in flies. Instead, different dopaminergic neurons convey valence in a context-dependent and flexible manner, reflecting the genetic heterogeneity of the dopaminergic neuronal population.

Disclosures: L. Guyton: None. **C. Rohrsen:** None. **A. Kumpf:** None. **K. Semiz:** None. **F. Aydin:** None. **B. deBivort:** None. **B. Brembs:** None. **L. Guyton:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.12/PP5

Topic: G.03. Motivation

Support: Appalachian Colleges Association Professional Leave Fellowship
University of the South Faulty Research Grant
University of the South Undergraduate Research Mentoring Grant
Sewanee Undergraduate Research Fellowship

Title: The dopamine receptor antagonist haloperidol disrupts behavioral responses of both sea urchins and sea stars

Authors: E. HOWELL, A. R. LANCASTER, J. BESH, A. B. RICHARDSON, E. GOMEZ, S. A. HARNEW-SPRADLEY, ***C. SHELLEY**;
Biol., Univ. of the South, Sewanee, TN

Abstract: Sea urchins and other echinoderms occupy an unusual position in animal neurobiology as they lack a brain or a brain-like structure, but phylogenetically they are more closely related to vertebrates than virtually all other invertebrates. Despite the lack of a brain and having an apparently symmetrically pentaradial nervous system, echinoderms are capable of complex, coordinated directional behavioral responses to different sensory stimuli. However very little is known about the molecular and cellular mechanisms underlying these behaviors in adult echinoderms. In many animals, dopaminergic systems play key roles in motivating and coordinating behavior and although the dopamine receptor antagonist haloperidol has been shown to inhibit the righting response of the sea urchin *Strongylocentrotus purpuratus* with an IC50 of 11 µM, it is not known if this inhibition is specific to this behavior, in this species, or whether dopaminergic systems are needed in general for echinoderm behaviors. We found that the dopamine receptor antagonist haloperidol inhibited multiple different behavioral responses in three different echinoderm species. Relative to vehicle alone, 100 µM haloperidol slowed the righting response of the sea urchin *Lytechinus variegatus* and of the sea star *Luidia clathrata* three-fold. It additionally reduced the lantern reflex frequency of *S. purpuratus* three-fold, reduced the shell covering response of *L. variegatus* three-fold, and slowed the immersion response of *L. variegatus* three-fold, but did not affect the immersion response of *S. purpuratus* or *L. clathrata*. Our results suggest that dopamine is needed for the neural processing and coordination of multiple different behavioral responses in a variety of different echinoderm species.

Disclosures: E. Howell: None. **A.R. Lancaster:** None. **J. Besh:** None. **A.B. Richardson:** None. **E. Gomez:** None. **S.A. Harnew-Spradley:** None. **C. Shelley:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.13/PP6

Topic: G.03. Motivation

Support: R01-MH117791

Title: Ventral tegmental area dopamine and orchestration of diverse fear conditioned behaviors

Authors: *A. CHU, S. ANZELLOTTI, E. L. RUSSELL, M. A. MCDANNALD;
Boston Col., Chestnut Hill, MA

Abstract: In Pavlovian fear conditioning, a neutral cue is paired with an aversive stimulus, such as foot shock. One result of this predictive relationship is the fear-conditioned cue will elicit defensive behaviors. The most commonly measured behavior is freezing, a 'passive' behavior defined by the absence of movement. Recently our laboratory has shown that a fear-conditioned cue can elicit 'active' defensive behaviors, characterized by rearing, jumping, and increased movement (Chu et al. 2022, in progress). The goal of the present study was to examine a role for ventral tegmental area dopamine neurons in organizing these diverse fear behaviors. To do so, we deleted ventral tegmental area (VTA) dopamine neurons and tested rats in a Pavlovian fear discrimination procedure. Subjects were 32, Th-cre rats (16 female). A Casp3 group received VTA dopamine neuron deletion via bilateral infusion of cre-dependent Caspase 3 (n=16, 8 female). A Control group received bilateral infusion of cre-dependent EYFP, leaving VTA dopamine neurons intact. Following recovery, rats received fear discrimination in a conditioned suppression setting. Rats were trained to nose poke for a food reward, then received 12 sessions in which a danger cue predicted foot shock while a safety cue did not. Poke-reward and cue-shock contingencies were independent. A TTL-triggered GiGE camera captured frames at sub-second resolution around cue presentation (5 s pre-cue, 10 s during cue, 5 s post-cue). Ethograms of cue-elicited behaviors spanning passive defensive, active defensive, and reward are constructed by hand scoring and using a convolutional neural network. We will present a complete histological and behavioral analyses revealing VTA dopamine's role in orchestrating diverse fear conditioned behaviors.

Disclosures: A. Chu: None. **S. Anzellotti:** None. **E.L. Russell:** None. **M.A. McDannald:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.14/PP7

Topic: G.01. Fear and Aversive Learning and Memory

Title: Nucleus accumbens dopamine receptor signaling in punished food seeking

Authors: *A. C. TOBUREN[1], G. M. JOYNER[2], P. VENTO[1];
[1]Psychology, [2]Univ. of South Carolina, Columbia, SC

Abstract: Addiction is characterized by deficits in punishment learning and impaired cost-benefit decision-making. The nucleus accumbens (NAc) has long been implicated in addiction-related behavior and plays major roles in motivation, reward-seeking, and associative learning. Within the NAc, dopamine D2 receptor expressing neurons have been shown to encode risky decision-making and activation in this neural subpopulation is associated with avoidance. Still, it remains unclear the manner in which activation of NAc dopamine receptor subtypes contribute to suppressing reward seeking under punishment. To test this, we implanted cannulae bilaterally in the NAc core and microinfused the D2 agonist, quinpirole, to simulate high dopamine states and whether that is sufficient to induce compulsive reward seeking in the presence of punishment (footshock). Specifically, rats were trained to lever press for a small (safe) reward or a large (punished) reward option that was immediately accompanied by brief footshock, to determine whether modulation of NAc D2 receptors alters decisions in whether to persist choosing the large, punished alternative or rather shift choices toward the safer, small reward alternative. Results show there was no significant difference between quinpirole (0.3µg or 1µg) and vehicle (0.5µl PBS) conditions in the amount of footshock rats were willing to endure to receive the large reward. This indicates that activation of NAc D2 receptors alone is not sufficient to induce compulsive reward seeking under punishment. Ongoing experiments are investigating whether activation of NAc D1 receptors, or a combination of D1/D2 activity is required to cause resistance to punishment in seeking of natural or drug rewards.

Disclosures: A.C. Toburen: None. **G.M. Joyner:** None. **P. Vento:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.15/PP8

Topic: G.01. Fear and Aversive Learning and Memory

Title: Stimulation of the rostromedial tegmental nucleus induces long lasting avoidance behavior

Authors: *J. WATSON[1], M. LOPEZ DE LEON[3], E. CARLSON[3], P. VENTO[2];
[2]Psychology, [1]Univ. of South California, Columbia, SC; [3]Psychology, Univ. of South Carolina, Columbia, SC

Abstract: Increased activity of midbrain dopamine neurons has been linked to approach and reinforcement learning; however, it is less understood how reductions in dopamine neuron activity relate to behavioral inhibition and avoidance. The rostromedial tegmental nucleus (RMTg) sends dense inhibitory projections to dopamine neurons in the ventral tegmental area (VTA), and inactivation of the RMTg > VTA circuit profoundly impairs punishment-induced suppression of reward seeking in rats. Recently, our lab found that optogenetic RMTg > VTA stimulation suppresses lever pressing (FR5) for food reward when delivered immediately after completion of each food-seeking trial. This stimulation-induced suppression of responding for food became more robust after repeated testing across 4 light-paired sessions, yet normal food

seeking resumed almost immediately after cessation of optical stimulation in three subsequent "recovery" sessions. To test whether more enduring effects of RMTg > VTA stimulation may persist when rats are given another food alternative, we developed a two-flavor choice paradigm where rats are trained to lever press (FR1) for two reward options (vanilla or chocolate flavored pellets) prior to undergoing optical stimulation paired with one of the two flavor choices. Here, we found that pairing RMTg > VTA stimulation with real-time responding for one of the two flavor options caused a rapid and robust shift to the opposite (non-paired) flavor that persisted for at least 10 sessions (over ~2 weeks) after stimulation was discontinued. Together, these data suggest an important role for the RMTg > VTA pathway in both behavioral inhibition and conditioned avoidance, with exciting implications for future interventions aimed at promoting adaptive decision-making.

Disclosures: J. Watson: None. **M. Lopez De Leon:** None. **E. Carlson:** None. **P. Vento:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.16/PP9

Topic: G.03. Motivation

Support: National Institute on Alcohol Abuse and Alcoholism Intramural Research Program Grant No. ZIA-AA000401 (to AH)
Postdoctoral Research Associate Training fellowship from the Center on Compulsive Behaviors (to PTP)

Title: Investigating the contributions of nucleus accumbens shell D1R and D2R spiny projection neurons to risky decision making.

Authors: *S. PERRY, R. SANDON VELIZ, K. CODEN, H. CHOI, N. SCHWAB, N. SPITZ, J. SCHAFFER, M. AUTHEMENT, P. PIANTADOSI, V. ALVAREZ, A. HOLMES;
Natl. Inst. of Alcohol Abuse and Alcoholism, Rockville, MD

Abstract: The ability to conduct cost-benefit analyses to guide actions is disturbed in neuropsychiatric conditions such as substance use and anxiety disorders. Although the neural basis for such dysfunction is unclear, activity in the nucleus accumbens shell (NAcSh) has been implicated in both adaptive and maladaptive action selection patterns. The NAcSh is composed primarily of spiny projection neurons (SPNs) expressing either the dopamine 1 receptor (D1R) or dopamine 2 receptor (D2R). These two largely distinct populations of projection neurons may work competitively to bias actions, yet how these neurons dynamically signal during complex decision making remains largely unknown. Here we characterized the function of NAcSh D1R and D2R SPNs during a "risky" decision making task whereby mice had to flexibly apportion their actions as a function of potential footshock punishment. Mice were initially trained in the absence of punishment to differentiate between two touchscreen windows, one that provided a

large volume of reward and another that provided a small volume. Mice were then exposed to a risky decision task (RDT), whereby a minor footshock was paired with selection of the large reward option across three discrete trial blocks of ascending shock probability (0, 50, 75%). Throughout training and testing, bulk GCaMP6m-based fluorescence was monitored in D1R or D2R SPNs using fiber photometry. Activity in each SPN population ramped prior to high value choices during safe decision making. During the RDT, pre-choice D1R SPN activity diminished as a result of punishment whereas D2R SPN activity was less affected. Surprisingly, optogenetic inhibition (using Halorhodopsin) of each population time-locked to the pre-choice period did not affect decisions. These findings suggest that, while SPNs encode aspects of reward-seeking and punishment, their activity is not required for task engagement or shifts in action-selection following punishment. We next evaluated how one prominent input to the NAcSh, the basolateral amygdala (BLA), affected D1R and D2R SPN activity and decision making. To address this, we combined fiber photometric recording of NAcSh SPN populations with BLA terminal inhibition (using Halorhodopsin) or excitation (using ChrimsonR). Preliminary data suggest that excitation or inhibition of BLA terminals in the NAcSh bidirectionally affected the activity of SPN populations. Interestingly, despite bidirectional effects on neural activity, both manipulations resulted in animals engaging in riskier decisions on the RDT. Ongoing experiments are attempting to uncover the complex ensemble dynamics of D1R and D2R SPNs using 1-photon imaging.

Disclosures: **S. Perry:** A. Employment/Salary (full or part-time):; National Institute of Alcohol Abuse and Alcoholism. **R. Sandon Veliz:** None. **K. Coden:** None. **H. Choi:** None. **N. Schwab:** None. **N. Spitz:** None. **J. Schaffer:** None. **M. Authement:** None. **P. Piantadosi:** None. **V. Alvarez:** None. **A. Holmes:** None.

Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.17

Topic: G.03. Motivation

Support: NIH Grant R01MH132019

Title: D1/d5 dopamine receptor agonists disrupt food consumption in a mouse effort-related choice task

Authors: T. VAN KRALINGEN[1], M. R. MASI[2,3], Y. LI[1], J. C. BARROW[1,2], *G. CARR[1,2]; [1]Drug Discovery and Develop., Lieber Inst. For Brain Develop., Baltimore, MD; [2]Pharmacol. and Mol. Sci., Johns Hopkins Univ., Baltimore, MD; [3]Drug Discovery and Develop., Lieber Inst. for Brain Develop., Baltimore, MD

Abstract: Activation of the D1 and D5 dopamine receptors with nonselective agonists has been shown to modulate feeding behavior in multiple experimental systems. Although D1/D5 agonists

have been tested in combination with D1/D5 antagonists in effort-related choice paradigms, to our knowledge, D1/D5 agonists have not been tested in a concurrent fixed-ratio/chow effort-related choice (ERC) task. The mouse ERC task utilized in this study provides the opportunity for food-restricted mice to earn a high value food reinforcer (strawberry milk) through a fixed-ratio 4 (FR4) schedule or consume freely available standard chow. We tested the effects of the D1/D5 agonist SKF 83959 (3, 6, 10 mg/kg) on consumption in the ERC task. We found that SKF 83959 (all doses) significantly decreased consumption of both the strawberry milk and chow, consistent with the previously reported appetite-suppressing effects of other D1/D5 agonists in different feeding assays. We are currently investigating whether structurally distinct D1/D5 agonists produce effects similar to SKF 83959 in the ERC task and whether the effects of D1/D5 nonselective compounds can be attributed to D1 activity, D5 receptor activity, or whether activation of both is required.

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Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.18/PP10

Topic: G.03. Motivation

Title: Gaba/glutamate co-transmission stabilizes network output and is abundant in the rodent and primate habenula

Authors: *L. RIOS[1], I. CHAMBERS[1], N. RODRIGUEZ SOSA[5], Y. SHARMA[1], A. HAJEISSA[1], R. RAJESH[1], V. NARLA[1], N. WANG[6], C. TAN[2], K. GLEASON[1], C. A. TAMMINGA[3], S. SHABEL[4];
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Abstract: The lateral habenula (LHb) responds bidirectionally to aversive and rewarding stimuli, and its hyperactivity is hypothesized to contribute to depression. Studies in rodents showed that inputs from the basal ganglia and ventral tegmental area to the lateral habenula co-release GABA with glutamate; the balance of GABA and glutamate at these synapses may regulate LHb activity and plasticity in downstream circuits. In the present study, we investigated whether the magnitude of co-release of GABA and glutamate in the LHb is conserved in primates and how simulated GABA/glutamate co-release affects output in neural networks. Our data indicate that the majority of GAD-expressing synaptic terminals in the mouse, rat, monkey, and human LHb also express Vglut2, consistent with abundant and conserved co-release of GABA and glutamate from individual terminals onto LHb neurons. Simulations showed that, in addition to reducing neuronal output, co-release of GABA with glutamate also stabilizes neuronal output over a wide

range of input activity - an effect which appears to depend on the spatial, but surprisingly not the temporal, overlap of GABA/glutamate co-release from individual terminals. Increased activity from synchronized, but not unsynchronized, GABA/glutamate co-releasing inputs increased network output. These data suggest that GABA co-release in excitatory inputs stabilizes the basal activity of mammalian LHb neurons, thus preventing maladaptive plasticity in downstream circuits, while also allowing changes in activity from synchronized inputs to propagate and induce adaptive plasticity.

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Poster

PSTR362. Dopamine and Reward

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR362.19/PP11

Topic: G.03. Motivation

Support: K08MH110610

Title: Single Session Behavioral Economic Analysis Reveals Asymmetry of Social Reward Learning in Mice

Authors: *G. RAMIREZ OVALLE[1], T. HIETAMIES[2], N. ESHEL[3], R. C. MALENKA[5], B. D. HEIFETS[4];
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Abstract: Social motivation deficits are at the center of several neuropsychiatric disorders. Yet, there is no translational assessment to quantify social motivation and test the impact of novel therapies. Here we combine Behavioral-Economic Demand (BED) analysis and Social Operant Conditioning (SOC) to extract social motivation (the intrinsic value of social reward and the effort expended to obtain that reward). Previous BED research has successfully extracted intrinsic values of rewards (e.g. drugs) in human and animal models, but not for social interaction. Previous SOC research has used multi-day testing at discrete Fixed Ratios (FR) of social interaction, an approach that allows limited assessment of effort, motivation, and effects of single drug doses. Here we present a single session method to quantify social motivation and show that social motivation can be bidirectionally modulated. We used 20 female C57BL/6J in-house bred adult mice. All underwent 2 weeks of isolation at 8 weeks old before social magazine training. Mice learned to activate the nose poke for temporary access to a sex-matched conspecific stranger mouse (group housed), then perform single separate sessions at FR-1 and FR-5, and finally single sessions with FR 1,3,5,10 and 30. Subjects were singly housed and met different partners in each session to conserve the novelty effect. We verified that the amount of

social self-administration was similar between multi-day and single day sessions ($T=1.8_{19}$, $P=0.09$ and $T=0.24_{19}$, $P=0.8$, respectively). Using Hurst and Roma (2016) equations, we generated BED curves and calculated the essential value (motivation) of social self-administration. Compared to single test/partner mouse pairs, social motivation was enhanced by a single session where test mice were paired with groups of partner mice ($T=4.0_{14}$, $P=0.001$). In contrast, multiple sessions of pairing with an inanimate object (toy) were required to reduce social motivation (mixed-effects: $F=15.7_{4,77}$,$P<0.0001$). Finally, social motivation was quickly reinstated to high levels after extinction, when a mouse was put back in the partner's side. We demonstrate here a translational, and efficient method to assess social consummatory behavior, expended effort, and estimated intrinsic social reward value in mice using an in-house developed behavioral apparatus. Social motivation modulation is asymmetric, requiring a single session in upward modulation and several sessions in downward modulation.

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Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.01/PP12

Topic: G.04. Emotion

Support: CNU 2017-0376-02
NRF 2020M3E5D9080734
NRF 2020R1A2C2102134
NIH Grant P30 GM103328

Title: Selenbp1 over-expression in the prefrontal cortex underlies negative symptoms of schizophrenia

Authors: *S. S. KIM[1], S.-W. KIM[1], M. BUI[1], Y. KIM[2], M. KIM[1], J.-C. PARK[3], N.-H. KIM[3], G. PYEON[4], Y. S. JO[4], J. JANG[5], H.-Y. KOH[5], C.-H. JEONG[1], M. KANG[1], H. KANG[6], C. A. STOCKMEIER[7], J. SEONG[8], D. WOO[2], J.-S. HAN[3], Y.-S. KIM[1];
[1]Chungnam Natl. Univ., Deajeon, Korea, Republic of; [2]Korea Inst. of Toxicology, Daejon, Korea, Republic of; [3]Konkuk Univ., Seoul, Korea, Republic of; [4]Korea Univ., Seoul, Korea, Republic of; [5]Korea Inst. of Sci. and Technol. (KIST), Seoul, Korea, Republic of; [6]Chung-Ang Univ., Seoul, Korea, Republic of; [7]Univ. of Mississippi Med. Ctr., Jackson, MS; [8]Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: The selenium-binding protein 1 (SELENBP1) has been reported to be upregulated in the prefrontal cortex (PFC) of schizophrenia patients in postmortem reports. However, no causative link between SELENBP1 and schizophrenia has yet been established. Here, we provide evidence linking the upregulation of SELENBP1 in the PFC of mice with the negative symptoms

of schizophrenia. We verified the levels of *SELENBP1* transcripts in postmortem PFC brain tissues from patients with schizophrenia and matched healthy controls. We also generated transgenic mice expressing human SELENBP1 (hSELENBP1 Tg) and examined their neuropathological features, intrinsic firing properties of PFC 2/3-layer pyramidal neurons, and frontal cortex (FC) electroencephalographic (EEG) responses to auditory stimuli. Schizophrenia-like behaviors in hSELENBP1 Tg mice and mice expressing *Selenbp1* in the FC were assessed. *SELENBP1* transcript levels were higher in the brains of patients with schizophrenia than in those of matched healthy controls. The hSELENBP1 Tg mice displayed negative endophenotype behaviors, including heterotopias- and ectopias-like anatomical deformities in upper-layer cortical neurons and social withdrawal, deficits in nesting, and anhedonia-like behavior. Additionally, hSELENBP1 Tg mice exhibited reduced excitabilities of PFC 2/3-layer pyramidal neurons and abnormalities in EEG biomarkers observed in schizophrenia. Furthermore, mice overexpressing *Selenbp1* in FC showed deficits in sociability. These results suggest that upregulation of SELENBP1 in the PFC causes asociality, a negative symptom of schizophrenia.

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Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.02/Web Only

Topic: G.04. Emotion

Title: The Alexisomia and Alexithymia among Patient with Schizophrenia Spectrum and Other Psychotic Disorders (F2)

Authors: *G. TUMUR-OCHIR[1], T. URTNASAN[2], G. JAVZANDULAM[1], E. BATKHUYAG[3], B. VANCHINDORJ[1];
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Abstract: Background: Schizophrenia is a severe mental disorder characterized by disturbances in perception, thought processes, and emotional expression. However, the essential nature of schizophrenia remains to be fully clarified. Two emotional constructs, alexithymia and alexisomia, have gained attention in schizophrenia research. This study aims to explore the association between alexithymia and alexisomia in patients with Schizophrenia Spectrum and Other Psychotic Disorders (F2), emphasizing their impact on emotional experiences and social functioning. **Methods:** A hospital-based cross-sectional study was conducted among psychiatric patients with F2 diagnoses in the treatment wards of the National Center for Mental Health from April to July 2022. The level of alexisomia and alexithymia was assessed using the Shitsu-

Taikan-Sho Scale (STSS) and the Toronto Alexithymia Scale (TAS-20). Pearson's correlation analysis was performed to detect associations between variables. Statistical analysis was conducted using SSPS 21.0. **Results:** A total of 68 patients diagnosed with F2, aged 20-66 years (mean age: 42.05±12.25), with 51.5% being male, were recruited for the study. The STSS total score was 57.0±12.4 points, with subscale scores of DIB, OA, and LHM being 23.5±7.5, 15.9±5.4, and 17.5±4.8 points, respectively. The TAS-20 total score was 55.1±5.8 points, and subscale scores for DDF, DIF, and EOT were 14.4±2.7, 16.3±4.8, and 24.5±3.0 points, respectively. A strong positive correlation was observed between the total scores of alexisomia and alexithymia ($r = 0.636$, $p < 0.01$). The subscales DIB and OA were significantly positively correlated with DDF and DIF (DIB $r = 0.521$, $r = 0.497$, $p < 0.001$; OA $r = 0.343$, $p < 0.01$, $r = 0.246$, $p < 0.05$, respectively). The subscale LHM was correlated only with subscale DIF ($r = 0.367$, $p < 0.05$). **Conclusions:**This study represents the first attempt, to our knowledge, to assess the level of alexisomia and alexithymia in a clinical population in Mongolia. Significant moderate to strong correlations were observed between the subscales of STSS and TAS-20. Further research is needed to elucidate the complex relationship between alexithymia and alexisomia in schizophrenia and determine the underlying neurobiological mechanisms. Longitudinal studies examining the impact of these constructs on treatment response and functional outcomes are warranted. Addressing alexithymia and alexisomia in schizophrenia patients may lead to more comprehensive and personalized interventions to improve emotional well-being and social integration in this population.

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Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.03/PP13

Topic: G.04. Emotion

Title: Mouse Models of Autism Demonstrate Deficits in Empathy

Authors: S. TUY[1,2], P. REZAIE BOROON[1], M. ISKAKOVA[1], G. SINGH[1], C. WU[1,3], M. THOMAS[4], B. A. REIN[5], R. C. MALENKA[5], *M. L. SMITH[1];
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Abstract: The ability to empathize is critical to social communication and refers to the ability to understand a social partner’s sensory and emotional state. Autism Spectrum Disorder (ASD) is commonly associated with deficits in social communication, often deeming those with ASD as unempathetic. However, little is known about the behavioral and neural mechanisms contributing to ASD-related deficits in distinct forms of empathy. We recently developed the “social transfer”

model of empathy in the mouse, where “bystander” mice rapidly adopt a social partner's sensory and affective state, a key component of empathy. During the social transfer of pain, bystander mice socially interact with a familiar mouse experiencing pain (due to a hind paw injection of Complete Freunds Adjuvant—CFA). Remarkably, after a 1-hour social interaction, bystanders demonstrate pain behavior matching that of CFA-injected mice, including hypersensitivity and behavioral despair. In the current studies, we investigated whether mouse strains used to model ASD (BALB/C, BTBR, Shank3 KO) acquire the social transfer of pain. BALB/C, BTBR, and Shank3 KO mice did not demonstrate any behavioral changes following a 1-hour social interaction with a conspecific in pain, indicating a deficit in empathy-like behavior. In the future, we aim to further characterize behavioral empathy deficits in ASD mouse models, investigate the underlying neural circuit mechanisms, and explore methods to rescue empathy in ASD models.

Disclosures: S. Tuy: None. **P. Rezaie Boroon:** None. **M. Iskakova:** None. **G. Singh:** None. **C. Wu:** None. **M. Thomas:** None. **B.A. Rein:** None. **R.C. Malenka:** None. **M.L. Smith:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.04/PP14

Topic: D.05. Auditory & Vestibular Systems

Support: MH111684

Title: Dissecting the Molecular Mechanisms Underlying Epilepsy Aphasia Syndrome

Authors: *K. HOSSAIN;
Duke Univ., Durham, NC

Abstract: Dissecting the Molecular Mechanism Underlying Epilepsy Aphasia Syndrome Kazi Hossain, Scott Soderling Epilepsy Aphasia Syndrome (EAS) are a spectrum of childhood disorders which include epilepsy as well as cognitive and language disorders. CNKSR2 is an X-linked gene involved in EAS. Novel work involving a Cnksr2 KO mouse line developed in our laboratory models’ aspects of EAS present in clinical patients with mutations to the gene. We have shown that deletion of CNKSR2 in mice causes increased neuronal activity, impaired learning and memory and seizures. Mutations also lead to increased levels of anxiety as well as loss of ultrasonic vocalizations (USV). Previously we have shown that Cnksr2 is expressed in the cortex, striatum, and cerebellum, with localizations in excitatory and inhibitory post synapses. Here we demonstrate that temporal and brain specific deletion of Cnksr2 in excitatory neurons lead to loss of USVs and increased levels of anxiety in male mice. Retro orbital deletion of CNKSR2 during postnatal day 21 in CamKII positive cells does not induce anxiety but leads to the loss of USVs. Also brain regional deletion of CNKSR2 in excitatory cells within the cortex and hippocampus leads to the loss of USVs and elevated levels of anxiety. We also delete CNKSR2 within excitatory cells of the Anterior Cingulate Cortex (ACC) and observe an effect

on song initiation in adult mice. Together our results provide evidence that deletion of CNKSR2 in the cortex is sufficient to drive anxiety and loss of USVs in adult male mice. ACC targeted deletion also separates USVs and anxiety. These findings further help us understand the role of CNKSR2 mutations within EAS.
CNKSR2_EMX_CreA1: Soluble Turbo IDA2: Soluble Turbo IDA3: Soluble Turbo IDB1: CNKSR2 TurboIDB2: CNKSR2 TurboIDB3: CNKSR2 TurboIDCNKSR2_Gad2_CreC1: CNKSR2 Turbo IDC2: CNKSR2 TurboIDC3: CNKSR2 TurboIDS1: Soluble Turbo IDS2: Soluble Turbo IDS3: Soluble Turbo IDdetergents: SDS, no bromophenol blueglassware exposed to detergents: trueph unknownestimation method: wild guesspurification method: magnetic streptavidin pulldown expected complexity: more than 20 proteins

Disclosures: K. Hossain: None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.05/Web Only

Topic: G.01. Fear and Aversive Learning and Memory

Support: Japan Science and Technology Agency (JST) Presto (18068712)
Japan Science and Technology Agency (JST) Moonshot (20343198)
Japan Society for the Promotion of Science (JSPS), Grant-in-Aid for Scientific Research (B) (18H02714 and 22H01111)

Title: Fear in action: Fear conditioning and alleviation through body movements

Authors: *M. ALEMANY[1], M. E. WOKKE[2], T. CHIBA[3], T. NARUMI[4], N. KANEKO[5], H. YOKOYAMA[5], K. WATANABE[6], N. KANEKO[5], K. NAKAZAWA[5], H. IMAMIZU[5], A. KOIZUMI[1];
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Abstract: Acquisition of fear memories enhances survival especially when the memories guide defensive movements to minimize harm. Accordingly, fear memories and body movements have tight relationships in animals: Fear memory acquisition results in adapting reactive defense movements, while training active defensive body movements to avoid threat reduces fear memory. However, evidence in humans is scarce because their movements are typically marginalized in experiments. In the present study, we tracked participants' whole-body motions while they underwent fear conditioning in a virtual 3D space. First, representational similarity analysis of body motions revealed that participants obtained distinct spatiotemporal movement

patterns through fear conditioning. Second, subsequent training to actively avoid threats with naturalistic defensive actions led to a long-term (24 hrs) reduction of physiological and embodied conditioned responses, while passive extinction or vicarious training only transiently reduced the responses followed by their spontaneous return. Together, our results highlight the intrinsic role of body movements in human fear memory functions, suggesting the potential for improving fear memory interventions through embodiment.

Disclosures: **M. Alemany:** None. **M.E. Wokke:** None. **T. Chiba:** None. **T. Narumi:** None. **N. Kaneko:** None. **H. Yokoyama:** None. **K. Watanabe:** None. **N. Kaneko:** None. **K. Nakazawa:** None. **H. Imamizu:** None. **A. Koizumi:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.06/PP15

Topic: A.05. Axon and Dendrite Development

Support: NIH Grant R01MH115466
NIH Grant UL1 TR001857

Title: Exploring the link between white matter microstructure and infant emotional regulation

Authors: ***Y. ZHANG**[1], L. BANIHASHEMI[1], A. VERSACE[1], A. SAMOLYK[1], M. TAYLOR[1], G. ENGLISH[1], V. J. SCHMITHORST[2], V. K. LEE[2], R. STIFFLER[1], H. ASLAM[1], A. PANIGRAHY[2], A. E. HIPWELL[1], M. L. PHILLIPS[1];
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Abstract: Low soothability can be assessed in infancy, which indicates emotional regulation capacity and can predict future aggression, disruptive behavioral disorders, and deficits in social engagement. White matter tracts undergo critical development in the first years of life. Therefore, during this period, the microstructural characteristics of major white matter tracts that connect emotion-related cortical and/or subcortical regions, including the forceps minor (FM), cingulum bundle (CG), and uncinate fasciculus (UF), are potential markers for early emotional regulation capacity. In this study, we aimed to identify the relationships between the neurite density index (NDI) and orientation dispersion index (ODI) of these tracts with soothability in 3-month-old infants. Multishell diffusion MRI was collected in 50 3-month-old infants and NDI and ODI were estimated using the NODDI Matlab toolbox. Mean NDI and ODI were extracted from the FM and the left and right CG and UF using DSI Studio through a tract-based approach and were independently modeled with the 3-month Soothability subscale of the Infant Behavior Questionnaire. Sociodemographic and clinical factors (infant age and biological sex; caregiver age, socioeconomic status, parental depression, affective instability, and anxiety) were included as covariates for both the infant and caregiver. Both the left UF NDI and ODI were negatively correlated with 3-month soothability ($\beta = -0.321$, $p = 0.023$; $\beta = -0.521$, $p < 0.001$). Greater

neurite density and dispersion of the left UF in 3-month-old infants were associated with impaired soothability. We suggest that a greater microstructural complexity of the left UF in infancy, probably caused by insufficient pruning, can lead to reduced efficiency of signal transmission among the lateral orbitofrontal cortex, amygdala, and anterior temporal lobe, thus contributing to lower soothability. Previous studies reported infants with greater fractional anisotropy or fiber density in the left UF show higher risk of developing autism spectrum disorders, supporting our findings that greater NDI is associated with impaired emotional regulation capacity. These findings indicate that microstructural characteristics of infant white matter tracts are potential indicators of future psychopathology.

Disclosures: Y. Zhang: None. **L. Banihashemi:** None. **A. Versace:** None. **A. Samolyk:** None. **M. Taylor:** None. **G. English:** None. **V.J. Schmithorst:** None. **V.K. Lee:** None. **R. Stiffler:** None. **H. Aslam:** None. **A. Panigrahy:** None. **A.E. Hipwell:** None. **M.L. Phillips:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.07/PP16

Topic: C.01. Brain Wellness and Aging

Title: Examining the Effects of Social Reintroduction on Neuropathology in 129s Mice on Account of Social Isolation

Authors: *A. H. YOUNG, M. LAMARCHE, N. ZABALA, J. M. FLINN;
Psychology, George Mason Univ., Fairfax, VA

Abstract: Social isolation is prevalent among the elderly and disabled, which negatively impacts cognition and behavior. Whether these deficits continue after social reintroduction remains an open question. We used 129s strain mice (n=63) to investigate the longevity of this damage. This strain was chosen to prevent aggression. The cohort was first divided into two conditions: group housed (n=20) with 3 members of the same sex and socially isolated (n=43). After 8 weeks of isolation, the social isolation condition was then split into two conditions: continually socially isolated (n=22) and socially reintroduced (n=21), producing 3 total conditions. Behavioral tests including Nesting, Burrowing, Morris Water Waze (MWM), Open Field (OF), Elevated Zero Maze (EZM), and Circadian Rhythm (CR) were run at 12 and 20 weeks for all conditions. Nesting, Burrowing, OF, and EZM were analyzed using a two-way ANOVA. MWM and CR were analyzed using repeated measures ANOVA. The 12-week OF test yielded a significant result between housing conditions for distance-traveled, p=.041. Group housed mice (M=2769.9, SD=2005.3) traveled farther than isolated mice (M=1766.9, SD=1688.9). Significant results were found at the 12-week CR analysis for both the sex, p=.003, and housing, p=.010, conditions. Group housed mice displayed less disruption than isolated mice, and males displayed less circadian disruption compared to females. EZM yielded significant results at the 20-week

measurement for head-dips in the housing condition, *p*=.023. Reintroduced mice performed better (M=8.6, SD=2.8) than isolated mice (M=4.3, SD=3.4). Entries-into-open-arms also yielded a significant effect between housing conditions, *p*=.009. Reintroduced mice showed higher entries-into-open-arms (M=7.6, SD=3.3) than isolated mice (M=3.9, SD=1.6). Significant sex differences were found for percentage-of-time-spent-in-open-arms, with males (M=61.5, SD=32.1) spending more time than females (M=19.4, SD=8.9), *p*=.024. No other significant sex differences were found in this study. These results indicate that social reintroduction may mitigate neurological deficits, particularly increased anxiety, and circadian disruption, caused by isolation. The significance of sex differences indicate that male mice may be less vulnerable to deficits caused by social isolation. These results demonstrate the need to provide social enrichment to individuals prone to isolation, like the elderly and disabled.

Disclosures: **A.H. Young:** None. **M. Lamarche:** None. **N. Zabala:** None. **J.M. Flinn:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.08/PP17

Topic: G.04. Emotion

Support: NIH Grant R01

Title: Investigating empathy behavior associated ensembles using SHANK3 autism mouse model

Authors: *S.-N. QIAO[1], Y.-H. JIANG[2];
[2]Dept. of Genet., [1]Yale Univ., New Haven, CT

Abstract: Empathy is defined as that an individual can percept other's mental state and respond with spontaneous and appropriate emotion or action. Empathy is an essential social behavior across mammal species. Aberrant empathy is considered as one of the hallmarks for autism spectrum disorder (ASD) and other psychiatric disorders. Studies from both human and rodents have suggested that anterior cingulate cortex (ACC) and basomedial amygdala (BLA) associated ensembles are implicated in empathy. However, the molecular and circuit mechanisms underlying remain largely unknown. *SHANK3* is one of the most replicated causative ASD genes from ASD genomics studies. Our lab has developed and characterized the first *Shank3* complete deletion model, *Shank3* $^{\Delta e4\text{-}22}$ mouse. This mouse model recapitulates the core behavioral features of ASD including abnormal social behaviors. We hypothesized that *Shank3* $^{\Delta e4\text{-}22}$ ASD model has impaired empathy which is due to *Shank3* deficits in ACC-BLA circuit. We used fear transfer behavioral assay to assess the empathy behavior in *Shank3* $^{\Delta e4\text{-}22}$ (KO) mouse mode. The pair of an observer mouse (**OB**) and a wild-type (WT) demonstrator mouse (**DE**) are placed in a fear-conditioning chamber whereas only DE given electric shocks and OB observing the process. Then OB mice are assayed 24 hours later in the same chamber. Unexpectedly, we found that KO

OB mice are significantly freezing longer than WT OB mice. WT OB mice significantly increased freezing at the following day but KO OB mice did not. It suggested that *Shank3* deficiency impaired affective empathy. This atypical empathy phenomenon in KO OB mice is persistent when: 1) lacking social familiarity between OB and DE and earlier shocking experience of OB, 2) using tone-cued fear transfer paradigm. Interestingly, the male mouse pairs showed more severe phenotype than female. Further conditional inactivation of *Shank3* specifically in ACC via Cre virus in *Shank3*$^{\Delta e4\text{-}22}$ floxed mice resulted in less freezing time. In contrast, conditional deletion of *Shank3* in BLA leads to increased freeze time. Moreover, the *in vivo* Ca imaging showed that *Shank3* deficient neurons in ACC are more hyperactive than WT at baseline level. But during fear transfer process, the ACC neuron activity in KO mice is lower compared to WT. Together, it support a critical role of *Shank3* in ACC-BLA circuit driven fear transfer in ASD model. *Shank3* deficient mice could offer a unique opportunity to dissect the neural ensembles responsible for impaired empathy in ASD with cellular resolution. Future plan includes elucidating the circuit mechanism underlying and offer a foundation for the circuit based behavioral treatment for ASD.

Disclosures: S. Qiao: None. **Y. Jiang:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.09/PP18

Topic: G.04. Emotion

Support: CIC/ SCIENTIFIC RESEARCH COORDINATION

Title: Anxiety: oral consumption of clonazepam & ethanol after reward devaluation

Authors: *L. MANZO[1], A. C. TAFOLLA[2,3], R. A. VEGA[2], S. JAIMES[2];
[1]Univ. Michoacana De San Nicolas De Hidalgo, Morelia, Mexico; [2]Univ. Michoacana de San Nicolas de Hidalgo, Morelia, Mexico; [3]Univ. Latina de America, Morelia, Mexico

Abstract: Anxiety is an adaptive response which detects and protects an individual against danger. Rats increased preference for ethanol after sessions of appetitive extinction, but no after acquisition (reinforced) sessions (Manzo et al., 2014). Drinking was not influenced by appetitive extinction in control groups with postsession access to water rather than ethanol. Because ethanol has anxiolytic properties in tasks involving reward loss, these results were interpreted as anti-anxiety self-medication. The present experiment tested the potential for self-medication with the prescription anxiolytic clonazepam a benzodiazepine with an addictive profile used in anxiety disorders. To test this hypothesis, Wistar male rats, exposed to a 32% to 4% sucrose devaluation received a two bottle, 2-h preference test immediately after consummatory training. Were given a two bottles, one bottle contained 1 mg/kg of clonazepam, 2% of ethanol, or water for different groups (the second bottle contained water for all groups).Three additional groups received the

same postsession preference tests, but were always exposed to 4% of sucrose during consummatory training. Rats showed suppression consummatory behavior after reward devaluation relative to unshifted controls. This effect was accompanied by a selected increase in both ethanol and clonazepam oral intake during the initial downshift sessions. Downshifted animals with access to water or unshifted controls with access to the anxiolytics failed to exhibit any changes in preference during the postsession changes in preference. Similar results were observed in terms of absolute consumption and relative consumption to body weight. These results sowed for the first time that a prescription anxiolytic supports enhanced voluntary consumption during periods of emotional distress triggered by reward loss. These results support the hypothesis that anti-anxiety self-medication may provides insights into a better understanding the early stage of addictive behavior.

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Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.10/PP19

Topic: G.04. Emotion

Support: JSPS KAKENHI 21K06440
JSPS KAKENHI 21H05817
JSPS KAKENHI 20H05955
JSPS KAKENHI 17KK0190
LOTTE Foundation
Mishima Kaiun Memorial Foundation
Takeda Science Foundation

Title: Identification of subtypes of ultrasonic vocalizations associated with chocolate eating in rats using machine learning

Authors: *K. MURATA[1], Y. IKEDO[1], T. RYOKE[1], K. SHIOTANI[2], H. MANABE[3], K. KURODA[1], H. YOSHIMURA[1], Y. FUKAZAWA[1];
[1]Univ. of Fukui, Eiheiji-cho, Japan; [2]Ritsumeikan Univ., Shiga, Japan; [3]Nara Med. Univ., Nara, Japan

Abstract: The measurement of emotional responses in experimental animals is essential for understanding the neural mechanisms underlying sensory perception. Pleasurable emotions associated with food play a crucial role in learning and motivation, particularly in relation to high-nutrient food. However, direct assessment methods for positive emotions in animals are still limited. Ultrasonic vocalizations (USVs) emitted by rats serve as an indicator of emotional states. Negative emotions, such as pain, elicit long 22 kHz USVs, while positive emotions, like social play and food anticipation, trigger short 50 kHz (30-100 kHz) USVs. In this study, we

examined the applicability of 50 kHz USVs as a measure of pleasurable emotions related to food consumption. Adult male Sprague-Dawley rat pairs were repeatedly presented with chocolate in a soundproof recording chamber. Their USVs were recorded by an ultrasound microphone (M500-384, Pettersson Elektronik) and detected by DeepSqueak. The number and spectrogram of 50 kHz USVs were compared between the 10-minute period before chocolate presentation (anticipatory phase) and the 10-minute period after chocolate presentation (consumption phase). The repeated chocolate presentation resulted in an increase in USVs during both the anticipatory and consumption phases. Regarding spectrogram, a decrease in frequency range during the consumption phase compared to the anticipatory phase was observed. Based on the USV data from the 10 rat pairs, a machine learning model using logistic regression was developed to differentiate between anticipatory and consumption phase USVs. Subsequently, another USV recording was performed using 22 rat pairs, divided into two groups: one receiving chocolate (11 pairs) and the other not (11 pairs). The USVs classified as 'consumption phase'-type occurred specifically during the post-chocolate presentation period in the 11 pairs receiving chocolate, while they were rarely observed in the absence of chocolate presentation. Furthermore, the spectrogram of the 'consumption phase'-type USVs predominantly exhibited an inverted U shape or flat shape in the 40 kHz frequency range. This study demonstrates the existence of USV subtypes associated with chocolate consumption and the ability to classify these subtypes using a machine learning model. Future neurobiological experiments should address whether the 'consumption phase'-type 40 kHz USVs can be served as an index of positive emotion accompanied by palatable eating.

Disclosures: K. Murata: None. **Y. Ikedo:** None. **T. Ryoke:** None. **K. Shiotani:** None. **H. Manabe:** None. **K. Kuroda:** None. **H. Yoshimura:** None. **Y. Fukazawa:** None.

Poster

PSTR363. Emotional Processing in Models of Disorder

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR363.11/PP20

Topic: G.04. Emotion

Support: JSPS KAKENHI 20H05955
JSPS KAKENHI 21H05817
JSPS KAKENHI 17KK0190

Title: Optimized recording and assessment system of ultrasonic vocalization for odontogenic pain in rats

Authors: *T. RYOKE[1], K. MURATA[2], Y. IKEDO[2], R. SAKAI[3], H. YOSHIDA[2], J. AKUTSU[2], M. SHIMADA[2], S. MATSUDA[2], K. KURODA[2], K. SANO[2], H. YOSHIMURA[2], Y. FUKAZAWA[1];
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Abstract: Odontogenic pain represents a globally common condition and stands as the most prevalent form of orofacial pain. Objective and quantitative evaluation of odontogenic pain is essential for understanding the pathophysiology of odontogenic pain and determining the effectiveness of treatments. Rats use ultrasonic vocalization (USV) in the 20-90 kHz range for social communication, with 22 kHz USV indicating negative emotions. USVs have been used as emotional indicators in disease models. However, the number of USV calls emitted in a particular recording condition can be influenced by multiple factors such as sociality (solo or group-housed) and the diurnal cycle. In this study, we established an efficient recording condition for USV recording by comparing solo or paired recording and evaluating the effect of the circadian rhythm. Then, we explored the utility of USV as an indicator of odontogenic pain using adult male Sprague-Dawley rats (8-12 weeks old). with experimental pulpitis. To investigate the influence of social interaction on USV emissions, we first fabricated a new USV recording chamber that enabled simultaneous and separated recording of a pair of rats. The two rats were separated by a transparent acrylic panel, and USVs emitted from individual rats were automatically identified by comparing the audio sound pressure at a particular time point recorded by two ultrasonic microphones separately placed over the recording chamber. USVs were recorded under two conditions: one where the rat was isolated and the other where cohabiting with a cage mate. We continuously recorded USVs for 24 hours consisted of the light (12 hours) and dark (12 hours) periods. To evaluate the effect of pulpitis-induced pain on USVs, we exposed the tooth pulp of a maxillary incisor in one of the paired rat and compared the number and phonetic features of USVs before and after the experimental pulpitis. Pulpitis was histologically confirmed by HE stains after USV recording. The number of USVs per rat was significantly higher in cohabiting condition with a cage mate than in the solo condition (n=4 for each condition, p=0.0312, t-test). The number of USVs during the dark period was higher than that during the light period (n=6). Collectively, we decided to record USVs in cohabited condition during the dark period for latter experiments. The number of 22 kHz USVs was increased in 4 out of 6 rats by the experimental pulpitis. These results suggest that 22 kHz USVs can be served as an indicator of odontogenic pain in rats, which enables noninvasive, objective, and quantitative evaluation of pain.

Disclosures: **T. Ryoke:** None. **K. Murata:** None. **Y. Ikedo:** None. **R. Sakai:** None. **H. Yoshida:** None. **J. Akutsu:** None. **M. Shimada:** None. **S. Matsuda:** None. **K. Kuroda:** None. **K. Sano:** None. **H. Yoshimura:** None. **Y. Fukazawa:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.01/PP21

Topic: G.05. Mood Disorders

Support: QU College of Arts and Sciences

Title: Assessing sex differences in inflammation-induced deficits in effort-related decision making

Authors: L. A. O'CONNOR[1], E. M. BISSELL[1], M. W. CHO[2], H. L. JACK[2], J. N. MILLER[2], T. M. PANTALENA[2], H. G. VAN BLARCOM[1], C. E. ZANESKY[2], *J. L. HAIGHT[2];
[1]Biol., [2]Psychology, Quinnipiac Univ., Hamden, CT

Abstract: Inflammation is associated with reductions in motivation that can be seen in human individuals with psychiatric illness, including mood disorders. Lipopolysaccharides (LPS) are molecules that induce inflammatory responses and have been used in animal models to further study the relationship between inflammation and mood disorders. Recently, it has been demonstrated that LPS-induced inflammatory responses can modify effort-related motivational behaviors in male rats, but female rats were not assessed. For this within-subjects pilot study, we explore the relationship between inflammatory responses induced by LPS and its effect on effort-related choice in both male and female rats. For this study, food restricted males (n=5) and females (n=6) were trained on an effort choice task in which the rats had to choose between consuming a freely available, low value food (rat chow), or exerting effort to work for a more palatable, high value reward (sucrose pellets). Trained rats were then tested using the same effort choice task following LPS or saline administration. Overall aggregate and sex difference analyses, as well as new time-course analyses, showed a similar reduction of lever pressing among both sexes following LPS administration. This change in behavior is consistent with reduced effort, demonstrating a negative correlation between inflammation and effort related behavior in both male and female rats.

Disclosures: **L.A. O'Connor:** None. **E.M. Bissell:** None. **M.W. Cho:** None. **H.L. Jack:** None. **J.N. Miller:** None. **T.M. Pantalena:** None. **H.G. Van Blarcom:** None. **C.E. Zanesky:** None. **J.L. Haight:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.02/PP22

Topic: G.05. Mood Disorders

Support: NIH R01MH051399
NIH R01MH129306
Hope for Depression Research Foundation

Title: Sexually divergent traits in approach/avoidance strategies transmute the psychedelic experience

Authors: *G. ROJAS[1], A. MINIER-TORIBIO[1], A. GODINO[1], T. MARKOVIC[1], T. LIN[1], A. WARREN[2], F. MARTINEZ-RIVERA[1], D. WACKER[2], E. NESTLER[1];

[1]Nash Family Dept. of Neurosci. and Friedman Brain Inst., [2]Dept. of Pharmacol. Sci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Sex is a biological variable that displays specific neurobehavioral features, including gene expression patterns, hormonal fluctuations, neurotransmission dynamics, and decision-making processes. For instance, while females preserve learned associations that prioritize avoiding negative outcomes over reward seeking, males exhibit lower sensitivity to adversity and greater sensitivity towards reward to maximize goal-directed approach. This ultimately leads to differential approach and avoidance (A/A) strategies with specific neurobiological contributions. The serotonin system has recently been implicated in A/A outcomes, and emerging studies highlight psychedelic drugs as a timely tool to manipulate these processes. In this study, we assess sexual divergence in decision-making during conflict in mice using the platform-mediated avoidance task (PMA) (Bravo-Rivera et al., 2014) to comprehensively assess active avoidance, reward approach, freezing (anxiety-related behavior), and learning and memory. Furthermore, as PMA extinction learning resembles exposure-based therapy, it illustrates the clinical relevance of our experiments. Thus, we combine the PMA extinction phase with adjunct exposure of the psychedelic drug LSD to investigate the contribution of the serotonin system in the extinction of A/A biases. Compellingly, only female mice are sensitive to LSD treatment. LSD prevents the extinction of avoidance (time on platform during tone) in females-- possibly by facilitating the prioritization of aversive memory association. Overall, these preliminary findings suggest a complex interaction of psychedelic action, sexual dimorphism, and A/A biases. Ongoing experiments using RNAscope and fiber photometry aim to reveal female/male differences in underlying neurotransmission dynamics in a sex and cell-specific manner. As the use of psychedelics continues to expand globally for therapeutic, recreational, and religious rituals, this work provides a neurobehavioral profile of sex-specific alterations in decision-making elicited by LSD, offering potential novel neurobiological targets to treat individuals with a history of maladaptive decision-making and psychedelic use.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.03/PP23

Topic: G.05. Mood Disorders

Support: NRF-2019M3C7A1031742
NRF-2020R1A2C2005868

Title: Validation of a modified sub-chronic variable stress animal model for studying sex differences in depression

Authors: *W. KIM, S. OH, C. CHUNG;
Biol. Sci., Konkuk Univ., Gwangjin-gu, Seoul, Korea, Republic of

Abstract: Sex influences the development of mood disorders; however, current animal models mostly focus on male rodents, neglecting sex differences. Sub-chronic variable stress (SCVS) is a depression model that has been reported to induce depressive symptoms only in female mice but occasionally in males in our laboratory. In this study, we modified the SCVS model by reducing stress intensity to validate behavioral changes by using various tests related to depression. We found that the modified SCVS induced depressive symptoms in female C57BL/6 mice, while both male and female mice displayed anxiety-like behavior in the novelty-suppressed feeding test. Notably, further analysis of the tail suspension test (TST) using K-mean cluster analysis and receiver operating curve (ROC) analysis revealed increased mobility per bout, initial mobility, and overall immobility in females and brief but frequent struggling in males. We also examined the impact of the modified SCVS on the medial prefrontal cortex (mPFC) and found distinct changes depending on the subareas of mPFC. In the infralimbic cortex, SCVS-exposed males showed a higher firing threshold, impaired firing activity on strong stimuli, reduced excitatory amplitude, and increased inhibitory frequency. In the prelimbic cortex, only females exhibited increased excitability and reduced inhibitory frequency. Our study highlights the potential of the modified SCVS model to explore sex differences in depression, as evidenced by various criteria in the TST and alterations in mPFC activity. By considering both sexes, this model provides a valuable tool for further research on mood disorders.

Disclosures: W. Kim: None. **S. Oh:** None. **C. Chung:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.04/PP24

Topic: G.05. Mood Disorders

Support: Stowers Institute for Medical Research

Title: Reward omission and social frustration impair subsequent odor discrimination

Authors: *D. U. MIKHAEL[1,2], R. GARG[1], M. S. UDDIN[1], C. RON YU[1];
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Abstract: Reward omission and social frustration impair subsequent odor discrimination.
Authors: David Mikhael, Md Sahab Uddin, Rahul Garg, C. Ron YuStowers Institute for Medical ResearchKansas City, MO
In humans, social injustice can contribute to feelings of hopelessness, powerlessness, and social isolation, and can lead to erratic behaviors, even violence. Chronic mild stressors including social frustration can affect rational decision-making, cause long-term cognitive and neural

impairments, and can lead to depression. Studies using animal models have shown impaired sociability and aspects of depressive-like behaviors but so far have not characterized early behavioral symptoms. The neural mechanisms underlying the behavioral responses are not known. Here, we develop a paradigm in mice to characterize behavioral changes upon exposure to short-term unfair treatment. In this paradigm, a pair of mice first receive an unconditioned reward while observing each other in chambers separated by a transparent window. To induce mild stress, the observer cannot access the reward but has to watch while the other does. The treatment leads the observer mouse to investigate the other chamber at an increased frequency and duration. It also increases the fruitless effort to access the reward. Moreover, in subsequent tests, learned odor association is impaired by the treatment. Together, these results show that mice exhibit frustration after a single disfavored experience, which impacts additional cognitive functions.

Disclosures: D.U. Mikhael: None. **R. Garg:** None. **M.S. Uddin:** None. **C. Ron Yu:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.05/PP25

Topic: G.05. Mood Disorders

Support: UF Informatics Institute Seed Fund 2021

Title: A characterization of behavioral and physiological impacts of social defeat stress in rats

Authors: *S. PICKERNELL, G. GRANUM, T. THOTA, V. CHIN-QUEE, V. QUACH, G. HEY, S. MURALIDHARA, P. SIDWELL, D. P. DEVINE;
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Abstract: Social stressors play an important role in the etiology of major depressive disorder (MDD). Accordingly, social stress has been modeled in preclinical studies using the social defeat model in rodents. Patterns of dominant and submissive behaviors have been examined across repeated training and testing days in mice, but not yet in rats. We are investigating the changes in dominance and submissive behaviors in both the resident and intruder rats across daily training sessions, the impacts of repeated social stress on social and physical environmental interactions, and its impacts on physiological and neurobiological alterations. Resident rats were housed in isolation for seven days. Then, in the social defeat training sessions, the behaviors of the resident rats stabilized by the second session. This indicates that little social defeat training is needed to establish consistency in dominance characteristics. Likewise, the intruder's submissive behavior also stabilized by the second session of social defeat. Following repeated social defeat, the intruder rats' social interactions and engagements with physical stimuli were assessed in a novel complex environment. The intruder rats displayed greater and more efficient agonistic interactions following repeated social defeat compared to the non-stressed control group. The

stressed intruder rats also spent more time in the socially isolated activity compared to the control rats that engaged with socially conducive physical stimuli. Additionally, we assessed correlations between behavior and physiological biomarkers in the stressed and control rats when exposed to the mild stressor of a novel circular corridor. We found no significant differences in distance locomoted in the task nor in the thymus and adrenal weights between the groups. The socially stressed group displayed significantly greater plasma corticosterone levels compared with the control group. We are currently analyzing RNAscope images to identify differences in mRNA expression between the stressed intruder and control groups.

Disclosures: S. Pickernell: None. **G. Granum:** None. **T. Thota:** None. **V. Chin-Quee:** None. **V. Quach:** None. **G. Hey:** None. **S. Muralidhara:** None. **P. Sidwell:** None. **D.P. Devine:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.06/PP26

Topic: G.05. Mood Disorders

Support: 5R01MH106500-09

Title: Prenatal stress and THC exposure impacts maternal and adolescent behaviors

Authors: *J. OLUSAKIN[1,2], M. DEWAN[3], M. LOBO[2];
[1]Univ. of Maryland Sch. of Medicin Program In Neurosci., Baltimore, MD; [2]Neurobio., [3]Med., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Cannabis is the most used illicit substance worldwide. The commonly cited reasons for continued cannabis use in chronic users is to cope with elevated stress (and/or) anxiety levels, pain and nausea. Pregnant women will often use cannabis to control nausea or stress/anxiety, and the effectiveness of cannabis in controlling these symptoms is likely due to the regulation of dopaminergic signaling in the reward-related brain areas. The main psychoactive component of cannabis, delta-9-tetrahydrocannabinol (THC), can readily cross the placenta during gestation, and is also secreted in the maternal milk during lactation. In addition, both clinical and preclinical studies have shown that chronic exposure to cannabis during gestation and lactation can induce behavioral teratogenic consequences. Little is known about the compound effects of prenatal exposure to stress and cannabis on long-term behaviors and cognition in adolescent offspring. In this study, pregnant dams underwent 10 days of chronic witness defeat stress (CWDS) from embryonic day 0 (E0) and concurrent administration of subcutaneous i.p injections of 2 mg/kg THC from E0 till birth. Following the 10 days of CWDS, we tested dams for anxiety-like behaviors - open field (OFT) and elevated plus maze (EPM), and social interaction (SI). At adolescence, postnatal day 35 (P35), we tested the offspring exposed to prenatal stress and THC on effort-related choice task using the Y-maze barrier test, a read-out for

motivation. In the Y-maze barrier test, adolescent mice exposed to prenatal stress and THC were presented with the option of either a high effort/high reward or a low effort/low reward food choice. Our analysis of anxiety-like behaviors in the dams revealed a significant interaction effect of THC and stress and particularly a main effect of stress suggesting exposure to THC alone was not sufficient to induce anxiety-like behavior however a combined exposure with stress significantly heightened anxiety-like behaviors in pregnant dams. In the adolescent mice, the effort-related task in the Y-maze barrier testing, revealed an interaction effect between prenatal THC and stress but no main effects of stress and THC. This suggest that a combined exposure to prenatal stress and THC results in maladaptive effort-related motivated behaviors at adolescent ages. We are currently investigating sex-specific effects of effort-related task at adolescent timepoints in mice exposed to prenatal stress and THC.

Disclosures: **J. Olusakin:** None. **M. Dewan:** None. **M. Lobo:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.07/PP27

Topic: G.05. Mood Disorders

Support: NIDA F32 Grant F32DA056191-02
NIDA R01 Grant 5R01DA047843-05
NIMH R01 Grant 5R01MH106500-09

Title: The Effects of Chronic Stress on BLA-VP Circuit Activity

Authors: *G. V. VIRATA, R. R. CAMPBELL, D. MARTINEZ, S. KEY, R. CHANDRA, M. LOBO;
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Abstract: Chronic stress is a major contributing factor to the onset of various psychiatric disorders, but it is unclear how it affects circuits in the brain, leading to the development of symptoms, including those of major depressive disorder (MDD). Dysregulation of the ventral pallidum (VP), a region within the brain's reward system, is associated with motivational deficits and MDD-associated symptoms, including anhedonia and social avoidance. However, it is unclear how chronic stress hinders VP activity. The basolateral amygdala (BLA) is an afferent VP region involved in emotional processing and learning that undergoes stress-induced changes in neural activity. Therefore, we focused on examining how stress-induced alterations to the BLA-VP circuit affect social avoidance in mice. We hypothesized that chronically stressed, socially avoidant mice will have decreased BLA-VP circuit activity. Using chronic social defeat stress (CSDS) and chronic witness defeat stress (CWDS) paradigms, we assessed the effects of chronic stress on immediate early gene expression in the BLA and VP of male and female mice. *Fos* mRNA expression was decreased in the VP but increased in the BLA of stressed mice

compared to unstressed controls, immediately following the last session of chronic stress and following social interaction. To assess the effects of chronic stress on neural activity within VP-projecting BLA neurons, we infused retroAAV-GFP into the VP of male mice and examined changes in Fos protein expression following CSDS and a social interaction test. A decrease in Fos protein expression was observed in these VP-projecting BLA cells in stressed mice compared to unstressed controls. On-going experiments include Fos protein BLA-VP activity analysis in male and female CWDS mice. Ultimately, these data will illustrate the impacts of chronic stress on neural circuitry and provide insight into mechanisms underlying MDD-related symptoms.

Disclosures: G.V. Virata: None. **R.R. Campbell:** None. **D. Martinez:** None. **S. Key:** None. **R. Chandra:** None. **M. Lobo:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.08/PP28

Topic: G.05. Mood Disorders

Support: Lundbeck Foundation Neurosurgical Scholarship
Helga og Peter Kornings Foundation

Title: The neuroanatomy and connectivity of the ventral striatum and ventral capsule area in the Göttingen minipig

Authors: *A. POULSEN, H. ZAER, B. SOEGAARD, A. N. GLUD, J. H. SORENSEN, D. ORLOWSKI;
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Abstract: A significant number of patients suffering from major depressive disorder (MDD) do not benefit from current treatment options, thus experiencing treatment-resistant depression (TRD). Novel treatment modalities, such as deep brain stimulation (DBS) have been evaluated for TRD, with ambiguous results that may be due to the complex biopsychosocial nature of MDD and the lack of a standardized large animal model. One proposed DBS target is the ventral striatum/ventral capsule (VS/VC), which is involved in emotional and behavioral circuitry. The Göttingen minipig (GM) is an established non-primate, large animal model that is increasingly used in neuroscience. However, the GM VS/VC is not well described. We aimed to describe the neuroanatomy and connectivity of the VS/VC in the GM and to compare the findings with humans and other species. 4 female GMs (19-21 kg, aged 8-9 months) received MRI-guided stereotaxic unilateral injection with the retro- and anterograde neuronal tracers FluoroGold (FG) and biotinylated dextran amine (BDA), respectively, in the VS/VC. Postmortem tissue was cryosectioned into 40 µm coronal sections for connectivity analysis and Nissl staining. Tissue from previous studies were labelled with anti-CB, -SP, -MBP, -DARPP32, -TH and -ChAT

antibodies for the immunohistochemical description of the VS/VC. Injection sites were confirmed using a Leica DM5000B microscope to be the core and dorsal pole of the nucleus accumbens (Acb), the internal capsule (IC) and the ventral pallidum (VP). We report an abundance of VS/VC projections, including retrograde connections from the CA1 subregion of the ventral hippocampus and amygdala to the Acb and VP. Furthermore, all injected areas received input from the ventral tegmental area, homologous to the human mesolimbic pathway. The GM IC is traversed by bidirectional fiber connections between cortical areas and the thalamus, resembling the basal ganglia loops described in the human and non-human primate brains. Postmortem tissue analysis demonstrated little injection trajectory reflux. GMs were injected very close to the anterior commissure, thus fiber cross-over contribution from this white matter tract cannot be denied. FG tracing was well distributed throughout the brains. BDA did not distribute quite as far, thus the anterograde tracing data from the VS/VC is not conclusive. The GM VS/VC has a connectivity and cytoarchitectural organization similar to previous descriptions of other species,with anatomy and circuitry closely resembling the rodent, human and non-human primate brains. Our findings will establish the anatomical foundation for a GM model for DBS in the VS/VC.

Disclosures: **A. Poulsen:** None. **H. Zaer:** None. **B. Soegaard:** None. **A.N. Glud:** None. **J.H. Sorensen:** None. **D. Orlowski:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.09/QQ1

Topic: G.05. Mood Disorders

Title: Voluntary wheel running promotes resilience to the behavioral effects of unpredictable chronic mild stress in male and female mice

Authors: E. ELIAS, A. Y. ZHANG, A. WHITE, ***M. T. MANNERS**;
Rowan Univ., Glassboro, NJ

Abstract: Besides significant benefits to physical health, exercise promotes mental health, reduces symptoms of mental illness, and enhances psychological development. Exercise can offset the impact of chronic stress, which is a major precursor to the development of mental disorders. The effects of exercise on chronic stress-induced behaviors are contradictory in preclinical studies, primarily due to the lack of data and sex-specific investigations. We sought to evaluate the effects of exercise on chronic stress-induced behavioral changes in both male and female mice. Mice were subjected to an Unpredictable Chronic Mild Stress (UCMS) paradigm with accessibility to running wheels for 2 h daily. Physiological and behavioral evaluations were conducted throughout the stress paradigm to determine if exercise blunts the effects of UCMS. Chronic stress induced voluntary wheel running (VWR) and weight loss in male and female mice. Compared to males, increased VWR was reported in females who also regained their

weight lost by the end of the UCMS protocol. Exercise promoted resilience to stress-induced hyponeophagia in the novelty-suppressed feeding test and increased sucrose consumption. Exercise induced a sex-specific reduction in immobility and avoidance behavior in the tail suspension and open field tests and increased exploratory behavior in the light-dark test. These results indicate that exercise can promote resilience to the behavioral effects of chronic stress in males and females and can affect behavior independent of chronic stress.

Disclosures: E. Elias: None. **A.Y. Zhang:** None. **A. White:** None. **M.T. Manners:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.10/QQ2

Topic: G.05. Mood Disorders

Support: Rutgers Research Council

Title: Identifying a Novel Approach to Stress-Induced Eating in Mice

Authors: V. VARGAS, *M. E. BOCARSLY;
Rutgers NJMS, Newark, NJ

Abstract: Individuals experiencing stressful circumstances often turn to increased food consumption as a coping mechanism. There exists the potential of this seemingly harmless strategy turning into an addictive behavior, and excess consumption of palatable foods is a fundamental factor of health-related disorders in humans. Despite this danger, the relationship between stress and food consumption has not been well studied, and it is unclear which physiological pathways are driving it. We seek to understand if “stress-eating” can be characterized through behavioral and biological means. Here, we developed and characterized a mouse model of stress-induced eating utilizing a mild stressor, with the future goal of using it to understand the underlying neural circuitry.
For this study, female mice were placed in a novel environment and allowed to consume a palatable food (Trix cereal) for another hour. Food intake was measured. After 6 days, mice were randomly divided into experimental (n=20) and control (n=20) groups. Mice in the experimental group were exposed to a tea strainer filled with crushed palatable food for 15 minutes. The mice were able to interact with the tea strainer, but not consume the food, which could potentially induce a stress response. The control group was exposed an empty strainer. Following the 15-minute experimental period, the mice were allowed to consume the palatable food for 15 minutes, and intake was recorded. In a separate trial, following 15 minutes of tea strained exposure, mice were allowed to explore an elevated zero-maze for 10 minutes. Time spent on the open and closed arms was scored, as a measure of anxiety-like behavior. Finally, on a separate occasion, following 15 minutes of tea strained exposure, tail blood was taken for measurements of corticosterone, the stress hormone.Intake of palatable food was higher in the experimental

mice compared to the control group. Anxiogenic behavior was used as a readout of stress. Relative to control mice, the experimental mice spent more time on the closed arms of the elevated zero-maze. Serum corticosterone levels were measured as a biological indicator of stress; compared to control mice, experimental mice exhibited higher levels of serum corticosterone.Together, these data demonstrate that we can model stress-induced eating in rodents. Future directions include the investigation into dopamine signaling in the striatum.

Disclosures: V. Vargas: None. **M.E. Bocarsly:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Topic: G.05. Mood Disorders

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Hope for Depression Research Foundation

Title: Stress Regulation of Nucleus Accumbens D1- and D2-Medium Spiny Neurons During Approach-Avoidance Conflict

Authors: *A. MINIER-TORIBIO[1], A. GODINO[1], T. LIN[2], L. M. HOLT[1], T. MARKOVIC[1], T. GYLES[1], A. TORRES-BERRIO[1], L. LI[1], A. V. AUBRY[1], R. DURAND-DE CUTTOLLI[1], C. AZIZIAN[1], C. J. BROWNE[1], G. ROJAS[1], A. LIN[3], F. J. MARTÍNEZ-RIVERA[1], S. J. RUSSO[1], E. J. NESTLER[1];
[1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [2]Hunter Col., New York, NY; [3]Herricks High Sch., New Hyde Park, NY

Abstract: Balancing the pursuit of rewards (approach) with avoidance of threats is vital for survival and well-being. Major depressive disorder (MDD) disrupts this balance, leading to blunted approach and excessive avoidance. Despite its prevalence, the neurobiology of this maladaptation remains elusive. Here, we investigated the MDD-associated approach-avoidance imbalance using mouse models at multiple levels: behavior, cellular, and molecular. We induced behavioral abnormalities in mice using chronic social defeat stress (CSDS), resulting in a spectrum of resilient (RES) vs. susceptible (SUS) phenotypes. Approach-avoidance biases were assessed using a platform-mediated avoidance (PMA) task, where mice avoid a tone signaling a footshock by forfeiting access to a lever signaling saccharine-water reward (approach). Although no significant differences were observed during conditioning, SUS mice displayed heightened aversive sensitivity indicated by excessive freezing to the tone. Subsequent extinction training revealed elevated freezing and avoidance, along with blunted approach in SUS mice. Building upon these findings, we studied the role of the nucleus accumbens (NAc), a key brain region

signaling approach, avoidance and stress. While traditionally thought to have opposing roles, recent evidence suggests that D1- and D2-medium spiny neurons (MSNs) in the NAc may signal both rewards and threats depending on location and exposure. Using fiber photometry, we measured the calcium activity of D1- and D2-MSNs during PMA in stress naïve and CSDS mice. Our results indicate that in naïve mice both D1- and D2-MSNs increase activity during shock acquisitions and avoidance responses. Only D2-MSNs, however, increase activity during the conditioning but not extinction phase (no shock) tone presentations and during reward consumptions in both phases. Chemogenetic manipulations show that inhibiting D1-MSNs enhances avoidance and approach, while inhibiting D2-MSNs reduces avoidance without affecting approach. In CSDS mice, avoidance was bidirectionally regulated by D1- and D2-MSNs, with D2 MSNs promoting avoidance extinction in RES mice. Ongoing studies explore molecular changes associated with approach-avoidance biases and stress sensitivity by employing RNA-sequencing targeting the NAc and other reward circuitry regions. Our findings shed light on the neurobiology underlying approach-avoidance associated with chronic stress and identify potential therapeutic targets for alleviating behavioral abnormalities in MDD.

Disclosures: **A. Minier-Toribio:** None. **A. Godino:** None. **T. Lin:** None. **L.M. Holt:** None. **T. Markovic:** None. **T. Gyles:** None. **A. Torres-Berrio:** None. **L. Li:** None. **A.V. Aubry:** None. **R. Durand-de Cuttolli:** None. **C. Azizian:** None. **C.J. Browne:** None. **G. Rojas:** None. **A. Lin:** None. **F.J. Martínez-Rivera:** None. **S.J. Russo:** None. **E.J. Nestler:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Program #/Poster #: PSTR364.12/QQ4

Topic: G.05. Mood Disorders

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Office of Naval Research (ONR) N00014-19-1-2149
The Hope for Depression Research Foundation (HDRF)
the Pritzker Neuropsychiatric Research Consortium

Title: Early Life Stress Responses in Selectively Bred Rats: How Genetic Temperament Determines Development of the HPA Axis

Authors: *P. MARAS[1,2], E. K. HEBDA-BAUER[2], S. J. WATSON[2], H. AKIL[2];
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Abstract: Heritable personality traits are a strong predictor of vulnerability or resilience to developing psychiatric disorders, including anxiety and depression. An individual's underlying temperament, however, is strongly influenced by environmental factors, and stressful life events, especially those experienced during early life, provide another major risk for mood disorders. Understanding the complex ways genetic and environmental factors interact to regulate emotion

has remained a challenge. To this end, our laboratory has developed a genetic model of temperament by selectively breeding rats based on locomotor response to novelty, a behavioral trait that corresponds to stable differences in emotionality and stress reactivity. Rats bred for high (bHR) or low (bLR) locomotor responses reflect externalizing or internalizing temperaments, respectively, and previous studies have demonstrated that bHRs and bLRs respond quite differently to stress in adulthood. Importantly, the hypothalamic-pituitary-adrenal (HPA) axis, the primary mediator of stress responses, undergoes significant functional organization during early postnatal life, and differences in HPA development may set the stage for lifelong differences in stress reactivity. The goal of the current set of experiments was to characterize the functional development of the HPA axis in bHRs and bLRs. Through a series of experiments, we compared plasma corticosterone (CORT) levels following an acute isolation stress and discovered that, compared to bLRs, bHRs had elevated CORT responses throughout much of early life, evident by postnatal day 12. Even though bHRs had a higher peak stress response, CORT levels in both lines returned to baseline by 60 minutes after the end of isolation, indicating similar efficiency in negative feedback processes. Furthermore, CORT levels in response to an adrenocorticotropic hormone challenge were comparable in bHRs and bLRs, suggesting that their differences in CORT responses to stress reflect differences in how upstream neural circuits respond to the stressful stimuli. Using hairpin chain reaction-based fluorescent *in situ* hybridization (HCR FISH) methods, ongoing experiments are comparing mRNA expression of c-fos (marker of neural activation), as well as multiple stress-related molecules, following isolation stress in bHR and bLR pups. Together, these data will shed light on how underlying genetic differences can shape the development of the stress system and ultimately determine risk for stress-related psychiatric disorders.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.13/QQ5

Topic: G.05. Mood Disorders

Support: CONACyT CB-241247

Title: Chronic mild stress and cafeteria diet combination exacerbate microglia and c-fos activation but not experimental anxiety or depression in menopause model

Authors: E. ESTRADA-CAMARENA[1], N. M. VEGA-RIVERA[1], S. TREVIÑO[2], N. CERVANTES-ANAYA[1], E. BECERRIL VILLANUEVA[3], *J. CHAN MONROY[4]; [1]Neuropsicofarmacología, Inst. Naciohnal de Psiquiatría Ramón de la Fuente, cd de México, Mexico; [2]Química, BENEMERITA UNIVERSIDAD AUTONOMA DE PUEBLA, BENEMERITA UNIVERSIDAD AUTONOMA DE PUEBLA, Mexico; [3]Dept. of

Psychoimmunology, Natl. Inst. of Psychiatry. Mexico, Mexico, Mexico; [4]Ramon de la Fuente Muniz, Mexico, Mexico

Abstract: Menopause transition could be a period where the vulnerability to develop both psychiatric and metabolic disorders, and both can be enhanced by stressful events worsening their effects. The present study aimed to evaluate whether a cafeteria (CAF) diet combined with chronic variable stress exacerbates anxious- or depressive-like behavior, neuronal activation, affects cell proliferation and survival as well as microglia activation in middle-aged ovariectomized (OVX) rats used as a model of menopause. In addition, body weight as an index of weight gain, lipid profile, insulin resistance as an index of metabolic changes, corticosterone as an index of HPA axis activation, and pro-inflammatory interleukins IL-6, IL- β and TNFα as an index of systemic inflammation were measured. CAF diet increases body weight, lipid profile (LDL, cholesterol, and triglycerides), and insulin resistance. CVS increases corticosterone and reduces HDL but does not modify the effect of CAF alone. CAF produces anxious-like behavior while CVS induces depressive-like behaviors; however, combining both factors did not further increase these behaviors. CVS increases serum TNFα independently of diet; no further changes were detected. CAF and CVS enhanced the percentage of Iba- and *c-fos* positive cells in the dentate gyrus hippocampus; noticeably, the combination of CAF and CVS further increased the percentage of iba- and *c-fos* positive cells in the ventral, but not dorsal, hippocampus region. CVS could contribute to metabolic alterations induced by CAF and the development of anxious and depressive-like behaviors by generating a slight neuroinflammation process and neuron activation in a hippocampal region-specific manner.

Disclosures: E. Estrada-Camarena: None. **N.M. Vega-Rivera:** None. **S. Treviño:** None. **N. Cervantes-Anaya:** None. **E. Becerril Villanueva:** None. **J. Chan monroy:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Program #/Poster #: PSTR364.14/QQ6

Topic: G.05. Mood Disorders

Support: NIMH Grant 3R01MH106500-09S1

Title: Impact of social defeat stress on microglia-neuron interactions in the nucleus accumbens

Authors: *D. FRANCO[1], B. M. SIEMSEN[1], G. KUMAR[1], S. L. KEY[1], M. E. FOX[2], M. LOBO[1];
[1]Univ. of Maryland, Baltimore, Baltimore, MD; [2]Pennsylvania State Univ. Col. of Med., Hershey, PA

Abstract: Chronic stress is a known risk factor for neuropsychiatric disorders, and it has been shown to alter neuron and myeloid cell structure and function in brain reward areas. The nucleus accumbens (NAc), a hub for integrating reward and motivation, exhibits molecular and cellular

alterations that are found in postmortem tissue of patients with major depressive disorder and drive motivational deficits in rodents. Moreover, exposure to chronic stress increases peripheral cytokines in individuals with post-traumatic stress, anxiety, and major depressive disorders and disrupts bidirectional CNS myeloid-neuronal communication in rodents exposed to social stress. Preclinical work from our lab has shown that exposure to chronic social defeat stress (CSDS), a validated animal paradigm of social stress, yields dendritic atrophy in NAc dopamine receptor-1 expressing medium spiny neuron (D1-MSNs) in mice that display negative affective behavior. Given that microglia play a mediating role in regulating neuronal dendritic adaptations after social stress, we subsequently characterized microglia and D1-MSN interactions in the NAc after 10 days of CSDS. We observed a cell-subtype specific reduction in microglia-D1-MSN contact in the NAc, a negative correlation between microglia-D1-MSN contact and CSDS-induced decreases in social interaction, and reduced microglia complexity in the D1-MSN microenvironment of mice with low social interaction. However, while it is evident that CSDS alters microglia-D1-MSN contact and morphology, the molecular mechanisms driving these cell-subtype specific, stress-induced changes in the NAc microenvironment remain unclear. Preliminary data from recent RNA-seq analyses from our lab using female mice that underwent Chronic Witness Defeat Stress (CWDS), a validated paradigm of indirect social stress, demonstrated enhanced Vtn (vitronectin) in D1-MSNs from female mice associated with low social interaction. Specifically, vitronectin appears to play a driving role in a network of genes altered by chronic stress. Given that vitronectin, an ECM glycoprotein, has been shown to induce microglia reactivity and increase inflammation-associated surface proteins, it is a promising molecular messenger mediating D1-MSN and microglia cross-talk. Ongoing work seeks to further characterize the role of vitronectin in neuron-microglia interactions in the context of social stress. Identifying microglia mechanisms contributing to altered neuronal dendritic morphology and negative affective behaviors can pinpoint novel therapeutic targets for stress-related disorders and improve current treatments.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.15/QQ7

Topic: G.05. Mood Disorders

Support: EU-MSCA Serotonin and Beyond GA-no. 953327

Title: Alterations of cognitive behaviours and prefronto-thalamic circuits after early life exposure to fluoxetine

Authors: *N. N. SOTO[1,2], A. DE STASI[1,2], A. AGUIRRE[1,2], P. GASPAR[1,2], A. BACCI[1,2];
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Abstract: The prefrontal cortex (PFC) plays a significant role in the regulation of higher order cognitive functions and emotional processes, by integrating sensory, associative, and emotional inputs from different cortical and subcortical structures. A critical developmental stage of the PFC occurs in the first two postnatal weeks, during which a specific subtype of deep-layer pyramidal neurons (PNs) transiently expresses the serotonin transporter (SERT) in a time sensitive manner. In this developmental period (P2-P11), the PFC is highly sensitive to environmental stressors including exposure to selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine (FLX). Perinatal fluoxetine treatment (PNFLX) in rodents results in PFC hypo-excitability, altered SERT+ PN firing, and increased anxiety- and depressive-like behaviours in adulthood. In addition, SERT+ PNs exhibit a dense bidirectional connectivity with the mediodorsal nucleus of the thalamus (MD), thereby constructing an important part of the prefronto-thalamic loop. This loop has been shown to play a key role in cognitive, goal-oriented behaviors and sustained PFC activity. Moreover, there is evidence that lesions of the MD result in cognitive impairments. However, while cognitive functions are impaired in nearly all psychiatric disorders, their underlying circuit pathophysiology remains vastly unknown. Here, using anatomical labeling, *in vivo* and *in vitro* electrophysiology, and a battery of cognitive tests, we sought out to dissect the effects of PNFLX on the prefronto-thalamic loop and cognitive functions. We found that PNFLX affects the learning phase in a cognitive flexibility task and alters thalamocortical inputs onto PFC-PNs. We are presently assessing potential sex-specific alterations of PNFLX on cognitive functions and their related circuits. These experiments help define a causal link between specific circuit alterations and cognitive impairments in a mouse model of depression.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.16/QQ8

Topic: G.05. Mood Disorders

Title: Unraveling the Complexity of Depression: The Differential Impact of Ketamine on Learned Helplessness and Anhedonia

Authors: N. MOJAHED[1], C. BARTSCH[3], S. AAFLAQ[4], J. T. JACOBS[5], E. QASEM[2], *J. NORDMAN[1];
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Abstract: Depression is a devastating condition, reportedly affecting approximately 9% of the US population, leading to an annual economic toll of around $210 billion. It has been observed that early life stress can significantly influence the probability and severity of depression, triggering intense feelings of despair, irritability, anger, lack of pleasure, and even suicidal thoughts or tendencies. However, developing effective treatments has been challenging, necessitating further understanding of the fundamental neural mechanisms involved in depression. Ketamine, an NMDA receptor antagonist primarily recognized as a sedative and a substance prone to misuse, has recently emerged as a promising fast-acting antidepressant, providing effects that can persist for weeks after a single administration. Although the FDA has greenlighted the use of ketamine for treatment-resistant depression, its efficacy remains a topic of debate. Recent studies by our lab using on a mouse model of traumatic stress revealed that a single dose of ketamine has unexpected and occasionally contradictory impacts on depression-like behaviors. For instance, while it can alleviate learned helplessness, it does not seem to affect anhedonia. Moreover, we find a single dose increases traumatic stress-induced aggression but does not impact mobility, fear memory, or anxiety-like behavior. Interestingly, learned helplessness and anhedonia engage different brain regions and neural pathways: learned helplessness is connected with dysfunction in the cortical-amygdala pathways, while anhedonia impacts reward pathways involving the ventral tegmental area. This suggests that ketamine specifically targets the cortical-amygdala pathways impacted by early life stress, aligning with findings that these pathways are influenced by glutamatergic transmission. These results underscore that depression is a complex, multi-faceted disorder involving unique neural pathways, and that the effectiveness of ketamine treatment may vary depending on the specific depression subtype, such as learned helplessness or anhedonia. Future research will focus on pinpointing the precise targets of ketamine and exploring how its distinctive effects may be linked to early life stress.

Disclosures: **N. Mojahed:** None. **C. Bartsch:** None. **S. Aaflaq:** None. **J.T. Jacobs:** None. **E. Qasem:** None. **J. Nordman:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.17/QQ9

Topic: G.05. Mood Disorders

Support: TÜBİTAK Grant 121K260
Boğaziçi BAP Grant 22B07M2

Title: Behavioral and neural effects of intermittent environmental enrichment or social isolation in adult Wistar rats

Authors: D. AYKAN, A. AKKAYA, *G. UNAL;
Bogazici Univ., Istanbul, Turkey

Abstract: Environmental enrichment and social isolation are two well-studied sensory-motor manipulations that lead to several behavioral, neural, and molecular alterations in rodents. Enriched environment entails improvements in cognitive performance, depressive- and anxiety-like states, and nervous system injuries via different plasticity mechanisms. Rodent social isolation paradigms, in contrast, often produce deteriorating effects in cognitive and affective systems. Both manipulations are generally studied with either short-term or chronic exposure. Here, we investigated whether intermittent enrichment or isolation in rats lead to significant behavioral or neural alterations. We combined behavioral testing with immunohistochemistry to quantify parvalbumin-positive (PV+) cells in the hippocampal formation and basolateral amygdala. Twenty-four experimentally naïve adult male Wistar rats were assigned to an enriched condition (EC; n = 8), impoverished condition (IC; n = 8), or standard condition (SC; n = 8). All animals were initially housed in standard cages of 4 animals for 5 consecutive days. Intermittent differential housing was maintained by transferring EC animals to an enriched cage and SI animals to individual isolation cages for 2 days at the end of the 5-day period spent in standard cages. This procedure (i.e. 5 days of standard housing followed by 2 days of differential housing) was repeated for a total of eight weeks. Behavioral testing at the end of the 8-week procedure revealed that the EC animals displayed behavioral despair in the forced swim test as compared to IC and SC animals. Overall locomotor activity between groups was not different as assessed in the open field test. In contrast, IC and SC groups displayed anxiety-like behaviors in the elevated plus maze by spending significantly more time in the closed arms of the apparatus, while EC animals showed no difference. In the Morris water maze (MWM), EC animals had shorter latency to locate the escape platform on the second training day, which indicates an enhanced spatial memory performance. The groups did not differ on following training days or in the probe trial of MWM. Ex vivo immunohistochemistry for parvalbumin revealed that the IC group had fewer PV+ cells in CA1 compared to both EC and SC, which had similar PV+ cells. The IC animals also had fewer PV+ cells in CA3 and dentate gyrus compared to the EC group. The three groups did not show a difference in the basolateral amygdala. These results indicate that intermittent exposure to environmental enrichment or social isolation can be sufficient to alter limbic circuits and lead to performance changes in different cognitive and affective systems.

Disclosures: D. Aykan: None. **A. Akkaya:** None. **G. Unal:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.18/QQ10

Topic: G.05. Mood Disorders

Title: Ketamine promotes active response states by exciting cortical neurons via suppressing inhibitory interneurons

Authors: *W. CUI[1], C. SHEN[1], P. CHEN[1], M. GARFINKEL[1], W.-C. XIONG[1,2], L. MEI[1];
[1]Neurosciences, Case Western Reserve Univ., Cleveland, OH; [2]Louis Stokes Cleveland Veterans Affairs Med. Ctr., Cleveland, OH

Abstract: Major depression (MDD) is a prevalent and devastating psychiatric disorder. Ketamine, an anesthetic for many years, has gained tremendous attention because of its antidepressant effects; nevertheless, the underlying cellular mechanisms of ketamine are not well understood. To this end, we simultaneously monitored the activities of both projection neurons and GABAergic interneurons in the prefrontal cortex (PFC) in free-behaving animals and identified projection neurons that are associated with the active response (AR) state and promoted by ketamine. By pathway-specific c-Fos mapping, chemogenetic manipulation and calcium imaging, we showed that the activity of PFC neurons projecting to the NAc are associated with the AR state, and is necessary and sufficient for AR behaviors. Intriguingly, GABAergic interneurons display an opposite firing pattern to that of projection neurons: inhibited in the AR state but increased in the passive response (PR) state; they are suppressed by ketamine. Finally, neurons innervated by GABAergic inhibitory neurons increase firing in the AR state and are inhibited by ketamine. These results indicate a critical role of GABAergic interneurons in controlling behavioral states and ketamine promotes AR state by suppressing the inhibitory transmission onto projection neurons, revealing a cellular mechanism of ketamine's antidepressant effects.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.19/QQ11

Topic: G.05. Mood Disorders

Support: INPRFM NC123240.1

Title: Effect of social isolation on epileptogenesis induced by amygdaloid kindling depressive-like behavior and, sleep.

Authors: *A. VALDÉS-CRUZ, L. CASTILLO-VILLEGAS, S. SIMON, M. LOE-MARTÍNEZ, I. ROMERO-ELIZALDE;
Inst. Nacional de Psiquiatría Ramón de la Fuente Muñiz, México, Mexico

Abstract: Early age social isolation (EASI) in rodents is the most well characterized animal model for early stressful experiences and their neurobehavioral consequences, this model induced depressive like behavior in adult age. The objective of this work was to analyze the effect of EASI over the expression of depressive-like behavior and epileptogenesis induced by amygdaloid kindling (AK), and sleep patterns. EASI rats on postnatal day twenty-one were

introduced to social isolation by housing them singly, in adulthood, postnatal day 85-95, two types of experiments were conducted, for epileptogenesis by AK, and to sleep patterns. Swimming forced test (SFT); 5 minutes duration was used to measure depressive-like behavior. EASI rats were compared against naive rats and implanted rats (sham). For the induction of epileptogenesis through AK, 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. Spikes frequency and after discharge duration per behavioral AK stage, focal and generalized seizure susceptibility were analyzed. For sleep studies, animals were implanted electrodes in right and left hippocampus, both prefrontal cortices, and neck muscles to record the electromyogram. Four six hours polysomnographic records were done on consecutive days. Total time (TT) and number of phases (NP) of wakefulness, slow waves sleep (SWS) and rapid eye movement sleep (REM) stages were analyzed. FST test showed greater immobility time, and minor swim time in EASI group ($p<.05$). In AK experiments the EASI rats show an increase in focal seizure susceptibility ($p<.05$), an increment in epileptiform spike frequency during generalized seizures ($p<.05$) and a reduction in the after-discharge stage IV duration ($p<.05$). On sleep EASI showed lower TT in wakefulness ($p<.002$) and SWS ($p<.001$), and higher NP in SWS ($p< .01$) and REM ($p<.01$). EASI have negative effects on seizures severity, and susceptibility, also a permanent effect on SWS and REM sleep. The pro-depressive process starts in the initial stages of development, which could provoke a dysfunction of cerebral structures, as prefrontal cortex, and limbic system, both involved into depression-epilepsy comorbidity that maintained until adult age.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.20/QQ12

Topic: G.05. Mood Disorders

Support: VIEP-BUAP 2023 to CA in Neuroendocrinología (BUAP-CA-288)
CONACYT No. 885936

Title: Orchiectomy has a differential effect in the high- and low- yawning sublines subjected to the forced swim test

Authors: *D. BRAVO DURÁN[1], J. R. EGUIBAR[2], C. CORTES[3], A. B. SILVA-GÓMEZ[4]; [1]Behavioral Neurophisiology Laboratory, Inst. of Physiol., Benemerita Univ. Autonoma De Puebla, Puebla, Mexico; [2]Intl. Office, [3]Behavioral Neurophisiology Laboratory, Inst. of Physiol., [4]Fac. of Biol. Sci., Benemerita Univ. Autonoma de Puebla, Puebla, Mexico

Abstract: It has been proposed that testosterone might provide a protective benefit against anxiety and depression, so the incidence of depression in men might increase with an age-related decline in testosterone plasma levels. In our laboratory, we selectively inbred two sublines from Sprague-Dawley (SD) rats, the high-yawning (HY) with 20 yawns/h and low-yawning (LY) with just 2 yawns/h. These sublines differ in the stress and anxiety responses in different psychobiological paradigms, being the LY rats more susceptible to stress with respect to HY, that had a resilient phenotype. The aim of this study was to evaluate the levels of depression, through immobility time in the forced swimming test (FST), in high- and low-yawning rats that were orchiectomized in adulthood (3 months of age). We used adult male HY and LY rats that were housed in standard conditions with free access to rodent pellets and purified water. Two weeks after the orchiectomy, all subjects had two swim sessions on two consecutive days, the first with 15 min duration and, 24 h later, a second session of 5 min. We scored the following behaviors: immobility, swimming, climbing, and diving. Our results show that orchiectomized LY rats exhibit a significant increase in the immobility time ($p < 0.05$), and a concomitantly increased in swimming ($p < 0.05$) and climbing times ($p < 0.05$) with respect to HY rats. In conclusion, these findings indicate that the absence of testosterone, by remotion of the testis, has a greater effect in the LY rats, which are genetically more susceptible to depression.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Topic: G.05. Mood Disorders

Support: Vulnerable Brain Project
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Title: Exploring the effects of ketamine and ketogenic diet on activity-based anorexia vulnerability

Authors: *C. AOKI, R. FELSHER, C. CARRASCO, D. SAHIN, Y. LIN, H. ZARDUS, I. LOTIA;
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Abstract: Anorexia nervosa (AN) is a mental illness with symptoms of severe food restriction and excessive exercising that, together, cause severe body weight loss. This mental illness has the highest mortality rate, yet is still without any accepted pharmacological treatments. AN emerges most often during adolescence and among females, with the age of onset being a strong predictor of its relapse rate (Walsh, doi:10.1176/appi.ajp.2012.12081074). We use an animal

model of AN, called activity-based anorexia (ABA) to test pharmacological treatment options that could dampen anorexia-like symptoms of excessive exercise, voluntary food restriction and severe weight loss (Aoki, '20, Animal Models of Eating Disorders). We have shown that these three measurements of anorexia-like symptoms can be ameliorated in female mice, if the first experience of ABA in mid-adolescence (ABA1) is accompanied by a single intraperitoneal injection of ketamine (Chen et al., '18, Int. J of Eating Disorders, v51, p1020). We recently discovered that ABA is more severe when ABA1 is delayed from mid-adolescence to late adolescence (LateAdolABA1, Aoki & Santiago, Frontiers in Behav Neurosci, doi.org/10.3389/fnbeh.2022.990354). This suggests that ABA1 that is experienced during mid-adolescence evokes greater plasticity towards recovery than when experienced during late adolescence and this age-dependent difference in plasticity may be the reason late onset of AN is a predictor of high relapse rate. We also learned recently that ketamine injections on three consecutive days during a second experience of ABA in late adolescence (ABA2), following the first experience of ABA in mid-adolescence (ABA1) can reduce severity of anorexia-like symptoms acutely and also during the third experience of ABA (ABA3), 15 days later in adulthood (ms undergoing revision). Here, we combined these two new findings to test whether the more severe effects of LateAdolABA1 can also be ameliorated by ketamine. There is limited but compelling clinical reports indicating that ketogenic diet may ameliorate severity of anorexia nervosa symptoms and relapse (Scolnick et al., Frontiers in Psychiatry v11, p763; Calabrese et al., '22, Eating & Weight Disorders, v27, p3751). Therefore, we are also testing whether ketamine in combination with ketogenic diet shows stronger ameliorative effects upon ABA severity than ketamine alone. To this end, we are generating LateAdolABA1, which experience ABA2 and ABA3 in adulthood, with ABA2 being the phase for ketamine treatments. Additional animals will be acclimated to the ketogenic diet during recovery from ABA1, then treated with ketamine during ABA2.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Topic: G.05. Mood Disorders

Support: UWRF Undergraduate Stipends and Expenses Grant

Title: The effect of adolescent social isolation on nicotine conditioned place preference, stress coping behavior, and serotonin neuron activity.

Authors: M. DAVIS[1], K. OSTROVIAK[1], A. KIRCKOF[3], D. JENSEN[1], E. AUGUSTINE[1], S. SYMALLA[1], *D. G. EHLINGER[2];

[1]Psychological Sci., [2]Univ. of Wisconsin-River Falls, River Falls, WI; [3]The Univ. of Kansas, Lawrence, KS

Abstract: Adolescence is a sensitive period in brain development marked by increased susceptibility to the effects of chronic stress, which may enhance vulnerability to neuropsychiatric conditions such as depression and substance use disorders. In this study, we used an animal model to examine the effect of adolescent social isolation stress on coping behavior, nicotine reward, and serotonin neuron activity. During adolescence (postnatal day P35-P49) or adulthood (P60-74), male and female C57BL/6J mice were exposed to either social isolation (SI) stress or standard rearing (SR) conditions and four nicotine exposures (0.35mg/kg) during a nicotine conditioned place preference (CPP) procedure. On approximately P49 (adolescent) or P74 (adulthood), stress-coping behavior was examined following a 6-minute forced-swim test (FST), and brains were processed for immunofluorescence of serotonin neurons and c-fos activity. Our behavioral results show that adolescent SI mice more rapidly develop nicotine CPP compared to SR mice and adult mice, exhibit increased levels of immobility in the FST, and that prior nicotine exposure during social isolation decreases immobility in the FST. Ongoing research is examining stress-induced functional (c-fos expression) differences in the brains of SI versus SR mice in response to the FST via immunofluorescence of the dorsal raphe ascending serotonergic system. Collectively, our results suggest that adolescent social isolation stress enhances the rewarding effects of nicotine and negatively impacts stress-coping behavior. Ongoing analyses will help determine neurological correlates of adolescent susceptibility to the negative effects of chronic social isolation stress and inform our understanding of adolescent brain development and vulnerability.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Program #/Poster #: PSTR364.23/QQ16

Topic: G.05. Mood Disorders

Support: FRM ARF202110013920
FRM EQU201903007809
ANR-17-EURE-0022

Title: The role of the cingulo-habenular pathway in chronic pain-induced anxiodepressive-like behaviors

Authors: *V. P. MATHIS[1], S. H. JOURNÉE[1], R. WAEGAERT[1], M. GAIKWAD[1,2], M. THOUAYE[1], S. HUGEL[1], Q. LEBOULLEUX[1], N. WILLEM[1], L. BECKER[3], S. OZKAN[1], P.-E. LUTZ[1,4], I. YALCIN[1,2];

[1]Inst. of Cell. and Integrative Neurosciences, Strasbourg, France; [2]Dept. of Psychiatry and Neurosci., Laval Univ., Québec, QC, Canada; [3]Dept. of Anesthesiol., Ctr. for Clin. Pharmacol., St. Louis, MO; [4]Douglas Mental Hlth. Univ. Inst., Montreal, QC, Canada

Abstract: *Rationale*: Uncontrolled and persistent pain strongly associates with anxiety and depressive disorders, and is among the most common cause of disability impairing the quality of life. Over the last 10 years, our group has established and validated paradigms designed to model this comorbidity, in mouse. We then exploited this model to uncover individual brain structures and molecular mechanisms affected by chronic pain. Among candidates, the anterior cingulate cortex (ACC), a structure common to the default mode, salience and reward networks, appears critical in pain and emotional processing. However, how these changes propagate to downstream structures of the ACC is yet to be studied. Given its role in aversion, anxiety and depression, combined with its unidirectional anatomo-functional connection with the ACC, the lateral habenula (LHb) seems to be an interesting ACC target. In this study, we thus aimed at deciphering the role of ACC neurons projecting to LHb in chronic pain and its comorbidity with depression. *Methods*: Using calcium imaging recordings in freely moving male mice, we first compared the Ca^{2+} dynamics of ACC and LHb neurons as well as ACC neurons projecting to the LHb ($ACC_{->LHb}$) while neuropathic male mice performed anxiodepressive tests or experienced aversive tasks. Then, using optogenetic approaches, we activated the $ACC_{->LHb}$ pathway in naïve (acute and chronic stimulation) or inhibited it in our neuropathic model and in a chronic variable stress model. Finally, combining vTRAP and RNAseq approaches, we described the chronic pain induces molecular adaptations in $ACC_{->LHb}$ neurons. *Results*: Altogether, these experiments revealed that $ACC_{->LHb}$ neurons present a unique Ca^{2+} signature in response to aversive events. Furthermore, chronic but not acute activation of the $ACC_{->LHb}$ pathway induces strong anxiodepressive-like behaviors while its acute inhibition is sufficient to prevent the anxiodepressive-like consequences induced by chronic pain but not by stress. Finally, combining vTRAP and RNAseq approaches, we identified differentially expressed genes in $ACC_{->LHb}$ neurons in animals showing depressive-like behavior following neuropathic pain Overall, these results highlight the important role of the cingulo-habenular pathway in chronic pain-induced emotional disorders and place it as a target of interest for future treatments.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.24/QQ17

Topic: G.05. Mood Disorders

Support: NIH Grant ZIAMH002881 to Zheng Li
CCB- Grant to Aijaz Naik

Title: Mapping Frustration-Induced Brain Network Alterations in a Novel Mouse Model of Frustrative Non-Reward

Authors: *A. NAIK[1,3], X. MA[1], M. R. MUNYESHYAKA[1], E. LEIBENLUFT[2], Z. LI[1];
[1]Section on Synapse Develop. Plasticity, [2]Natl. Inst. of Mental Hlth. (NIMH/NIH), Bethesda, MD; [3]Ctr. on Compulsive Behaviors, Natl. Inst. of Hlth., Bethesda, MD

Abstract: Deficient or poor emotional regulation is a symptom of many psychiatric disorders, including bipolar disorder and disruptive mood dysregulation disorder (DMDD). DMDD represents a condition wherein children aged between 6-18 years' experience ongoing severe irritability and frequent, intense temper outbursts. Irritability, defined as proneness to anger that can reach a pathological extent, is one of the most common reasons youth presents for psychiatric evaluation and care. Aberrant responses to omission of an expected reward (Frustrative non-reward, FNR) are central to the pathophysiology of irritability. FNR is a cross-species RDoC construct. The development of preclinical FNR models could advance mechanistic studies of this important yet relatively understudied clinical phenomenon of irritability. Here, we used FNR as a conceptual framework to develop a novel behavioral paradigm in mice which we named Alternate Poking Reward Omission, APRO. After APRO, mice were examined with a battery of behavioral tests and processed for whole brain c-Fos imaging. Our findings demonstrate that mice, regardless of sex, increased locomotion, and aggression towards conspecifics after FNR, with no changes in anxiety-like, depression-like, or non-aggressive social behaviors. FNR induced significant neural activity changes in 13 subregions of thalamus, iso-cortex and hippocampus including the prelimbic, ACC, hippocampus, dorsal thalamus, cuneiform nucleus, pons, and pallidum areas. FNR also shifts the brain network towards a more global processing mode. Our novel FNR paradigm produces a frustration effect resembling the symptoms observed in youth with severe irritability. The novel behavioral paradigm and brain regions identified to be selectively activated by it lay the groundwork for further mechanistic studies of frustration and irritability in rodents.

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Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

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Program #/Poster #: PSTR364.25/QQ18

Topic: G.05. Mood Disorders

Title: The interaction between chronic pain and depression on the onset and exacerbation in parallel rodent strains

Authors: *Y. CAI, Q. GUO, Y. ZHANG;
Fudan Univ., Shang Hai, China

Abstract: his study was aimed to comprehensively investigate the interaction between chronic pain and depression on the onset and exacerbation with each other, and compare different rodent strains/species in two validated pain/depression models. The trigeminal neuralgia (TN) and learned helplessness (LH) models were established on three independent strains. The TN model was prepared by chronic constriction injury to the unilateral infraorbital nerve (CION); the LH model was induced by inescapable electric foot-shocks. The depressive/anxiety-like behaviors were assessed by the open field test, elevated plus maze, active avoidance shuttle box test and forced swimming test; the mechanical allodynia was assessed by the von Frey test. The antidepressant clomipramine and the analgesic pregabalin were i.p. injected to determine whether treatment of one disease could attenuate the other which was originated from the former. Moreover, CION+LH combined stress was applied to observe the potential exacerbating effect. For Wistar rats and C57BL/6J mice, the TN model developed depressive/anxiety-like behaviors and the LH model developed hyperalgesia behavior. Comparatively, SD rats showed a higher resilience to distress. Clomipramine counteracted the LH induced depressive/anxiety disorders and LH developed hyperalgesia; pregabalin attenuated CION induced hyperalgesia and CION developed depressive/anxiety disorders. They couldn't challenge the nociceptive or depressive behavior derived from the state they don't target. Finally, aggravated depressive-like and hyperalgesia behaviors were found under the combined stress compared with LH alone. These findings reveal that pain and depression could develop into each other, but different resilience exists among strains. Combined stress partially exacerbates pain and depression.

Disclosures: **Y. Cai:** None. **Q. Guo:** None. **Y. Zhang:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.26/QQ19

Topic: G.05. Mood Disorders

Support: Centre National de la Recherche Scientifique, contract UPR3212
University of Strasbourg, contract UPR3212
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ANR, ANR-20-CE37-0014
Région Grand-Est, FRCR CLueDol

Title: Alterations of the habenulo-tegmental pathway in a mouse model of chronic pain-induced depression

Authors: P.-A. DERRIEN, B. MULLER, V. ANDRY, R. WAEGERT, Q. LEBOULLEUX, N. WILLEM, M. KREMER, Y. GOUMON, I. YALCIN, *M. BARROT, **J. KAUFLING**;
INCI - CNRS UPR3212, Strasbourg, France

Abstract: Neuropathic pain is a chronic condition caused by an injury or disease of the somatosensory system and around 30% of these chronic pain patients will develop a major depressive disorder (MDD) during their lifetime. Dopaminergic (DA) neurons of the ventral tegmental area (VTA), part of the mesolimbic system, are involved in various functions, such as motivation and reward, and alterations in these neurons have been reported in chronic pain and depression. The lateral habenula (LHb), another key region in mood regulation sends projections to VTA DA neurons, with a minor direct excitatory and a major indirect inhibitory pathway. Our project was to identify whether VTA DA neurons, and in particular those receiving LHb inputs, may be altered in the comorbidity of chronic pain and depression. Using single-cell recordings of optogenetically identified VTA DA neurons in anesthetized mice, we observed alterations in the basal activity of DA neurons located in the anterior part of the VTA in a cuff mouse model of neuropathic pain-induced depressive-like behaviors. Using mass spectrometry, we then observed no change in mice expressing pain-depression comorbidity compared to control mice for basal dopamine concentration in the VTA and NAc (nucleus Accumbens), a major VTA DA neurons output. To go further and assess the consequences of the development of pain-depression comorbidity on dopamine release in the NAc induced by overall stimulation of VTA DA neurons, we used a longitudinal approach. Using fiber photometry and Dlight, a dopamine biosensor, we thus recorded the time course of variation in DA release in the NAc induced by optogenetic stimulation of VTA DA neurons in awake animals. Finally, to investigate specific alterations of the LHb-VTA pathway in the model of chronic pain-induced depression, we recorded VTA DA neurons calcium activity and NAc dopamine release using fiber photometry, upon optogenetic stimulation of LHb in anesthetized mice. Overall, our data reveal differential alterations in several subpopulations of VTA DA neurons in the chronic pain depression comorbidity.

Disclosures: P. Derrien: None. **B. Muller:** None. **V. Andry:** None. **R. Waegert:** None. **Q. Leboulleux:** None. **N. Willem:** None. **M. Kremer:** None. **Y. Goumon:** None. **I. Yalcin:** None. **M. Barrot:** None. **J. Kaufling:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

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Program #/Poster #: PSTR364.27/QQ20

Topic: G.05. Mood Disorders

Support: R25 - NS080686
GM - 122646

Title: Neural Pathophysiological Mechanisms of Premenstrual Dysphoric Disorder (PMDD)

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Abstract: Premenstrual dysphoric disorder (PMDD), a cyclical disorder with significant impacts on women's mental health, is characterized by increased sensitivity to stress during the luteal phase. This exciting new data and review consolidates recent findings on the neural pathophysiology of PMDD, focusing on hormonal involvement, immune factors, genetic susceptibility, and stress responses. Despite early hypotheses, recent evidence suggests that PMDD is not necessarily linked to abnormal estrogen or progesterone levels. Instead, a negative correlation has been found between premenstrual estrogen levels and anxiety in PMDD. Furthermore, a higher allopregnanolone/progesterone ratio is observed in women with PMDD compared to controls, pointing to a potential dysfunction in progesterone metabolism. Emerging evidence points to genetic susceptibilities, specifically variants in the estrogen receptor alpha gene, in PMDD pathophysiology. Dysregulations in the hypothalamic-pituitary-adrenal (HPA) axis, characterized by lower cortisol and higher allopregnanolone levels, have also been identified during the luteal stage in PMDD patients. Animal models have helped elucidate the intricate interplay between fluctuating sex steroids and stress response within the dopaminergic system. Our studies reveal a correlation between electrophysiological signatures in the ventral tegmental area (VTA) across estrous cycles and susceptibility to alterations in dopamine-related social interaction behaviors during stress acquisition, aligning with clinical observations in depression, anxiety, and substance use disorders. This work underscores the complex neural pathophysiology of PMDD, emphasizing the need for continued research into this disorder. An improved understanding of PMDD's neurobiological underpinnings will facilitate the development of targeted and effective treatments for affected women.

Disclosures: S. Sheik: None. **M. Shanley:** None. **A.K. Friedman:** None.

Poster

PSTR364. Animal Models of Mood disorders: Circuits and Behavior Paradigms

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR364.28/QQ21

Topic: G.05. Mood Disorders

Title: A neuro-immune circuit mediates cancer cachexia-associated apathy

Authors: S. STAROSTA[1], A. ZHU[2], M. FERRER GONZALEZ[5], J. HOU[2], F. LUCANTONIO[2], R. MUNOZ CASTANEDA[5], S. EVANS[2], A. V. KRAVITZ[6], P. OSTEN[5], T. JANOWITZ[5], M. PIGNATELLI[3], ***A. KEPECS**[4];
[1]Washington Univ. in St. Louis, St. Louis, MO; [2]Washington Univ. in St. Louis, St Louis, MO; [3]Psychiatry, Washington Univ. in St. Louis, ST. LOUIS, MO; [4]Neurosci. and Psychiatry, Washington Univ. in St. Louis, St. Louis, MO; [5]Cold Spring Harbor Lab., Cold Spring Harbor, NY; [6]Washington Univ. In St Louis, Saint Louis, MO

Abstract: Cachexia, a severe wasting syndrome associated with multiple inflammatory conditions, precipitates multi-organ dysfunction and is often fatal. Patients with cachexia often battle both physiological detriments and psychological hardships like clinical depression,

extreme fatigue, and apathy. The biological mechanisms linking cachexia to these symptoms, and distinguishing cachexia's physiological effects from end-of-life emotional distress, remain elusive. Using a mouse C26 carcinoma model that replicates impaired feeding and metabolic traits seen in humans, we explored the impact of cancer cachexia on motivational states and the underlying neural circuit mechanisms. We began with extensive behavioral phenotyping in an array of effort-guided, reward-guided and affective decision tasks. Cachectic mice exhibited heightened effort-sensitivity, apathy-like behavior, without other affective symptoms like anhedonia or despair. Brain regions affected by cachexia were identified through brain-wide, cellular resolution screening of the immediate early gene cFos. This screen pinpointed candidate regions involved in sensing inflammation states and regulating motivation and feeding. Based on this, we performed in vivo and ex vivo experiments to delineate a cytokine-sensing brainstem-to-basal ganglia circuit that produces the apathy-like symptoms. This circuit detects the inflammatory cytokine IL-6 at cachexia onset, and translates it into decreased mesolimbic dopamine, thereby increasing behavioral effort-sensitivity. We alleviated these symptoms through optogenetic stimulation of mesolimbic dopamine neurons, or with anti-IL6 treatment. These results indicate that cachexia-induced apathy is a unique aspect of the syndrome, distinct from anorexia yet potentially common across IL6-induced inflammatory conditions. Our findings uncover a central neural circuit that senses inflammation and orchestrates behavioral changes, providing mechanistic insights into the connection between chronic inflammation and affective symptoms relevant to psychiatric disorders like depression.

Disclosures: **S. Starosta:** None. **A. Zhu:** None. **M. Ferrer Gonzalez:** None. **J. Hou:** None. **F. Lucantonio:** None. **R. Munoz Castaneda:** None. **S. Evans:** None. **A.V. Kravitz:** None. **P. Osten:** None. **T. Janowitz:** None. **M. Pignatelli:** None. **A. Kepecs:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.01/QQ22

Topic: G.09. Drugs of Abuse and Addiction

Title: Plasticity of dopamine, acetylcholine, and behavioral dynamics across timescales during pscyhostimulant sensitization

Authors: *G. LANGE[1], F. GNAZZO[2], R. P. FAUST[3,4], J. A. BEELER[3];
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[3]Psychology, Queens Col. CUNY, Flushing, NY; [4]Queens Col. CUNY, New York, NY

Abstract: The complex behavioral changes during psychostimulant sensitization are partly mediated by dopamine (DA) and acetylcholine (Ach) interactions, whose understanding remains incomplete. Previous research showed that disturbing this bidirectional circuit can augment psychostimulant response and sensitization but it is unclear how. We monitored DA and Ach simultaneously in the nucleus accumbens shell of wild-type mice (n=16, 8 females) during

sensitization with cocaine (IP, 20 mg/kg) or amphetamine (IP, 2 mg/kg). We overcame the challenges of photometry in monitoring DA levels over longer timescales by developing a method that accurately adjusts for photobleaching while preserving psychostimulant-induced changes in dopamine levels and dynamics. This allowed us to observe increased DA concentrations during sensitization at different time scales, achieved via wavelet analysis to decompose DA signals into separate frequency domains. The results demonstrated effects of psychostimulants and sensitization beyond the typically observed "tonic" timescale: While total dopamine concentration increased, phasic dopamine fluctuation attenuates, and large transients become less likely.How does this observation relate to behavior? We recorded depth videos of the mice and used DeepLabCut and MoSeq to segment their behavior. Mice displayed not only increased locomotor activity but also repetitive grooming and reduced environmental exploration. A linear classifier trained on MoSeq syllables was able to distinguish between cocaine, amphetamine, and saline injections with high accuracy, superior to conventional scalar measures of locomotor activity that could only classify saline injections. Moreover, behavior between individual mice did not change into the same direction but diverged during sensitization. A linear classifier could classify whether an animal previously received psychostimulants with moderate accuracy on the held-out dataset. Ach activity displayed short events, occurring irregularly at a frequency of 1-2 Hz. Drug sensitization led to a significant reduction in the amplitude of these events, mirroring the DA concentration. Notably, our data suggests a relationship where Ach events frequently preceded large DA transients, suggesting that these two neurotransmitters may modulate each other during drug-induced behavior changes. These findings highlight the richness of behavioral changes during drug sensitization, beyond simple locomotion, and reveal the intricate dynamics between DA, Ach, and behavior.

Disclosures: G. Lange: None. **F. Gnazzo:** None. **R.P. Faust:** None. **J.A. Beeler:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR365.02/QQ23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DP1DA046587

Title: Three-dimensional genome architecture of mouse nucleus accumbens medium spiny neuron subtypes in cocaine use disorder

Authors: *J. M. CHITAMAN[1,2], R. S. JASROTIA[1,2], H. XU[1,2], Y. LI[1,2], V. MALYSHEVA[3], P. FRASER[1], J. FENG[1,2];
[1]Biol. Sci., [2]Program in Neurosci., Florida State Univ., Tallahassee, FL; [3]VIB-UAntwerp Ctr. for Mol. Neurol., Univ. of Antwerp, Antwerp, Belgium

Abstract: In the mammalian brain, three-dimensional (3D) genome organization regulates cell type-specific gene expression. This regulatory process involves long-range *cis*-chromosomal interactions (>20 kb) between gene promoter regions and noncoding DNA sequences (e.g., enhancers) that facilitate transcriptional programs required for cognition and adult neuroplasticity. Recently, it has become increasingly evident that alterations in these 3D gene regulatory networks are implicated in various neurological and neuropsychiatric conditions, including substance use disorders. However, the extent to which the use of illicit substances can affect 3D genome architecture in the adult brain remains unexplored. To address this, we applied chromosome conformation capture with high-throughput sequencing (Hi-C) and Promoter Capture Hi-C (PCHi-C) in medium spiny neurons (MSNs) of the nucleus accumbens (NAc) following intravenous cocaine self-administration in male mice. We first mapped hierarchical 3D genome architecture (i.e., chromatin compartments, topologically associating domains, and DNA loops) in functionally distinct NAc D1 and D2 MSN subpopulations. Additionally, we integrated D1 PCHi-C with other cell type-specific omics datasets (e.g., RNA-seq, ATAC-seq, H3K27Ac ChIP-seq) to characterize the effects of cocaine use on the promoter interactome in NAc D1 MSNs. We found that numerous cocaine-induced transcriptional changes at synaptic plasticity genes associated with addiction are accompanied by altered promoter interactions following cocaine use. Moreover, analysis of differentially accessible regions within the D1 promoter interactome revealed enrichment of binding motifs for several novel transcription factors, suggesting potential regulatory roles in aberrant gene expression associated with cocaine use disorder. Therefore, our study elucidates features of higher-order gene regulation in NAc medium spiny neurons after cocaine use and provides a framework to explore novel molecular mechanisms of cocaine action in the brain. These findings not only offer an unprecedented view of the 3D genome organization in discrete neuronal subpopulations of the adult brain, but may also pave the way for improved diagnostic and therapeutic targets for substance use disorders in the future.

Disclosures: J.M. Chitaman: None. **R.S. Jasrotia:** None. **H. Xu:** None. **Y. Li:** None. **V. Malysheva:** None. **P. Fraser:** None. **J. Feng:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.03/QQ24

Topic: G.09. Drugs of Abuse and Addiction

Support: Commonwealth of Pennsylvania CURE Addiction Center of Excellence: Brain Mechanisms of Relapse and Recovery (Childress)
NIDA U54 DA039002 Cocaine Cooperative Medication Development Center
NIDA R01 DA039215 (Childress, PI)
NIDA UG1DA050209 Clinical Laboratories with Integrated Neuroscience (Kampman and Childress MPI)

Title: The amygdala as fortune-teller: Poor drug use outcome in cocaine patients is linked to heightened amygdala resting functional connectivity with subcortical motivational ("GO!") regions, and lower connectivity with cortical regulatory ("STOP!") regions

Authors: *A. CHILDRESS, K. JAGANNATHAN, P. S. REGIER, T. R. FRANKLIN, R. R. WETHERILL, D. D. LANGLEBEN, M. J. GAWRYSIAK, K. M. KAMPMAN, C. P. O'BRIEN; Univ. PENN Perelman Sch. Med., Philadelphia, PA

Abstract: AIM: Our amygdalae "GO!" into action at the first whiff of danger or reward; they are the brain's critical "first responders". A bigger brain response (too much fear, too much reward motivation) is not always better. For example, in cocaine patients, an over-response of the amygdala and its interconnected motivational nodes (e.g., midbrain, ventral striatum, ventral pallidum) to drug reward cues predicts poor clinical outcome. Here, we examined whether the amygdala's functional connectivity with other motivational nodes - and with cortical regulatory ("STOP!") regions -- might also have 'fortune-telling' ability for drug use outcome, *even when the brain is at rest*.**METHODS:** Using BOLD fMRI (3T), we collected six-minute, eyes-open, resting scans in detoxified, treatment-seeking cocaine inpatients (n=25). Resting amygdala connectivity (L and R) data were analyzed within SPM 12. The resulting connectivity maps (r to Z transformed maps) were examined separately for GOOD (&lt 30% cocaine urines pos/missing across 12 outpt. weeks; n=9) and POOR (&gt 90% cocaine urines pos/missing; n=16)outcome subgroups; these were subsequently compared (t-test, p &lt 0.05; thresholded 2 &lt t &lt 4 for display).**RESULTS:** As found in prior resting data from cocaine populations, there was robust positive "intra-limbic" connectivity between the amygdala (both L and R) and multiple nodes of the motivational ("GO!") network. Importantly, individuals who proceeded to POOR (vs. GOOD) drug use outcomes had stronger positive amygdala resting connectivity with several subcortical motivational nodes (midbrain, ventral striatum, ventral pallidum), while those proceeding to GOOD (vs. POOR) outcomes demonstrated stronger inverse connectivity with dorsal cortical (regulatory) regions.**CONCLUSION**: These preliminary data suggest that amygdala's "fortune-telling" ability for clinical outcomes can be demonstrated even when the brain is "at rest", without evocative cue tasks. This offers a practical advantage, as resting functional connectivity scans can be collected without special task expertise, and they are a familiar feature in large datasets (e.g., ABCD). From a neuroscience perspective, these data underscore the critical function of the amygdala and its interconnected motivational - and regulatory -- circuitry in shaping the clinical outcome for psychopathologies related to amygdala function, whether in the domain of aversive (e.g., anxiety/stress disorders/PTSD) or appetitive (e.g., drug reward) motivation. These findings encourage targeted novel (behavioral, pharmacologic, or neuromodulatory) interventions to boost or to restore regulation of the amygdala.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.04/QQ25

Topic: G.09. Drugs of Abuse and Addiction

Support: R01AG072897
R21NS108128
R01AA025784

Title: Ca^{2+} Transients in the Secondary Motor Cortex are Closely Associated with Cocaine Taking

Authors: *Y. CHEN[1], H. FU[1], M. LANGE[1,3], A. KORADA[1,3], C. RAYANKI[1,3], D. LAI[2], S. FANG[4], C. GUO[1], Y.-Y. MA[1,5];
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Abstract: We recently reported that the risk of cocaine relapse was attributable to the hyperexcitability of cortical pyramidal neurons (CPNs) in the secondary motor cortex (M2). This effect was detected 45 days, but not 1 day, after intravenous self-administration (IVSA) of cocaine. The question to be addressed here was what causes the hyperexcitability of M2 CPNs. Our hypothesis was that M2 neurons were affected directly by cocaine taking behaviors. This hypothesis was tested by monitoring neuronal activity in M2 using in *vivo* MiniScope Ca^{2+} imaging from C57BL/6J mice when they have access to IV cocaine delivery (IV-C) contingent to active lever press (ALP) but not to inactive lever press (ILP). The association between the Ca^{2+} transient activity and ALP, ILP and IV-C was analyzed during two 15-min time windows (i.e., segment 0-15 min denoted S1, and segment 46-60 min denoted S4) of the 1-hr daily IVSA session on Day 1 (D1) and 5 (D5). Ca^{2+} activity of M2 neurons detected with significant linear association was categorized by the most significantly associated factor among ALP, ILP and IV-C. We found more neurons in M2 were classified as “IV-C” than those classified as “ALP” or “ILP”. Excitingly, we also found that ALP was correlated to the amplitude of the Ca^{2+} transients, particularly 1 min before the performance of ALP. This association was detected in both D1S1 and D1S4, but not in D5S1 or D5S4. In addition, IV-C was correlated to the frequency Ca^{2+} transients, particularly within 3 min after IV-C. This association was detected only in D1S1. Our data provide evidence of significant effects of cocaine taking behaviors on M2 neuronal activities.

Disclosures: Y. Chen: None. **H. Fu:** None. **M. Lange:** None. **A. Korada:** None. **C. Rayanki:** None. **D. Lai:** None. **S. Fang:** None. **C. Guo:** None. **Y. Ma:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Topic: G.09. Drugs of Abuse and Addiction

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Ministerio de Ciencia e Innovación / Agencia Estatal de Investigación Grant PID2020-117989RA-I00

Title: Pharmacological characterization of (R)-ketamine at the mu-opioid receptor: implications for abuse liability

Authors: *R. BUDINICH[1], M. LEVINSTEIN[1], J. BONAVENTURA[2,3], M. MICHAELIDES[1,4]; [1]Natl. Inst. on Drug Abuse, Baltimore, MD; [2]Departament de Patologia i Terapèutica Exptl., Univ. de Barcelona, Catalonia, Spain; [3]Neuropharm. and Pain Group, Inst. d'Investigació Biomèdica de Bellvitge, Catalonia, Spain; [4]Dept. of Psychiatry and Behavioral Sci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Ketamine and its optical isomers (R)-ketamine and (S)-ketamine are being used or investigated as antidepressant medications, with (R)-ketamine currently undergoing clinical trials. Due to the high comorbidity of depression and substance use disorder, the abuse liability of antidepressant medications is important to consider. Racemic ketamine ((R,S)-ketamine) has known abuse liability, which we previously found to be largely dependent on (S)-ketamine and mediated by the mu-opioid receptor (MOR) - the primary pharmacological target of drugs of abuse such as morphine, heroin, and fentanyl. Compared to (S)-ketamine, (R)-ketamine is generally considered to have low abuse liability, however (R)-ketamine also binds to and activates MORs. While we previously found that (R)-ketamine binds to MOR with 3-4x lower potency (Ki = 19 ± 5 µM; EC_{50} = 34 ± 14 µM) and ~20% lower efficacy (E_{max} = 39 ± 3 %) than (S)-ketamine (Ki = 7 ± 3 µM; EC_{50} = 9 ± 2 µM; E_{max} = 48 ± 3 %), the in vivo interactions of (R)-ketamine at the MOR and (R)-ketamine's abuse liability have not been thoroughly assessed at equipotent doses expected to engage MORs, even if such doses are far beyond the therapeutically efficacious doses of (R)-ketamine used in preclinical models of depression. We used [^{35}S]GTPγS autoradiography to determine the ability of (R)-ketamine to activate MOR. We then used mass spectrometry and receptor occupancy autoradiography to investigate brain and plasma drug concentrations as well as MOR occupancy in vivo after high doses that would be expected to confer abuse liability. We also used the intravenous self-administration (IVSA) procedure in rats to assess the abuse liability of (R)-ketamine. Finally, we assessed the physiological and behavioral consequences of repeated exposure to IVSA-relevant doses of (R)-ketamine on MOR density (using autoradiography) and sensitivity (using [^{35}S]GTPγS), and subsequent heroin self-administration behavior. The results from these experiments will be important to better understand the potential consequences of using (R)-ketamine as a treatment for depression.

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collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Received research funding from AstraZeneca, Received research funding from Redpin Therapeutics, Received research funding from Attune Neurosciences.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.06/QQ27

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant 5U01DA051947-03

Title: Heroin-induced genomic regulation of cells in the Ventral Pallidum and Nucleus Accumbens

Authors: *B. GRISSOM[1], R. R. CAMPBELL[3], M. E. CORTES-GUTIERREZ[4], S. MITRA[5], B. QAMAR[1], D. M. DIETZ[6], M. LOBO[7], S. A. AMENT[2];
[2]Dept. of Psychiatry and Inst. for Genome Sci., [1]Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Anat. and Neurobio., Univ. of Maryland Sch. of Medicin Program In Neurosci., Baltimore, MD; [4]Univ. of Maryland, IGS, Baltimore, MD; [5]Biomed. Sci., Marshall Univ., Huntington, WV; [6]SUNY At Buffalo, Buffalo, NY; [7]Anat. and Neurobio., Univ. of Maryland SOM, Baltimore, MD

Abstract: Opioid use disorder (OUD) is associated with long term changes to the mesolimbic dopamine reward system, but the cell type diversity within these brain regions and the molecular adaptations associated with substance use and addiction are poorly understood. To address this, we sequenced the nuclear transcriptomes (snRNA-seq) and chromatin accessibility states (snATAC-seq) of 279,219 cells from the ventral pallidum (VP) and nucleus accumbens (NAc) of rats in the context of heroin self-administration. The VP and NAc are interconnected basal ganglia regions with well-established roles in OUD. We generated a multimodal atlas for the diversity of neuronal and non-neuronal cells in each brain region, including ten transcriptionally distinct subtypes of spiny projection neurons in the NAc and >20 transcriptionally distinct neuronal subtypes in the VP. We characterized cell type-specific changes in gene expression and chromatin accessibility in rats that self-administered heroin vs. controls at 1 or 14 days of abstinence to gain insight into both acute and persistent effects on gene regulation. We integrated both data modalities to model gene regulatory networks mediating the effects of heroin on neuroplasticity and neuroinflammation. Our results provide insight into the gene regulatory mechanisms mediating the persistent effects of opioids on the brain. The identification of high-impact high-specificity transcriptional mechanisms will open promising new avenues for treatment research.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.07/QQ28

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Drug support program

Title: Assessing the impact of pre-exposure to the novel synthetic cathinone eutylone on the aversive effects of cocaine and MDMA.

Authors: *N. GHASEM ARDABILI, A. RILEY;
American Univ. DC, Wasington, DC

Abstract: Assessing the impact of pre-exposure to the novel synthetic cathinone eutylone on the aversive effects of cocaine and MDMA. Nina Ardabili, Shira Tan and Anthony L. Riley, Psychopharmacology Laboratory, Department of Neuroscience, Center for Neuroscience and Behavior, American University, Washington, DC 20026 Abstract **Background**: While animals readily avoid consumption of solutions paired with various drugs of abuse (indicative of the aversive effects of these drugs), a history with these compounds significantly attenuates this avoidance. Given that drug use and abuse are a function of the balance between the drug's rewarding and aversive effects, any reduction in its aversive effects increases abuse liability. The effects of drug pre-exposure have also been reported when the pre-exposed and conditioning drug are different, i.e., the cross-drug pre-exposure effect. The present study extended this analysis to eutylone, a relatively new synthetic cathinone reported to be used serially with other drugs. Eutylone exhibits a distinctive neurochemical profile, functioning as a dopamine (DA) reuptake inhibitor and a serotonin (5-HT) substrate releaser. Due to these dual mechanisms of action, it could possibly impact the aversive effects of other abused drugs that share these mechanisms of action. **Methods:** To test this, in the present experiment, adult male and female Sprague-Dawley rats were exposed to eutylone 20 mg/kg and 10 mg/kg, respectively, or its vehicle every 4^{th} day for a total of four to five injections prior to taste avoidance conditioning in which saccharin was paired with either vehicle, cocaine (20 mg/kg) or MDMA (3.2 mg/kg) every other day for a total of four injections. **Results:** Cocaine and MDMA each induced significant taste avoidance relative to controls [$F(2,18)=17.039$, $p<0.05$, males; $F(2,42)=296.386$, $p<0.05$, females], but there was no effect of eutylone pre-exposure on this avoidance as assessed in both one bottle [$F(2,18)=0.094$, $p=0.911$, males; $F(2,42)=0.228$, $p=0.797$, females] and two-bottle $F(2,18)=1.437$, $p=0.264$, males; $F(2,42)=0.145$, $p = 0.865$, females] tests.**Conclusions:** That eutylone failed to impact avoidance induced by cocaine and MDMA despite shared neurochemical activity (monoamine reuptake inhibition for cocaine and 5-HT substrate release for MDMA) suggests that eutylone's hybrid neurochemical activity results in a unique

interoceptive effect that does not overlap sufficiently with either cocaine or MDMA, preventing the attenuation of the acquisition of taste avoidance generally produced by drug history. Such effects may have implications for the continued serial use of these compounds.

Disclosures: N. Ghasem Ardabili: None. **A. Riley:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.08/RR1

Topic:

Support: K99/R00 DA048970
P30DA048742

Title: Identification of neurotensin receptor 1-expressing cells using genetically encoded reporters

Authors: *N. FOSTER, C. LEMCHI, E. MARRON FERNANDEZ DE VELASCO, L. SLOSKY;
Pharmacol., Univ. of Minnesota, Minneapolis, MN

Abstract: The neurotensin receptor 1 (NTSR1) is a G protein-coupled receptor (GPCR) that acts centrally to modulate dopamine, glutamate, and GABA neurotransmission. Dysregulation of neurotensin-NTSR1 signaling is implicated in the pathophysiology of schizophrenia, Parkinson's disease, and substance use disorders, making it an attractive therapeutic target for multiple brain diseases. Our understanding of these diseases and NTSR1 drug development efforts would be further by a mechanistic understanding of how NTSR1 regulates brain circuits. Critically, the regional and cellular distribution of NTSR1 has not been fully characterized. To address this, we generated NTSR1 fluorescent reporter mouse lines, using a knock-in NTSR1-Cre mouse and three Cre-inducible fluorescent reporter mouse lines that permit visualization of axons and/or cell bodies. In these lines, Cre recombinase drives expression of either cytosolic tdTomato ('tdTomato' mouse), a GFP-tagged ribosomal subunit ('TRAP' mouse), or an anti-actin nanobody conjugated to mKO ('Chromobody' mouse). We crossed NTSR1-Cre mice to fluorescent reporter lines to drive fluorophore expression selectively in NTSR1-expressing cells. Using widefield fluorescent imaging of fresh brain sections, we generated macro-level images for determination of NTSR1 regional distribution and we acquired higher magnification images of selected areas of interest, including the basal ganglia. To assess regional connectivity, fixed whole brains were cleared using the polyethylene glycol (PEG)-associated solvent system (PEGASOS) and imaged on the Cleared Tissue LightSheet (CTLS) microscope. Images were registered to Allen Mouse Brain Common Coordinate Framework. For each reporter line, Cre-dependent fluorescence was observed. Regional expression patterns of all three reporters were comparable. The TRAP reporter produced more punctate signal, permitting automated

identification and quantification of cell bodies. The tdTomato and Chromobody reporters produced more diffuse signal but permitted axon visualization. The cortex, hippocampus, thalamus, and midbrain were among the regions with the highest abundance of NTSR1-expressing cell bodies. Dense cortico-thalamic projections from NTSR1-expressing neurons were identified. A limitation of this approach is that cells with developmentally constrained expression of NTSR1 cannot be discriminated from cells with ongoing NTSR1 expression. We are currently using a viral approach in adult mice to delineate the contribution of developmental NTSR1 expression to the expression patterns observed in the reporter lines.

Disclosures: N. Foster: None. **C. Lemchi:** None. **E. Marron Fernandez de Velasco:** None. **L. Slosky:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.09/RR2

Topic: G.09. Drugs of Abuse and Addiction

Support: DA054368
DA053261
DA042888
DA07242

Title: The inhibition of MAG Lipase reduces opioid reward via CB1 receptors without altering analgesia

Authors: *A. MARTINEZ-RIVERA[1], R. N. FETCHO[2], L. BIRMINGHAM[3], J. XU[4], R. YANG[1], Y. PAN[4], L. A. BRIAND[3], F. S. LEE[1], A. M. RAJADHYAKSHA[1];
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Abstract: The interaction between the opioid and endocannabinoid systems suggests that targeting the endocannabinoid system (eCB) could be a viable approach to develop new pharmacological treatments in conjunction with opioid-based therapies. The eCB signaling is controlled by two main enzymes: monoacylglycerol lipase (MAGL), responsible for regulating 2-Arachidonoylglycerol (2-AG), and fatty acid amide hydrolase (FAAH), which regulates anandamide (AEA). Using conditioned place preference and self-administration experiments conducted with both male and female mice (C57BL/6 mice, 8-12 weeks old), we demonstrate that systemic inhibition of MAGL that enhances 2-AG levels, significantly reduces the rewarding effects of opioids (morphine and oxycodone), without altering their analgesic effects. Conversely, pharmacological inhibition of FAAH has minimal impact on opioid reward or analgesia. To identify the brain site of action, animals received JZL184 directly into the Ventral Tegmental Area (VTA) and as with systemic treatment, intra-VTA JZL184 attenuated morphine

reward without altering analgesia. The observed effects are mediated by CB1 receptors (CB1Rs), as systemic inhibition of CB1Rs using the inverse agonist AM251 (dose dependently), counteracted the JZL184-induced blunting of morphine reward. By using fiber photometry with calcium and dopamine (DA) fluorescent sensors, we found that JZL184 reduces the activity and DA neurotransmission related to opioid reward in the nucleus accumbens (NAc), a brain region associated with reward processing. These findings indicate that enhancing 2-AG counteracts the rewarding properties of opioids and presents a potential additional therapeutic strategy for opioid-related analgesic treatments. All experiments include a sample size of 8 or more animals, and differences among treated groups were established after performing the corresponding statistical analyses with p-values equal to or below 0.05.

Disclosures: **A. Martinez-Rivera:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Weill Cornell Medicine, Pritzker Neuropsychiatric Disorders Research Consortium-Cornell. **R.N. Fetcho:** None. **L. Birmingham:** None. **J. Xu:** None. **R. Yang:** None. **Y. Pan:** None. **L.A. Briand:** None. **F.S. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Weill Cornell Medicine. **A.M. Rajadhyaksha:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Weill Cornell Medicine, Pritzker Neuropsychiatric Disorders Research Consortium-Cornell.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.10/RR3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH Intramural Research Program

Title: Partial D2 receptor deletion causes a sex-dependent reduction of striatal dopamine signals

Authors: *E. S. SWANSON[1], V. A. ALVAREZ[1,2];
[1]NIH, Natl. Inst. of Mental Hlth. (NIMH), Bethesda, MD; [2]NIH, Natl. Inst. on Alcohol Abuse and Alcoholism (NIAAA), Bethesda, MD

Abstract: Dopamine signaling in the striatum is a crucial component of drug reinforcement that, when dysregulated, is thought to increase the risk for substance use disorders. In particular, low availability of the dopamine D2 receptor (D2R) has traditionally been linked to the development of substance use disorders in humans. Yet the mechanisms by which low levels of striatal D2Rs generate vulnerability are not fully understood. In this study, we directly manipulate the levels of striatal D2Rs in transgenic mice to explore the impact on dopamine signals in the striatum. Mice with low availability of D2Rs were generated through a single-allele deletion of the *Drd2* gene that was targeted to indirect-pathway spiny projection neurons in the striatum. These mice,

iSPN-Drd2 HETs, have a ~25-30% reduction in D2-like agonist binding in the striatum compared to littermate controls that are bred alongside these mice from the cross between Adora2a-cre x Drd2*loxP/wt* mice. Using fast-scanning cyclic voltammetry, we recorded *ex vivo* electrically-evoked dopamine signals in the dorsomedial striatum (DMS) of male and female mice of both genotypes. Relative to controls, we find that the magnitude of dopamine transmission in mice expressing partial D2R deletion is significantly reduced at baseline. In these mice, electrical stimulation produces an average peak signal amplitude of 26 ± 1.2 nA (n = 43 slices / 17 animals), while control mice reach 33 ± 1.8 nA (n = 35/12). Interestingly, we observe sex differences in the magnitude of the dopamine signals in the DMS. In male littermate control mice, the DMS shows larger dopamine transients (37 ± 2.5 nA, n = 17/6) compared to females (30 ± 2.2 nA, n = 18/7). The sex difference was present, although less pronounced in mice with low striatal D2Rs. Later, we block nicotinic acetylcholine receptors, GABAA and GABAB receptors, and kappa opioid receptors with a variety of drug antagonists while recording dopamine signals. In so doing, we aim next to identify systems that regulate the mechanism of D2R modulation of dopamine signaling in the striatum. This work will facilitate our understanding of the role D2Rs plays in drug reinforcement, and may shed light on the mechanism by which only certain individuals develop substance use disorders.

Disclosures: E.S. Swanson: None. **V.A. Alvarez:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant P41GM104603
NIH Grant R21HL131554
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Title: Restoring cocaine and methamphetamine-induced alterations of HS and CS composition and sulfate contents in the lateral hypothalamus and striatum ameliorates anxiety and drug seeking behaviors in mice during withdrawal.

Authors: *R. MACCIONI[1], M. K. SETHI[2], T. KAWAMURA[1], V. CANONIGO[1], J. CHEN[1], J. ZAIA[2], P. SANNA[1];
[1]Scripps Res. Inst., San Diego, CA; [2]Dept. of Biochem., Ctr. for Biomed. Mass Spectrometry, Boston Univ. Sch. of Med., Boston, MA

Abstract: Drug abuse is a major concern, with few therapeutic options. Heparan sulfate (HS) and chondroitin sulfate (CS) interact with a plethora of growth factors and their receptors and have profound effects on cellular signaling. Thus, targeting these dynamic interactions might represent a potential novel therapeutic modality. Here, we utilized mass spectrometry-based glycomics to understand the effects of cocaine and methamphetamine (METH) on HS and CS from two brain regions extensively involved in drug abuse: the lateral hypothalamus (LH) and striatum (ST). We observed that cocaine and METH significantly alter HS and CS abundances and sulfate contents and composition. In particular, repeated METH or cocaine treatments reduced CS 4-*O*-sulfation and increased CS 6-*O*-sulfation vs. saline-treated control mice. Since C4S and C6S exercise differential effects on axon growth, regeneration and plasticity, these changes likely contribute to drug-induced neural plasticity in these brain regions. Here, we observed that increasing CS *4-0* levels in the LH by adeno-associated virus (AAV) delivery of an shRNA to Arylsulfatase B (N-acetylgalactosamine-4-sulfatase, ARSB) ameliorated anxiety and prevented the expression of preference for cocaine in a novelty induced conditioned place preference test during cocaine withdrawal. Together, our study supports the role of HS and CS in drug abuse and suggests that manipulation of HS and CS proteoglycans can represent novel therapeutic strategies for stimulant abuse.

Disclosures: R. Maccioni: None. **M.K. Sethi:** None. **T. Kawamura:** None. **V. Canonigo:** None. **J. Chen:** None. **J. Zaia:** None. **P. Sanna:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.12/RR5

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 DA033641

Title: Multiomic single nuclei sequencing identifies potential molecular mechanisms for cocaine resistance and methamphetamine susceptibility in drug-sired male offspring

Authors: *S. E. SWINFORD-JACKSON[1], A. JADALI[2], B. N. PHAN[3], M. SARMIENTO[5], Y. ZHU[4], S. MANKAME[1], S. J. WOROBEY[1], T. J. SACKO[1], D. GANGEMI[1], A. R. PFENNING[3], K. Y. KWAN[2], R. P. HART[6], R. C. PIERCE[1];
[1]Brain Hlth. Inst. and Dept. of Psychiatry, Rutgers Robert Wood Johnson Med. Sch., Piscataway, NJ; [2]Dept of Cell Biol. and Neurosci. and Keck Ctr. for Collaborative Neurosci., Rutgers Univ., Piscataway, NJ; [3]Computat. Biol. Department, Sch. of Computer Sci. and Neurosci. Inst., [4]Dept. of Computat. Biology, Sch. of Computer Sci., Carnegie Mellon Univ., Pittsburgh, PA; [5]Ctr. for Neurobio. and Behavior, Perelman Sch. of Med., Univ. of Pennsylvania, Philadelphia, PA; [6]Dept. of Cell Biol. and Neurosci., Rutgers, The State Univ. of New Jersey, Piscataway, NJ

Abstract: Preclinical evidence indicates parental exposure to drugs of abuse alters behavior and physiology of offspring. We previously demonstrated that when male rats self-administered cocaine, their male, but not female, progeny displayed reduced cocaine self-administration. Contrary to our hypothesis, male offspring of methamphetamine-experienced sires self-administered more methamphetamine (0.1 mg/kg/infusion) and were more motivated for methamphetamine than saline-sired conspecifics. There was no difference in methamphetamine self-administration or motivation for methamphetamine in female offspring. Sires self-administered methamphetamine (0.033 mg/infusion) or cocaine (0.25 mg/infusion) and controls received yoked-saline delivery for 60 days and were subsequently mated with naïve females. The gene expression and open chromatin profiles of experimentally-naïve methamphetamine- and saline-sired male offspring were investigated by multiomic single nuclei RNA-sequencing and ATAC-sequencing of the nucleus accumbens from adult male F1 offspring. Differential gene expression was observed primarily in neuronal (dopamine D1 and D2 receptor-containing medium spiny neurons) and glial (especially microglia) subtypes and in pathways associated with neuronal transmission and synapse regulation. Differential transcription factor binding motifs included those that were bidirectionally regulated in cocaine- vs. methamphetamine-sired offspring. These results implicate potential mechanisms for the epigenetic inheritance of cocaine resistance or methamphetamine susceptibility in drug-sired male offspring, and further suggest putative molecular targets for modulating drug intake. Future experiments will functionally validate candidate genes to manipulate drug self-administration and interrogate the epigenetic profile in the sperm of methamphetamine sires for targets that may influence offspring gene expression.

Disclosures: S.E. Swinford-Jackson: None. **A. Jadali:** None. **B.N. Phan:** None. **M. Sarmiento:** None. **Y. Zhu:** None. **S. Mankame:** None. **S.J. Worobey:** None. **T.J. Sacko:** None. **D. Gangemi:** None. **A.R. Pfenning:** None. **K.Y. Kwan:** None. **R.P. Hart:** None. **R.C. Pierce:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR365.13/RR6

Topic: G.09. Drugs of Abuse and Addiction

Support: FCT - 2021 - PTDC/SAU-TOX/0067/2021
2020.07188.BD

Title: Rhogtpases in the spotlight: unveiling methamphetamine's effects on neuronal remodeling in the hippocampus

Authors: *A. TERCEIRO[1,2], A. LOBO[1], T. CANEDO[1], R. SOCODATO[1], L. CARVALHAIS[1], M. AROSO[1], M. SOUSA[1], A. MAGALHÃES[1], P. AGUIAR[1], J. RELVAS[1,3], T. SUMMAVIELLE[1,4];

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Abstract: The hippocampus plays critical roles in drug addiction. Methamphetamine (Meth), a potent psychostimulant, is known to induce long-lasting synaptic and morphological remodeling, in the brain reward system, hypothesized to be involved in the relapsing nature of this disease. Yet, the mechanisms regulating these processes are not clear. Here, we evaluate the role of RhoGTPases, important regulators of the actin cytoskeleton, on Meth-driven structural alterations. For this, we analyzed rhoA, rac1 and cdc42 activation *in vitro* through FRET assays in hippocampal neurons and found that Meth activated cdc42. Furthermore, inhibition of cdc42 activation *in vitro* prevented Meth-induced neuronal outgrowth and increase in dendritic spine density. We found that hippocampal neurons of WT mice exposed to a Meth binge regimen (4x5 mg/kg, 2h intervals), increase neurite length, ramification and dendritic spine density. Alongside these alterations, we observed increased cdc42 activation on hippocampal synaptoneurosomes 15 min after Meth administration in WT mice. We are now evaluating whether the *in vivo* deletion of cdc42, specifically in hippocampal neurons, is sufficient to prevent Meth-induced effects in neurons and behaviour. Overall, so far, our data demonstrates cdc42 as a crucial mediator of Meth-induced neuronal remodeling in the hippocampus and we expect to clarify if targeting RhoGTPases may contribute to improve treatment in addictive disorders.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.14/RR7

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01DA043461

Title: Molecular mechanisms associated with behavioral sensitization to intermittent ketamine in male and female rats

Authors: *S. SALAND, F. DUCLOT, M. KABBAJ;
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Abstract: With the growing number of clinical studies showing promising effects of repeated, low-dose ketamine treatment across various psychopathologies—including depression and drug addiction—it is critical to determine the potential addictive properties and their associated mechanisms in both sexes. Accordingly, the present work examined the abuse potential of repeated low to moderate doses of ketamine in male and female rats, and molecular profiles

associated with the development of behavioral sensitization in D1 and D2 dopamine receptor-expressing medium spiny neurons (MSNs) of the nucleus accumbens (NAc). Here, following bilateral intra-NAc infusions of a Cre-inducible ribosomal tagged virus, locomotor activity was measured in adult Drd1a-iCre and Drd2-iCre transgenic male and female rats following repeated administration of ketamine (10 or 20 mg/kg, *i.p.*) to evaluate the development of locomotor sensitization. Ketamine was administered six times at an interval of once every four days in males and females in either diestrus or proestrus. NAc tissue was collected four days after the final dose of ketamine in a drug-free state and processed via RiboTag affinity purification to obtain polyribosome-bound D1- or D2-MSN-specific mRNA likely undergoing active translation. Results showed that the magnitude of ketamine's locomotor activating effects was greater in female compared to male rats. Further, female rats demonstrated a heightened susceptibility to develop sensitization to the lower dose of ketamine (10 mg/kg), whereas both sexes developed sensitization when administered at 20 mg/kg. Following purification of polyribosome-bound D1-/D2-MSN-specific mRNA, qPCR was performed for directed evaluation of key plasticity-related related genes associated with the greater sensitivity of females to ketamine's locomotor-activating effects. Additionally, RNAseq was carried out for a broader exploration of sensitization-associated transcriptional profiles and MSN-specific transcriptomes in diestrus and proestrus females at baseline. Taken together, these findings are consistent with earlier reports of sex-dependent sensitivity to behavioral sensitization to intermittent low-dose ketamine and provide novel evidence of associated cell type-specific molecular profiles in female rats. Additional studies are warranted to further delineate the functional contributions of NAc D1- and D2-MSN populations to the abuse liability of low-dose ketamine in both sexes.

Disclosures: S. Saland: None. **F. Duclot:** None. **M. Kabbaj:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.15/RR8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH diversity supplement
NIH DA047441-01A1

Title: Epigenetic Mechanisms Underlying Drug-seeking Behavior: Role of HDAC3 in Regulating Gene Expression within the MHb Cholinergic Population for Cocaine-induced Relapse-like Behavior

Authors: *V. ALIZO VERA[1], M. A. WOOD[2];
[2]Univ. of California Irvine, [1]Univ. of California Irvine, Irvine, CA

Abstract: As a chronic neuropsychiatric disease, addiction is associated with specific molecular and functional neuronal plasticity changes that are triggered by repeated drug exposure leading to persistent changes in neuronal function and ultimately behavior. One powerful mechanism that may underlie aspects of this persistence is epigenetics. Epigenetics has been shown to establish stable changes in cell function. These stable changes in cell function can give remarkable changes at many levels of observation. Currently, we still know very little about the epigenetic mechanisms that could establish the persistent nature of drug-seeking behavior and whether such mechanisms may also be involved in reinstatement, or other relapse-like behaviors. This project is focused on examining the molecular and cellular mechanisms that may be involved in reinstatement. More specifically, the role of the medial habenula (MHb) in cocaine-induced reinstatement of drug-seeking behavior. Most studies investigating the MHb have focused on nicotine seeking due to the high concentration of nicotinic acetylcholine receptors found throughout the medial habenula-interpeduncular nucleus pathway. Recent studies have begun to implicate the MHb in cocaine-associated behaviors, yet the role of the MHb in regulating reinstatement of cocaine-seeking behavior remains largely unknown. In fact, the MHb is rarely included in reward circuitry diagrams. Our recent findings demonstrate that the MHb is engaged by cocaine-primed reinstatement and the activity of choline acetyltransferase (ChAT) expressing neurons in the MHb is sufficient to drive reinstatement. These results suggest that the MHb is a powerful regulator of relapse-like behaviors, which has important implications for understanding the reward pathways in the brain related to relapse. We will also examine the role of a histone deacetylase, called HDAC3, and a key HDAC3 target gene, called Nr4a2, in MHb-dependent reinstatement of drug-seeking. HDAC3 is a key negative regulator of memory formation and associative plasticity, which functions by repressing the expression of Nr4a2. NR4A2 is a transcription factor that regulates aspects of dopamine signaling during development. Both HDAC3 and NR4A2 are highly expressed in the MHb within ChAT expressing neurons, indicating these important regulators of memory processes have a central role in behaviors associated with MHb-dependent reinstatement. In this proposal, we will test the central hypothesis that the MHb is a key regulator of reinstatement of cocaine- seeking behavior and does so in an HDAC3-dependent manner.

Disclosures: V. Alizo Vera: None. **M.A. Wood:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.16/RR9

Topic: G.09. Drugs of Abuse and Addiction

Support: NSERC Canada Graduate Scholarship-Master's (CGS-M)

Title: Sucrose-induced locomotor sensitization occurs in female but not male rats.

Authors: *V. MATIC, K.-P. OSSENKOPP, M. KAVALIERS;
Psychology, Univ. of Western Ontario, London, ON, Canada

Abstract: Sensitization is the increase in effects of a drug that occur with its repeated administration. In the development of drug addiction, sensitization of the mesolimbic dopamine pathway is thought to mediate the pathological enhancement in drug "wanting". In rodents, the sensitization of locomotor activity (locomotor sensitization) is thought to depend on the mesolimbic pathway and is used as an index of motivational sensitization. Sucrose, a highly palatable food, enhances the development of locomotor sensitization induced by dopaminergic drugs of abuse and can facilitate behaviours typically associated with drug addiction (i.e., binge consumption, withdrawal), suggesting a common motivational pathway for both natural and drug reward. Sex differences in food reward have been identified in both humans and rodents. Compared to men, women have a greater prevalence of obesity and binge-eating disorder, experience stronger cravings for sweet foods, and have greater difficulty managing cravings. Similarly, in rodents, female rats show greater preference for sucrose in a two-bottle test and display more lever responses to obtain sucrose in a progressive ratio schedule task compared to males. The present study evaluated if sex differences exist in sucrose-induced locomotor sensitization. Age-matched male (n = 16) and female (n = 16) Long-Evans rats were given 30 min daily access to sucrose (0.3 M) or water for 9 consecutive days. Locomotor activity was assessed for 30 min on days 1, 5 and 9 immediately following fluid consumption. An effect of sucrose-induced locomotor sensitization was identified in females but not males. Female-sucrose rats displayed greater activity than male-sucrose rats on all assessment days ($p < .05$). On days 5 and 9, female-sucrose rats displayed greater activity than female-water rats ($p < .01$) while male-sucrose and male-water groups did not significantly differ. Female-sucrose rats showed an increase in activity from day 5 to day 9 ($p < .05$) while male-sucrose rats did not significantly differ between days. These results provide evidence of a sex difference in sucrose-induced locomotor sensitization which may reflect a female-biased difference in mesolimbic pathway activity in response to sucrose.

Disclosures: V. Matic: None. **K. Ossenkopp:** None. **M. Kavaliers:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.17/RR10

Topic: G.09. Drugs of Abuse and Addiction

Support: GNT1147207

Title: Investigating activity of dorsal striatal direct pathway during goal-directed and habitual action control following cocaine exposure

Authors: *I. CHEW[1], N. HOUGH[1], C. MITCHELL[1], E. CAMPBELL[1,2], S. FISHER[2], L. MANNING[1], C. DAYAS[1];
[1]Univ. of Newcastle, Newcastle, Australia; [2]Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia

Abstract: Goal-directed and habit-based action selection is controlled by separate brain systems involving the dorsomedial (DMS) and dorsolateral (DLS) striatum, respectively. Breakdown of the balance between these systems disrupts flexible decision making and is thought to contribute to neuropsychiatric disorders such as substance use disorder (SUD). Within the DMS and DLS, aspects of these functions are coordinated by direct pathway spiny projection neurons (dSPNs). Exposure to drugs is thought to impair goal-directed action control and/or accelerate habit learning. Here we aimed to understand how exposure to cocaine might influence the activity of dSPNs in the DMS and DLS during early and late phases of instrumental conditioning following a random interval (RI) schedule of training.
Male and female Drd1a-iCre rats between 10-12 weeks of age were injected with Cre-dependent GCaMP8f virus and implanted with fibre optic probes targeting the DMS (n=18) or DLS (n=10). Rats were allowed 6 weeks of recovery for viral expression, then food restricted and given intraperitoneal injections of cocaine (30mg/kg) or saline for 6 days prior to behavioural testing. An established protocol based on Corbit et al., 2014 was used to assess behavioural action control. Photometry signals aligned with behaviourally significant events (e.g. lever press (LP), magazine entry (ME), pellet delivery (PD)) were examined using standard summary statistical methods including peak and AUC.
Behavioural Data: Mixed-effects analyses suggested that animals became less goal-directed with extended training, assessed using the outcome-devaluation test ($p=0.0003$), and there was trend for a significant drug X training effect ($p=0.0986$). Cocaine animals were significantly less goal-directed during late training compared to early training (Bonferroni test, $p=0.0009$). We then used the GuPPY pipeline (Sherathiya et al, 2021) to analyse Ca2+ transients across specific event windows in DMS and DLS dSPNs. Two-way ANOVA of Ca2+ transients associated with LP, ME and PD revealed no effect of cocaine on DMS dSPN activity. However, a main effect of drug (AUC, $p=0.0451$) was observed for DLS dSPN activity around ME during early phase RI30 and during the late phase RI60 session associated with LP (peak, $p=0.0059$).
Overall, these findings indicate that extended training and exposure to drug tends to reduce goal-directed behaviour. From our photometry data, we showed that DLS dSPNs are more sensitive to cocaine exposure - a finding that might have relevance for understanding the development of inflexible behaviour in neuropsychiatric conditions.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.18/RR11

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA049531
Tufts University Graduate School of Biomedical Sciences
Tufts University Cummings Sch of Veterinary Medicine

Title: Involuntary Administration of Oxycodone during Pregnancy & High Motivation for Cocaine in Male Adult Offspring

Authors: *C. C. A. AARON[1], K. R. GILDAWIE[2], K. E. BUDGE[2], F. M. VASSOLER[2], E. M. BYRNES[2];
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Abstract: The recent opioid crisis has led to a drastic increase in women of reproductive age diagnosed with opioid use disorder. This trend has led to an increased number of children exposed prenatally to opioids, which may lead to long-term neurobiological changes. Our lab uses a voluntary paradigm of oxycodone (OXY) intravenous self-administration (SA) in female Sprague-Dawley rats during gestation. For these studies, we include both a saline-yoked control that receives an infusion of saline every time the OXY SA female presses for drug, as well as an OXY-yoked control that receives an infusion of OXY every time the OXY SA female presses for drug. Thus, the OXY-yoked females receive the same amount of OXY in a non-contingent manner (i.e. involuntary). All females are surgically implanted with a jugular catheter and trained to self-administer OXY in daily operant conditioning sessions (2h/day, 5 days/week for 2 weeks, 6h/day 5 days/week for 1 week; fixed ratio (FR1) schedule; 0.1 mg/kg/infusion). Once females are pregnant, they have daily access (7 days/week) to operant chambers and are allowed to self-administer OXY for 6h/day. On postnatal day 1 (PND1) litters are culled to 4 females and 4 males and cross-fostered to time-mated drug naïve donor mothers. For the current study, adult male and female offspring were trained on cocaine SA using both fixed ratio and progressive ratio schedules to assess reward learning and motivated responding. Preliminary data from our lab indicate that involuntary OXY, rather than the level of maternal oxycodone intake, affects adult responding for cocaine in a sex-specific manner. Thus, male but not female offspring of OXY-yoked dams demonstrate higher motivated responding for cocaine during PR. One mechanism underlying changes in offspring cocaine SA may be alterations in opioid regulation of the mesolimbic dopamine pathway. We will present data on the effects of prenatal OXY intake, both voluntary and involuntary, on the expression of mu and kappa opioid receptors in the ventral tegmental area (VTA) and nucleus accumbens (NAc) in adult offspring. Moreover, given the role of the dopamine transporter (DAT) in cocaine-mediated effects, we will also present data on the expression of DAT in the NAc of these adult offspring. Together, these findings will begin to determine the mechanisms underlying increased cocaine use liability in the male offspring of dams exposed to non-contingent OXY during pregnancy.
This work is supported by the National Institute on Drug Abuse R01 DA049531

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant F31-DA053111-02
NIH Grant RO1-AA026267-04

Title: Paradoxical GABAergic firing in the ventral tegmental area enhances adult morphine reward after adolescent nicotine exposure

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Abstract: Opioid use disorder is an immense source of preventable mortality and economic burden in the United States. An important, yet poorly understood, risk factor for opioid use disorder is previous nicotine use. Nicotine use often begins in adolescence. Epidemiological evidence suggests that adolescent nicotine increases the vulnerability to subsequent drug use, including to opioids. However, the neural mechanisms underlying this interaction remain unknown. To address this gap, we tested the effect of adolescent nicotine on adult morphine reward. We exposed both male and female adolescent mice to nicotine in their drinking water for two weeks (0.1 mg/mL on Days 1-5; 0.2 mg/mL starting on Day 6) from postnatal days 28-42, and probed both behavioral- and cellular-level adaptations to morphine four weeks later in adulthood. Adult mice that received nicotine during adolescence showed enhanced preference for the morphine-paired chamber in a conditioned place preference (CPP) paradigm relative to adolescent water controls. Adolescent nicotine mice also consumed more morphine in a morphine two-bottle choice task and demonstrated increased morphine locomotor sensitization in adulthood compared to controls. In contrast, mice that received two weeks of nicotine during adulthood did not show increased morphine reward. These data reveal that nicotine exposure during the vulnerable adolescent period has a long-term impact on drug reward. Notably, we also found that these behavioral changes corresponded with alterations in ventral tegmental area (VTA) GABA neurons. While VTA GABA neurons from control animals show canonical reduced action potential firing during morphine application in adulthood, GABA neurons from adolescent nicotine mice show paradoxical increased action potential firing. To determine whether this paradoxical firing causes the observed increase in morphine reward, we chemogenetically inhibited VTA GABA neurons during morphine CPP. In adolescent nicotine mice, VTA GABA neuron inhibition during morphine conditioning sessions normalized morphine preference to the control level, indicating that GABA neuron excitation in response to morphine is necessary for aberrant morphine reward after adolescent nicotine. Conversely, among adolescent water controls, inhibiting VTA GABA neurons increased preference for the morphine-paired chamber. These data reveal a novel mechanism by which adolescent - but not

adult - nicotine intake promotes morphine reward in adulthood and suggest that adolescent nicotine exposure profoundly alters reward circuitry well into adulthood.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.20/RR13

Topic: G.09. Drugs of Abuse and Addiction

Title: Nucleus accumbens medium spiny neuron subtypes are associated with human substance use phenotypes

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Abstract: The nucleus accumbens (NAc) plays an important roles in drug-taking and -seeking behaviors. The primary output pathways of the NAc consist of GABAergic medium spiny neurons (MSNs), usually expressing dopamine type 1 receptors (D1 MSNs) and dopamine type 2 receptors (D2 MSNs). These groups of MSNs in the NAc are known to have functional differences and additional evidence suggests that there are distinct subtypes within the major groups. However, details on MSN subtypes are limited and the potential roles of these populations in substance use disorders are largely unknown. The goal of the current study is to make use of the largest currently available rat NAc single nucleus transcriptomic data set to present high resolution subclustering of MSNs and polygenic enrichment for substance use phenotypes in MSN subtypes. Brains from male Brown Norway rats (n=11) were snap frozen and NAc samples were punched. Single nucleus RNAseq libraries were generated using the 10x Genomics platform. The R package Seurat was used for quality control and clustering. MSNs (n=47,591) were identified based on known marker genes and subclustered. MSNs from an independent rat NAc replication data set were mapped to the discovery data. Correlation of gene expression between clusters in the discovery and replication data sets was calculated by Pearson correlation. Gene-level summary statistics were obtained for genome-wide association studies of tobacco use disorder, opioid use disorder, alcohol use disorder, and alcohol consumption using MAGMA. Polygenic enrichment for each phenotype was scored for all nuclei with scDRS. Subclustering of MSNs resulted in identification of 37 transcriptomically-distinct populations, including D1 and D2 MSNs and separate non-D1/D2 populations. All subclusters were defined by expression of only 1-3 marker genes and were not statistically biased towards any individual sample. Mapping of an independent rat NAc data set onto the discovery data revealed the presence of all 37 subclusters in the replication samples, supporting their validity. Unique

populations of MSNs were significantly enriched for expression of genes associated with each of the substance use phenotypes analyzed. These data indicate that MSNs consist of numerous subtypes with unique gene expression profiles, which likely represent different functional niches within the NAc. Subtype-specific differences in polygenic enrichment for various substance use phenotypes suggest these phenotypes may engage distinct MSN populations. Future work should focus on targeted manipulation of the relevant MSN subtypes to determine their functional significance in the context of substance use.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

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the National Research Foundation of Korea(NRF) grant funded by the Korea government(MSIT) (2020R1A2C1006559)
the Ministry of Food and Drug Safety (23212MFDS217)

Title: Evaluation of the abuse liability of 4'-fluoro-4-methylaminorex in mice

Authors: J. KIM[1], J. KIM[1], S.-J. JEONG[1], E. HWANG[1], S. YOON[1], *C. YANG[2];
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Abstract: 4'-Fluoro-4-methylaminorex (4-FPO) is a recreational designer drug with psychostimulant effects as a derivative of aminorex. Previous studies demonstrated that, 4,4'-dimethylaminorex, a derivative of aminorex, causes the release of dopamine and serotonin in the brain. These neurotransmitters are believed by many to be responsible for the reinforcing effects of psychostimulants. Thus, there is a lot of interest in gaining a better understanding of how 4-FPO acts to produce addictive behaviors. In this research, we determined whether repeated exposure to 4-FPO produces behavioral sensitization to 4-FPO and 4-FPO-induced conditioned place preference (CPP) in mice. Results showed that systemic challenge with 4-FPO at a dose of 2 mg/kg produced a much larger increase in locomotor activity in 4-FPO-pretreated mice compared to saline-pretreated mice. Sensitization to the locomotor activating effect of 4-FPO was blocked by systemic injections of either the D1 receptor antagonist SCH23390 or the D2 receptor antagonist eticlopride. Also, 4-FPO at doses of 1 mg/kg and 2 mg/kg (i.p.) induced a

significant CPP. These results suggest the possibility that the mesolimbic dopamine system may mediate the reinforcing properties of 4-FPO.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.22/RR15

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant U01 DA051993

Title: Differential gene expression and chromatin accessibility in the medial prefrontal cortex as a function of individual variability in three measures of opioid addiction in rats

Authors: *S. X. LIU[1,2], Z. L. MATTI[1], P. MUELKEN[4], A. RAMAKRISHNAN[5], M. S. ESTILL[5], M. G. LESAGE[1,3,4], J. R. SMETHELLS[3,4], L. SHEN[5], P. V. TRAN[2], A. C. HARRIS[1,3,4], J. C. GEWIRTZ[1,6];
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Abstract: Characterizing molecular mechanisms underlying individual differences in vulnerability to opioid addiction is essential for developing more effective prevention and treatment strategies. Here, we investigated genome-wide transcription (RNA-seq) and chromatin accessibility (ATAC-seq) in the medial prefrontal cortex (mPFC) of male rats showing differential addiction vulnerability in 3 separate paradigms modeling initial response to morphine exposure (Withdrawal-Induced Anhedonia (WIA)), persistent use (Demand), and relapse (Reinstatement). Rats were assigned to "High" vs. "Low" addiction vulnerability groups based on a median split of their behavioral scores. RNA-seq and Ingenuity Pathway Analysis revealed greater changes in canonical pathways in Low-vulnerability (vs. Saline) rats in comparison to High -vulnerability (vs. Saline) rats across all 3 paradigms. These changes occurred in gene networks associated with neural signaling, neural development/protection, neuroinflammation, and metabolism, suggesting that adaptations in these pathways in the mPFC may contribute to resilience to opioid addiction. HOMER motif analysis of ATAC-seq data revealed changes in accessibility to a small set of transcription factor (TF) binding sites, some that were shared by the 3 paradigms and others that were unique to each. Changes in accessibility to the binding sites of AP-1 family members, including FOS, JUNB, and FRA, were significant in both High- and Low-vulnerability vs. Saline comparisons but not the High vs. Low comparison across all 3 paradigms. This suggests that one or more AP-1 TFs are highly responsive to the pharmacological effects of morphine across all stages of exposure. In contrast, the MADS box

motif (MEF2 family) and bHLH binding domains were differentially enriched in High vs. Low groups in the Demand and Reinstatement paradigms, suggesting an effect associated with vulnerability in these models. Finally, the Nuclear Receptor family (PGR, GRE) binding motif was significantly enriched only in the Reinstatement paradigm. In conclusion, we have identified changes in biological pathways and TF binding motifs that are either paradigm-specific or span all 3 paradigms, as well as changes that are more closely associated with addiction vulnerability than opioid exposure. These findings point to the involvement of distinct transcriptional and epigenetic mechanisms in vulnerability to opioid addiction versus opioid exposure and in different stages of the disorder.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR365.23/RR16

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH IRP Grant ZIAAA000550

Title: Brain connectivity changes to fast versus slow dopamine increases

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Abstract: The rewarding use of drugs such as methylphenidate (MP) depends crucially on how fast they raise dopamine in the brain, which is influenced by the speed at which they enter the brain. Yet how the rate of drug-induced dopamine increases impacts brain network communication remains unresolved. We manipulated route of MP administration to generate fast versus slow dopamine increases. We hypothesized that fast versus slow dopamine increases would result in a differential pattern of global brain connectivity (GBC) in association with regional levels of dopamine D1 receptors, which are critical for drug reward. Twenty healthy adults received MP intravenously (0.5mg/kg, producing fast dopamine increases) and orally (60mg, producing slow dopamine increases) during simultaneous [^{11}C]raclopride PET-fMRI scans on separate days (double-blind, placebo-controlled). We used a recently validated method to estimate striatal dopamine dynamics based on minute-to-minute changes in raclopride binding in response to MP. We then performed multiple regression to test how minute-to-minute changes in GBC were temporally associated with slow and fast dopamine increases. Connectivity patterns

across the brain were strikingly different in association with slow and fast dopamine increases, and whole-brain spatial patterns were negatively correlated with one another (rho = -.54, p_{spin} = .009, controlling for spatial autocorrelation). For the direct comparison of fast versus slow dopamine increases, GBC showed significant "fast > slow" associations in dorsomedial and dorsolateral prefrontal cortex, bilateral insulae, posterior thalamus and brainstem, dorsal caudate and precuneus; and significant "slow > fast" associations in ventral striatum, medial orbitofrontal cortex, and frontopolar cortex ($p_{FDR} < .05$). The degree to which dorsal prefrontal GBC tracked fast dopamine increases was significantly correlated with 'high' ratings to intravenous MP across individuals ('skipped' correlation: $r_{(19)}$= .73, Bonferroni-corrected p = .004). Further, as hypothesized, whole-brain GBC patterns to fast versus slow dopamine increases showed significant spatial correspondence with D1 receptor availability (as estimated by normative maps of [^{11}C]NNC-112 binding; rho = .22, $p_{spin} < .05$, controlling for spatial autocorrelation). In sum, different routes of MP administration produce strongly divergent patterns of brain connectivity. Fast dopamine increases are associated with connectivity patterns that may have relevance for the subjective experience of drug reward.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NIDA Grant R01DA054526
NIH/NIDA Avenir Award DP1DA056018

Title: Investigating the mechanisms of CNS HIV-1 infection using human iPSC-derived microglia xenografted mouse model

Authors: A. MIN, B. JAVIDFAR, M. DURENS, S. MARRO, L. DE WITTE, B. K. CHEN, T. SWARTZ, *S. AKBARIAN;
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Abstract: The persistence of latent viral reservoirs has rendered HIV-1 an incurable disease that has a propensity to rebound when antiretroviral therapy (ART) is stopped. The central nervous system (CNS) is seeded with HIV-1 within the first 2 weeks of acute infection, and it makes up a major viral reservoir. HIV-1 predominantly targets microglia in the CNS. Studies in human post-mortem brain have shown that HIV-1 infection causes reprogramming of the microglial transcriptome and 3D genome that favor up-regulation of genes linked to innate immune activation and inflammation. On the other hand, the mechanisms governing HIV-1 latency in vivo are largely unknown due to difficulty in distinguishing latently infected cells that are

transcriptionally silent. Extensive studies on CNS HIV-1 disease have also been limited due to restricted access to human brain tissue and limitations in existing cell cultures and animal models. To overcome this obstacle, we developed a novel chimeric mouse model where human induced pluripotent stem cells (iPSC)-derived microglia are xenografted into mouse brains. This use of iPSC allows a renewable source of microglial cells where prior studies have shown genotypic and phenotypic resemblance with primary microglial cells. Additionally, we genetically engineered a Cre-recombinase-dependent dual fluorescent reporter cassette into the iPSC, allowing us to permanently mark cells that have ever been infected with an HIV-1 clone that expresses Cre. This innovative molecular tool called the HIV-1 induced lineage tracing (HILT) enables us to track the frequency and kinetics of infected microglial cells. Peripheral engraftment of these mice with human PBMC enables infections to be initiated in the peripheral immune cells and is observed to spread to the CNS-engrafted microglia. The ability to sort single infected nuclei in this model will enable us to study latency mechanisms in the CNS at single-cell resolution. The mouse model also enables quantitative testing of molecular, epigenetic, and pharmacological interventions to reduce the HIV-1 reservoir in the brain and cure HIV-1.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Program #/Poster #: PSTR365.25/RR18

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grand T32 DA007237

Title: Adolescent social isolation stress influences oxycodone self-administration in mice

Authors: *E. BLACK[1], M. KNOUSE[1], E. A. BIRMINGHAM[2], L. A. BRIAND[3];
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Abstract: Adolescence is a crucial period for social development. As such, isolation during adolescence can lead to impaired socialization and stress-related problems during adulthood. Additionally, adolescent social isolation influences the propensity to develop substance use disorders later in life. Our lab has previously demonstrated that adolescent social isolation increases cocaine-seeking and motivation to self-administer cocaine in male and female mice. In contrast, previous studies suggest that other forms of early life adversity may confer resistance to opioid seeking. Therefore, the current study aimed to determine if adolescent social isolation alters opioid self-administration. Specifically, we examined the impact of adolescent social isolation on oxycodone self-administration in male and female mice. Consistent with previous results, adolescent-onset social isolation did not alter the ability of mice to learn operant self-

administration for food. In contrast to the previous increases in cocaine seeking, we found a main effect of housing condition on cocaine intake such that adolescent social isolation stress led to a decrease in oxycodone self-administration. This effect appears to be primarily driven by the female mice. However, this decrease in intake does not appear to be driven by a reduction in motivation for oxycodone as we do not see any effect of social isolation on responding on a progressive ratio schedule of reinforcement. Studies are ongoing to examine a more complete dose response curve for oxycodone in these mice. Further, due to the strong connection between the mesolimbic dopamine system and drug taking and seeking, we are currently investigating the impact of adolescent social isolation on dopamine dynamics in the nucleus accumbens. These studies begin to shed light on the complex interplay between different drug classes and biological sex.

Disclosures: E. Black: None. **M. Knouse:** None. **E.A. Birmingham:** None. **L.A. Briand:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR365.26/RR19

Topic: G.09. Drugs of Abuse and Addiction

Title: Examining the impact of post-weaning social isolation on oxycodone analgesia

Authors: *C. B. SCHULDT[1], E. M. BLACK[2], L. A. BRIAND[2];
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Abstract: *Examining the impact of post-weaning social isolation on oxycodone analgesia.*
Schuldt, C.B.[2], Black, E.M.[1], & Briand, L.A.[1,2]
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Early life stress, particularly during adolescence, induces behavioral alterations that can extend far into adulthood. For example, social isolation during adolescence increases cocaine seeking in both males and females. In contrast, recent work in our lab has shown that adolescent social isolation affects opiate self-administration in a sex-specific manner, increasing motivation for oxycodone in male mice but decreasing oxycodone intake in female mice. However, it is unclear if this decrease in females is due to an increase in oxycodone potency or a decrease in the rewarding properties of oxycodone. Along with its effects on reward, oxycodone is a powerful analgesic and chronic stress can alter this antinociception. Therefore, the current studies are aimed at examining the impact of adolescent social isolation on oxycodone analgesia in both males and females. Using a hot water tail flick assay, we examined both baseline pain responsivity and the nociceptive response to two doses of oxycodone during adulthood in mice that were either socially isolated or group housed at weaning. We did not see any effect of

adolescent social isolation on either baseline pain responsivity or the antinociceptive response to oxycodone in male mice. In contrast, adolescent social isolation appears to lead to a rightward shift in the antinociceptive dose response curve for oxycodone in female mice. Additional doses of oxycodone are being tested to confirm this effect as well as studies to determine the impact of chronic oxycodone on antinociception in mice following adolescent social isolation.

Disclosures: C.B. Schuldt: None. **E.M. Black:** None. **L.A. Briand:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

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Program #/Poster #: PSTR365.27/RR20

Topic: G.09. Drugs of Abuse and Addiction

Title: Selective Modulation of Hippocampal Theta Oscillations in Response to Morphine versus Natural Reward

Authors: *S. JAMALI[1], A. KALBASI[2], M. PARTO-DEZFOULI[3], M. DALIRI[4], A. HAGHPARAST[5];
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Abstract: Despite the overlapping neural circuits underlying natural and drug rewards, several studies have suggested different behavioral and neurochemical mechanisms in response to drug vs. natural rewards. The strong link between hippocampal theta oscillations (4-12 Hz) and reward-associated learning and memory has raised the hypothesis that this rhythm in hippocampal CA1 might be differently modulated by drug- and natural-conditioned place preference (CPP). Time-frequency analysis of recorded local field potentials (LFPs) from the CA1 of freely moving male rats previously exposed to a natural (in this case, food), drug (in this case, morphine), or saline (control) reward cue in the CPP paradigm showed that the hippocampal CA1 theta activity represents a different pattern for entrance to the rewarded compared to unrewarded compartment during the post-test session of morphine- and natural-CPP. Comparing LFP activity in the CA1 between the saline and morphine/natural groups showed that the maximum theta power occurred before entering the unrewarded compartment and after the entrance to the rewarded compartment in the morphine and natural groups, respectively. In conclusion, our findings suggest that drug and natural rewards could differently affect the theta dynamic in the hippocampal CA1 region during reward-associated learning and contextual cueing in the CPP paradigm.

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Poster

PSTR365. Addictive Drugs: Neural Mechanism

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.28/SS1

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA R01 DA045836
NIDA F31 DA059203

Title: Cortical interneurons inhibit heroin seeking

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Abstract: The infralimbic (IL) cortex exerts top-down control over drug-seeking behaviors in preclinical rodent models of substance use disorders. Approximately 15% of neurons in the IL cortex are inhibitory GABAergic interneurons. These interneurons tonically inhibit local IL pyramidal projection neurons to tightly regulate IL output within defined neural circuits. In this study, we used a preclinical rat model of opioid use disorder (OUD) involving heroin self-administration and chemogenetics to investigate the contribution of cortical interneurons to heroin motivation and relapse. To manipulate activity of cortical interneurons, we injected a DLX-promoter driven virus delivering either an inhibitory Gi-DREADD or excitatory Gq-DREADD into the IL cortex of male and female rats. Heroin self-administration training occurred over a period of 13 sessions, beginning with an FR1 schedule of reinforcement and advancing to VR5 and VR15 to engender higher rates of heroin seeking. A tone and light cue were paired with delivery of heroin infusions during training and used to subsequently trigger relapse in this model. After rats acquired heroin self-administration, we conducted within-subjects progressive ratio tests to assess heroin motivation. We found that neither activation nor inhibition of IL cortical interneurons during progressive ratio testing altered heroin breakpoint, suggesting that manipulating IL interneuron activity does not impact heroin motivation under these conditions. After self-administration, heroin and corresponding heroin cues were no longer available during operant extinction training, resulting in diminished levels of heroin seeking over the course of 7 extinction sessions. Thereafter, heroin cues were reintroduced to trigger relapse, or reinstatement of heroin seeking, still in the absence of heroin. Again, using a within-subjects design, we found that activating IL cortical interneurons with the Gq-DREADD did not alter heroin relapse in this model. However, inhibition of IL cortical interneurons with the Gi-DREADD increased reinstatement of heroin seeking triggered by the heroin cues, evidenced as an increase in heroin lever pressing after treatment with the DREADD-ligand (J60) compared to vehicle. Taken together, our results indicate that activity in IL interneurons is necessary to limit

heroin seeking under relapse conditions. Although it was not possible to drive this limiter function further using the Gq-DREADD, alternative strategies targeting this neuronal subpopulation may present viable new therapeutic interventions toward preventing relapse in individuals with OUD.

Disclosures: **R. Vareed:** None. **G. Giannotti:** None. **K. Glodoski:** None. **J. Peters:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.29/SS2

Topic: G.09. Drugs of Abuse and Addiction

Support: AA027915
Miami University

Title: Striatal cholinergic interneurons influence fentanyl seeking

Authors: *C. R. BEANE, S. C. MONROE, T. W. PERRY, A. K. RADKE;
Dept. of Psychology, Miami Univ., Oxford, OH

Abstract: The opioid epidemic is a significant public health issue in the United States with a growing number of overdoses related to illicit opioids such as fentanyl. One of the neural circuits implicated in opioid-related behaviors is the mesolimbic dopamine system, consisting of projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Striatal cholinergic interneurons (CINs) are known to modulate dopamine release and their activity has been shown to counter motivated and reward-related behaviors. CINs express μ opioid receptors (MORs), the primary target of exogenous opioids, but the role of CINs in opioid-related behaviors has not been tested. We hypothesized that opioids interrupt CIN firing through MOR binding, enhancing the impact of these drugs on dopaminergic function. To examine the role of CINs in opioid seeking, we first used a Cre-dependent transgenic approach to delete MORs on cholinergic (ChAT+) cells, resulting in ChAT-MOR Cre+ mice that had the MOR deletion (experimental group) and Cre- mice that had intact CIN MORs (control group). Opioid consumption was tested using a 2-hour, operant oral fentanyl self-administration paradigm over 28 sessions. During the last three days of fentanyl self-administration, 500 uM of quinine was added to the fentanyl to test aversion-resistant consumption. Following this study, we looked at the behavioral effects of site-specific excitation of CINs in the NAc by injecting the Cre-dependent viral vector AAV8-hSyn-hM3Dq-DIO-mCherry into the NAc shell of ChAT-Cre mice. Mice were trained on the oral fentanyl self-administration task and then tested using injection of a vehicle or clozapine N-oxide (CNO), counterbalanced on the final two sessions. In ChAT-MOR mice, we observed greater consumption in female vs. male mice. Cre+ mice lacking CIN MORs also responded less for fentanyl during quinine sessions. For the chemogenetic study, preliminary results suggest that excitation of NAc shell CINs does not influence fentanyl

consumption.. Together, our results suggest that cholinergic neurons may be involved in aversion-resistant opioid seeking and consumption.

Disclosures: C.R. Beane: None. **S.C. Monroe:** None. **T.W. Perry:** None. **A.K. Radke:** None.

Poster

PSTR365. Addictive Drugs: Neural Mechanism

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR365.30/SS3

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA027915
Miami University

Title: Cholinergic interneuron regulation of nicotine and alcohol self-administration

Authors: *D. G. LEWIS[1], N. BRUNS VI[2,4], K. L. PIKUS[2], M. H. DURFEE[2], R. A. ZEGARELLI[2], T. W. PERRY[3], C. R. BEANE[1], A. K. RADKE[3];
[1]Miami Univ. Dept. of Psychology, Oxford, OH; [2]Psychology, [3]Miami Univ., Miami Univ., Oxford, OH; [4]NIH, Bethesda, MD

Abstract: Alcohol and nicotine are common substances that are used and abused in society. Not only are these substances used independently of each other, but there is considerable overlap, with many people consuming both nicotine and alcohol. Alcohol and nicotine use are known to depend on striatal dopamine release, which is modulated by the release of acetylcholine from local cholinergic interneurons (CINs) onto dopaminergic terminals. We sought to determine whether CINs participate in self-administration of alcohol and nicotine, individually and concurrently, using a transgenic mouse with genetic deletion of the mu-opioid receptor gene Oprm1 in cholinergic (ChAT+) cells. 30 female and male mice (15 Cre- controls and 15 Cre+ experimental) were tested for consumption of 20% ethanol and a 30 μg/mL nicotine solution using a 24-hour intermittent access, two-bottle choice paradigm. The order of solution presentation was counterbalanced such that half of the mice received nicotine for two weeks, followed by ethanol for two weeks and the other half began with ethanol. Moreover, bottle positions were switched with each session to control for side preferences. For the final two weeks, all mice received a combination of ethanol and nicotine solutions. Consumption and preference for each solution (vs. water) were analyzed. Results demonstrated that Cre+ mice with CIN MOR deletion consumed similar amounts of nicotine as Cre- mice but had a lower preference for nicotine vs. water. For ethanol, there was a trend toward a sex-specific effect, with Cre+ males consuming less ethanol than Cre- wildtype males. When the combined solution was presented, Cre+ mice consumed more than Cre- mice. Finally, preference vs. water was highest for the nicotine solution among all mice. This research provides evidence that striatal CINs are involved in both ethanol and nicotine self-administration behaviors, potentially in a sex-

dependent manner. Future studies will examine potential sex-dependent influences of CIN MOR deletion on ethanol consumption using a study fully powered to detect sex differences.

Disclosures: **D.G. Lewis:** None. **N. Bruns VI:** None. **K.L. Pikus:** None. **M.H. Durfee:** None. **R.A. Zegarelli:** None. **T.W. Perry:** None. **C.R. Beane:** None. **A.K. Radke:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.01/SS4

Topic: G.09. Drugs of Abuse and Addiction

Support: T32DA007237
DP1DA051550

Title: Morphine driven m6A epitranscriptomic neuroadaptations in primary cortical neurons

Authors: *K. R. DABROWSKI, S. SILLIVAN;
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Abstract: Opioid overdose is the leading cause of accidental deaths in the United States. Current pharmacotherapies target the μ-opioid receptor; however, opioid exposure induces many other neuroadaptations, including epitranscriptomic regulation in the brain, highlighting additional avenues to explore therapies for opioid use disorders. Recent studies have demonstrated N6-methyladenosine (m6A) RNA methylation is significantly enriched in the brain and involved in learning, memory, and reward. m6A modifications and related enzymes have not yet been studied in opioid use disorder, despite being one of the most common RNA modifications. We detected significant regulation of enzymes involved in m6A modification in the orbitofrontal cortex of rats following heroin self-administration and rat primary cortical neurons following chronic morphine treatment, including AlkB Homolog 5 (Alkbh5). The m6a demethylase Alkbh5 functions as an m6A eraser, removing m6A modifications from mRNA. We hypothesized that chronic opioid treatment regulates m6A modifications in the cortex through modulation of Alkbh5. We profiled the shifts in m6A modifications of primary cortical neurons following chronic morphine treatment and Alkbh5 knock-down. We observed differential regulation of m6A modifications for 568 transcripts following morphine chronic treatment and for 2865 transcripts following Alkbh5 knock-down. There was a subset of 103 transcripts that were commonly regulated by both morphine and Alkbh5 knock-down with 92 transcripts being hypermethylated. This suggests that 16.2% of morphine regulated m6A modifications are mediated through the downregulation of Alkbh5 following chronic morphine treatment in primary cortical neurons. These commonly regulated transcripts were associated through gene ontology with serotonin secretion, synapse disassembly, neuron remodeling and immune response among others. Thus, we conclude that morphine can drive epitranscriptomic changes in

an Alkbh5 dependent manner. These epitranscriptomic changes can potentially mediate the neuroadaptations seen following opioid exposure.

Disclosures: K.R. Dabrowski: None. **S. Sillivan:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.02/SS5

Topic: G.09. Drugs of Abuse and Addiction

Support: HEAL Initiative

Title: Development of 3D bioprinted brain region specific neural circuitry models

Authors: *S. KUNDU[1], M. SONG[2], M. FERRER[1];
[1]Natl. Ctr. for Advancing Translational Sci., Natl. Inst. of Hlth., Rockville, MD; [2]Natl. Ctr. for Advancing Translational Sci., Nation Inst. of Hlth., Rockville, MD

Abstract: We are developing in vitro functional neural circuitry cellular models to study neurological and neurodegenerative diseases. In the current study, we describe a hydrogel-based approach to produce bioprinted 3D neural models using human iPSC derived neurons and astrocytes, in a fibrin-based extracellular matrix that enables the neurons to autonomously form networks and spontaneous synaptic connections. Functional assays in these 3D fibrin gel-based neural circuitry models were designed with genetically encoded fluorescence biosensors like GCaMP6f, dLight1.2 and iGluSnFr for calcium activity and neurotransmitter released assessment, respectively. This approach enables for real time measurements of neural network dynamic with optogenetic modulation by transfection of AAV mediated ChrimsonR-opsin and pharmacological perturbations. We adopted fiber photometric measurements to study dynamic spatial modulation of neural networks in real time in vitro. The fibrinogen gel is compatible with extrusion-based bioprinting technique allowing for the spatially controlled deposition of cellular constructs with brain region specific neuronal compositions, VTA-like (Dopaminergic neurons rich), PFC-like (Glutamatergic neurons rich), NAcc-like (GABAergic neurons rich), with various designed spatial arrangements to mimic brain network physiology. In this study, two spatial arrangements were bioprinted, linear and triangular, and their neural activity were compared. The dynamics of calcium oscillations as measured by fiber photometry, on these two spatial arrangements with three brain regions specific bioprinted constructs, differ within and between the system accordance with their spatial synaptic plasticity. The optogenetics modulation within and between our linear and triangular neural arrangements also showed significant activity differences. Finally, the pharmacological interventions with morphine, a mu opioid-receptor (μ-receptor) agonist, reveals that the triangular arrangement of VTA-like, PFC-like, NAcc-like neural populations produced calcium and dopamine responses that were similar to those observed in vivo, compared to its linear counterpart. Our neural biofabrication approach allows

for progressive inclusion of additional physiological complexity, such as vasculature, additional cell types like microglia, in designed spatial patterns to mimic different circuitry of the human brain in vitro.

Disclosures: S. Kundu: None. **M. Song:** None. **M. Ferrer:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.03/SS6

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH / NIDA IRP
DA000069

Title: Characterization of a highly selective and potent mu opioid receptor agonist

Authors: *J. GOMEZ[1], E. N. VENTRIGLIA[1], A. SULIMA[2], A. RIZZO[3], Z. M. GARÇON-POCA[3], D. P. HOLT[4], R. F. DANNALS[4], M. G. POMPER[5], J. BONAVENTURA[3], M. BAUMANN[1], K. C. RICE[2], M. MICHAELIDES[1];
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Abstract: Mu opioid receptor (MOR) agonists are the most potent analgesic medications; Nonetheless, these medications are associated with recognized negative consequences, such as respiratory depression and the potential for abuse. As such, there is a desperate need to develop novel MOR agonists with lower adverse effect profiles than current MOR-based medications. The aim of these studies was to characterize the pharmacological properties of a novel fluorinated etonitazene analog, called fluornitrazene (FNZ). Competitive binding assays against [^{3}H]DAMGO showed that FNZ had a Ki = ~1.0 nM. Similarly, [^{3}H]FNZ showed a Kd = ~1.3 nM. FNZ showed an EC50 of ~0.1 nM and Emax ~100% for cAMP and EC50 of ~10 nM and Emax ~100% for β arrestin. FNZ exhibits minimal interaction with efflux transporters and no major circulating metabolites. Using PET, we found that [^{18}F]FNZ showed rapid brain entry and quick washout, within 20-minutes. In the brain, [^{18}F]FNZ briefly accumulates in MOR-rich areas and this accumulation can be blocked by pre-treatment with naltrexone. *Ex vivo* uptake of [^{3}H]FNZ (1.0 µg/kg/iv) also shows an accumulation of FNZ in MOR rich areas that can be blocked by pre-treatment with naloxone. FNZ was found to be reinforcing in the intravenous self-administration paradigm. FNZ produces maximal antinociception at 10 µg/kg/sc with minimal catalepsy and no decrease in body temperature. In sum we found that FNZ is a selective and potent MOR agonist. The effective dose of FNZ to promote pain suppression was in a range insufficient to induce adverse effects such as catalepsy and hypothermia, suggesting a favorable

therapeutic profile. Ongoing efforts are aimed at further assessing the safety profile of FNZ to determine its risk for abuse potential and respiratory depression.

Disclosures: **J. Gomez:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent No. 63/452,879. **E.N. Ventriglia:** None. **A. Sulima:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent No. 63/452,879. **A. Rizzo:** None. **Z.M. Garçon-Poca:** None. **D.P. Holt:** None. **R.F. Dannals:** None. **M.G. Pomper:** None. **J. Bonaventura:** None. **M. Baumann:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent No. 63/452,879. **K.C. Rice:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent No. 63/452,879. **M. Michaelides:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); U.S. Provisional Patent No. 63/452,879.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.04/SS7

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Intramural Research Program

Title: Characterization of c-fos expression in the parabrachial nucleus during morphine withdrawal in mice

Authors: *K. SHIMODA, S.-C. LEE, J. ROSS, S. IKEMOTO;
Behavioral Neurosci. Res. Br., NIH, Natl. Inst. on Drug Abuse (NIDA), Baltimore, MD

Abstract: Opioid withdrawal produces strong negative affective symptoms. However, neural mechanisms of such emotion are poorly understood. Previous studies have shown that the central nucleus of the amygdala is highly activated during opioid withdrawal, specifically the capsular part (CeC). This pattern of subregion-specific activation raises the question of potential upstream brain areas for CeC activation during opioid withdrawal. One candidate source of excitatory drive of the CeC is glutamatergic neurons in the parabrachial nucleus (PBN). Calcitonin gene-related peptide (CGRP) neurons, which are glutamatergic in the external lateral parabrachial nucleus (elPBN), selectively project to the CeC and are suggested to contribute to the negative affective component of pain and the formation of fear memories. Additionally, the PBN contains high expression of mu opioid receptors (MOR). Thus, we hypothesized that the activity of a subpopulation of PBN neurons are modulated by opioid withdrawal and CeC-projecting elPBN CGRP-expressing neurons contribute to the activation of the CeC during opioid withdrawal. To

determine whether the activity of PBN neurons is modulated during opioid withdrawal, we examined the expression pattern of c-fos, a marker of cellular activation, in the PBN after induction of opioid withdrawal. To model opioid withdrawal in mice, we administered increasing doses of morphine over 5 days followed by naloxone for precipitated withdrawal. Using fluorescence *in situ* hybridization (RNAscope), we observed a robust increase in c-fos mRNA in the elPBN area compared to controls, and many c-fos activated elPBN neurons co-expressed MOR and CGRP mRNA. Additionally, there is a trend in differences of c-fos expressing neurons in the lateral superior subdivision of the PBN in naloxone treated mice compared to saline controls. In addition to the elPBN, we are examining whether c-fos expressing neurons colocalize with CGRP, MOR, and other cellular markers in other subregions of the PBN. Identifying the types of activated parabrachial neurons will help to understand their role in regulating aversive opioid withdrawal symptoms.

Disclosures: K. Shimoda: None. **S. Lee:** None. **J. Ross:** None. **S. Ikemoto:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.05/SS8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH MH111604
MSU DFI
MSU CNS Undergraduate Research Support
MSU Honors College Hymen and Miriam Stein Scholarship

Title: Investigating the role of GCG in the ventral tegmental area in morphine behaviors

Authors: *O. DODSON[1], C. RIVERA QUILES[2], M. S. MAZEI-ROBISON[3];
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Abstract: Although opioid dependence and addiction continue to constitute a major health and economic burden, our limited understanding of the underlying neurobiology limits better diagnostics and interventions. Dysregulation of the mesocorticolimbic reward circuit is acknowledged to contribute to various aspects of drug addiction, with alteration in the activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) known to contribute to the rewarding aspects of drug use. However, the molecular mechanisms underlying these changes in VTA DA function remain relatively unexplored. Thus, we used translating ribosome affinity purification (TRAP) to identify gene expression changes in mice that specifically occur in VTA DA neurons following chronic morphine exposure. We found that expression of several neuropeptides not traditionally described in the VTA are robustly induced by morphine exposure. Glucagon-like peptide-1 (GCG) was of particular interest as it was enriched in VTA DA neurons and its expression was robustly increased following chronic morphine exposure.

These data support increased GCG expression in the VTA following multiple types of opioid exposure and form a strong premise for studying GCG function. Thus, we hypothesize that activity of VTA GCG neurons contributes to morphine-elicited behaviors. To test this, we have begun to characterize the expression and functional impact of VTA DA neurons that co-express GCG using GCG-Cre mice and Cre-dependent viral vectors. Specifically, we are using DREADDs, designer receptors exclusively activated by designer drugs, to selectively activate or inhibit VTA-GCG neurons. We stereotaxically injected the excitatory DREADD hM3Dq (AAV-DIO-hM3Dq-mCherry) into the VTA of male and female wild-type and GCG-Cre mice and found that acute activation of VTA-GCG neurons via i.p. injection of clozapine-N-oxide (CNO, 0.3 mg/kg) does not affect general locomotor activity or elicit conditioned place preference or aversion (n = 5,9). We are now assessing whether activation of VTA-GCG neurons alters morphine-elicited behaviors (conditioned place preference, locomotor sensitization). Our preliminary data suggest there's a decrease in morphine-induced locomotion and morphine CPP in animals whose VTA-GCG neurons were activated. Studies are currently underway to assess these behaviors in a second cohort of mice. Together, these studies are expected to set the stage for future work investigating the role of specific VTA-DA^{GCG} circuits, their activity during behavior, and their potential as targets for therapeutic intervention.

Disclosures: O. Dodson: None. **C. Rivera Quiles:** None. **M.S. Mazei-Robison:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.06/SS9

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Intramural Research Program

Title: Parabrachial-to-amygdala circuits associated with opioid withdrawal

Authors: *S.-C. LEE, K. SHIMODA, J. ROSS, S. IKEMOTO;
Neurocircuitry of motivation section, Natl. Inst. on Drug Abuse, Baltimore, MD

Abstract: Opioid epidemic has been a major public health challenge in our society. Opioids, such as morphine and fentanyl, induce robust analgesic and euphoric effects, but chronic use induces strong opioid dependence. Termination of chronic opioid use induces aversive withdrawal symptoms, which is one of the main drives for opioid use disorder. Therefore, it is important to control withdrawal symptoms for the treatment of opioid addiction. However, neural mechanism of aversive effects induced by opioid withdrawal has been poorly understood. In this study we examined neuronal populations that could contribute to aversive effects induced by naloxone-precipitated opioid withdrawal. Mice received double daily injections of morphine over 5 days, then withdrawal was induced by the injection of naloxone. Using c-Fos as a proxy of neuronal activation, we examined amygdala and parabrachial regions, which have been

implicated in pain perception and aversion, following naloxone-precipitated morphine withdrawal. We found that the capsular part of central amygdala (CEC) and the interstitial nucleus of posterior limb of anterior commissure (IPAC) area strongly expressed c-Fos, while the medial and lateral nuclei of the central amygdala (CEM, CEL) showed modest or low c-Fos expression. Consistent with the connectivity in which the CEC receives dense glutamatergic projection from the external lateral part of the parabrachial nucleus (PBN), we found increased c-fos expression in the external lateral part following morphine withdrawal. To determine whether the PBN activates CEC neurons and induces aversive effects of opioid withdrawal, we examined whether such effects are reduced by silencing PBN neurons with the use of chemogenetic procedures. Treatment of CNO in wild-type mice with bilateral injections of AAV-CamKIIa-hM4Di into the PBN suppressed jumping behavior and reduced opioid withdrawal-induced conditioned place aversion (CPA). Moreover, we examined effect of inhibiting glutamatergic PBN neurons with Cre-dependent AAV-hsyn-DIO-hM4Di bilaterally injected into the PBN of vglut2-cre mice. CNO treatment reduced jumping behavior and attenuated CPA induced by withdrawal. In addition, such inhibition of PBN vglut2+ neurons also decreased c-Fos expression in the CEC, while not affecting the IPAC significantly. These results indicates that PBN afferents are critical for the activation of CEC during withdrawal and contribute to the aversive effects induced by opioid withdrawal. Future study will examine the contributions of more specific neural populations within the PBN, including mu-opioid receptor- and CGRP-expressing neurons.

Disclosures: **S. Lee:** None. **K. Shimoda:** None. **J. Ross:** None. **S. Ikemoto:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.07/SS10

Topic: G.09. Drugs of Abuse and Addiction

Title: Alterations of ghrelin-related intracellular signaling proteins following acquisition of oxycodone and cocaine self-administration in rats

Authors: *Z.-B. YOU, **A. AGARWAL**, A. TAYLOR, E. L. GARDNER;
NIDA-IRP/NIH/DHHS, Baltimore, MD

Abstract: Ghrelin, an orexigenic hormone, has recently been recognized as a critical biological substrate implicated in drug reward and motivation. We have found that both oxycodone and cocaine self-administration significantly elevates circulating ghrelin levels in drug-trained rats. Further, acquisition of such self-administration behaviors significantly upregulated ghrelin receptor (GHS-R) mRNA levels in dopamine neurons within the ventral tegmental area (VTA), a brain region critical to drug reward. We also found that antagonism of ghrelin signaling by JMV2959, a selective GHS-R antagonist, effectively inhibits oxycodone and cocaine self-administration and reinstatement of drug-seeking behaviors primed by these two addictive drugs.

However, the precise signaling mechanisms underlying the effects of ghrelin on these drug-motivated behaviors largely remains to be elucidated. In this study, we measured GHS-R and several downstream intracellular protein levels in the VTA, nucleus accumbens (NAS) and ventral hypothalamus from oxycodone- or cocaine-trained male Long-Evans rats using a Western Blot assay. In the VTA, we found that cocaine, but not oxycodone, self-administration in drug-trained rats significantly elevated GHS-R and phospho-CamK2 levels. Conversely, acquisition of oxycodone, but not cocaine, self-administration significantly decreased Phospho-CREB levels in this brain region. In the NAS, acquisition of oxycodone, but not cocaine, self-administration decreased phospho-ERK protein levels while other measured protein levels were not significantly affected. In the hypothalamus, acquisition of cocaine, but not oxycodone, self-administration decreased β-arrestin protein levels. Collectively, these findings reveal several region-specific adaptations of ghrelin-related intracellular signaling proteins in response to acquisition of drug-taking behaviors and suggest that oxycodone and cocaine may modulate different downstream signaling processes associated with GHS-R activation.

Disclosures: Z. You: None. **A. Agarwal:** None. **A. Taylor:** None. **E.L. Gardner:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR366.08/SS11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA048085

Title: Altered expectancy and reward related dopamine signaling in rat VTA following opioids

Authors: *C. LEHMANN[1], V. NAIR[2], K. MOUSSAWI[2];
[1]UPMC, Pittsburgh, PA; [2]Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Drug-associated cues acquire enhanced and persistent salience for users which is associated with cravings and increased likelihood of relapse. Development of effective treatments has proved difficult, as the underlying neurophysiology is poorly understood. However, midbrain dopamine neurons have long been implicated due to their causal role in value-learning. A popular hypothesis claims that the pharmacological effects of opioids and other drugs cause over-learning of the drug's value, resulting in inflated dopamine responses to drug-associated cues, but this hypothesis remains untested. Long-Evans rats (8 male, 2 female) were implanted with chronic microelectrode bundles in ventral tegmental area and jugular intravenous catheters for delivery of fast-acting opioid remifentanil. They were then classically conditioned to associate three distinct auditory tones with delivery of 10% oral sucrose at a well (sucrose cue), intravenous delivery of remifentanil (drug cue), or no consequence (neutral cue). Sessions consisted of two blocks of randomly interleaved trials of all three types. Quantity of sucrose and drug dose were fixed within blocks but varied between blocks. Single-unit

recordings were collected from VTA, classified with established functional clustering methods (Cohen et al., 2012), and analyzed in MatLab. In identified dopamine neurons, no significant difference was detected between firing responses to sucrose vs. remifentanil cues within the same unit and block (n = 77), regardless of associated sucrose volume or drug dose. However, firing responses to all cues as well as to sucrose reward were significantly elevated compared to neurons recorded in animals with no exposure to opioids. Negative prediction errors were preceded by a burst of firing in opioid-exposed rats but were otherwise identical to naïve comparison subjects. Contrary to predictions of current models of cue reactivity, opioid use appears to increase dopamine firing to all salient events, suggestive of a generally heightened motivational state. Our results do not support the hypothesis that the cue reactivity phenomenon is caused by greater dopamine firing to drug-related cues than natural-reward alternatives when both cues are learned in the same context. Thus, these data cast doubt on widespread models of addiction that propose selective enhancement of dopamine transmission to drug-associated stimuli, and represent a major advance in our understanding of cue reactivity and addiction.

Disclosures: C. Lehmann: None. **V. Nair:** None. **K. Moussawi:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.09/SS12

Topic: G.09. Drugs of Abuse and Addiction

Title: Kappa opioid receptor (KOR) induced aversion is mediated by G protein signaling

Authors: *A. BLOUNT, S. DARIRA, L. SUTTON;
Biol., Univ. of Maryland, Baltimore County (UMBC), Baltimore, MD

Abstract: The Kappa opioid receptor (KOR) system is a neuromodulator system that represents a potential alternative to current analgesics, but aversive side effects of KOR activation have hindered its drug development. Deciphering the intracellular signaling events activated by KOR that modulate the therapeutic and aversive effects may help aid in the development of novel compounds. In this project we interrogate the involvement of G protein signaling by targeting their endogenous antagonist, Regulators of G protein signaling [RGS]. To target the R7 family, we used R7BP knockout (KO) mice as R7BP is a membrane anchor for all R7 family members. Using a Conditioned Place Aversion test, we found that R7BP KOs have enhanced KOR-induced aversion indicating that the R7 family may modulate KOR-induced aversion. We further delineated which member of the R7 family is responsible for mediating this effect. Overall, our findings suggest that the R7 Rgs family mediates KOR-induced aversion.

Disclosures: A. Blount: None. **S. Darira:** None. **L. Sutton:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.10/SS13

Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA Grant DA057500

Title: Mu opioid receptor positive allosteric modulator BMS-986122 differentially alters pharmacological properties of agonists depending on the Gα subunit coupling to the receptor

Authors: *G. M. GRIEBLE, B. I. KNAPP, J. M. BIDLACK;
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Abstract: The mu opioid receptor (MOR) is a G-protein coupled receptor (GPCR) that is the pharmacological target of all opioid drugs currently used to treat pain. Like all GPCRs, MOR converts extracellular cues into intracellular signaling events by coupling to heterotrimeric G proteins, specifically through inhibitory-class G proteins of the $G\alpha_{i/o/z}$ family. Although all opioids currently in the market are orthosteric ligands, allosteric modulators of MOR have seen an increased interest over the past decade due to their ability to “fine tune” opioid signaling without the need to block the natural signaling tone of endogenous peptide agonists. While substantial advancements have been made in our understanding of the allosteric nature of GPCRs, the precise mechanisms of allosteric drug action for the MOR have yet to be determined. Classic pharmacological assays, such as the [^{35}S]GTPγS assay, allow for the general characterization of how allosteric modulators alter functional parameters such as potency (EC_{50}) and efficacy (E_{max}) of various MOR agonists. However, these assays suffer from having to rely on the G protein subtypes endogenously expressed by the specific cell lines used. Bioluminescence resonance energy transfer (BRET) technology allows for the functional characterization of a variety of MOR agonists and modulators for each of the six inhibitory-class $G\alpha_{i/o/z}$ subunits individually. Using an mVenus tagged Gβγ dimer and a nano-luciferase tagged GRK3ct, a downstream effector of Gβγ, we characterized the pharmacological properties of both full and partial agonists for MOR in the presence and absence of the positive allosteric modulator BMS-986122 for each Gα subunit. This study revealed unique allosteric effects based not only on the chemical identity of the orthosteric agonist but also the specific Gα subunit co-binding to the receptor. For instance, BMS-986122 at 10 μM robustly potentiated the potency of the full agonist DAMGO for MOR when coupled to $G\alpha_{oA}$, with an EC_{50} shift from 36 ± 9 nM to 9 ± 1 nM, but only weakly affected potency when the receptor coupled to $G\alpha_{i2}$, from 47 ± 4 nM to 26 ± 2 nM. Moreover, the PAM substantially increased the efficacy of the partial agonist (-)-pentazocine for $G\alpha_{i1}$ from 53 ± 2% to 103 ± 2%, with little change in potency for all $G\alpha_{i}$ subunits, but only modestly increased efficacy in the presence of $G\alpha_{oB}$, from 84 ± 1% to 106 ± 2%. Our results demonstrate that BMS-986122 differentially modulates the affinity and efficacy of orthosteric agonists based on which Gα subunit MOR signals through, allowing for a more comprehensive understanding of how allosteric modulation alters the distribution of conformational states of the receptor species.

Disclosures: **G.M. Grieble:** None. **B.I. Knapp:** None. **J.M. Bidlack:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.11/SS14

Topic: G.09. Drugs of Abuse and Addiction

Support: F31DA054759

Title: Regulation of neuronal activity in the paraventricular thalamus by chronic morphine

Authors: ***V. FRIEDMAN**, L. MU, X. LIU, H. YU, Q.-S. LIU;
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Abstract: High rates of opioid abuse and overdose deaths represent a substantial public health issue in the United States. Avoidance of withdrawal symptoms is a primary driver of continued opioid use and overdose. Understanding the neuroadaptations induced by chronic opioid exposure holds great promise for the identification of novel and efficacious therapeutics. The paraventricular thalamic nucleus (PVT), located in the dorsal midline thalamus, encodes behavioral states relevant to drug addiction, including arousal, hunger, reward-seeking, and aversion. Recent studies have pointed to a critical role of the PVT and its projections in regulating many behavioral effects of opioids. The Mu-opioid receptor (MOR) is expressed in many brain regions, which collectively mediate many cellular and behavioral effects of opioids, but the extent to which the MOR in the PVT contributes to morphine withdrawal-induced somatic signs remains unknown. In addition, several important questions remain poorly understood. To what extent do the changes in neuronal activity contribute to morphine withdrawal-induced somatic signs? Using mRNAscope in mice, we visualized co-expression of *oprm1* and *slc17a6* in PVT neurons, which indicated relatively uniform expression of MOR in glutamate neurons. Using *ex vivo* brain slice electrophysiology in mice, we have assessed how pharmacological activation of MOR in mouse PVT neurons stimulates acute hyperpolarization through G-protein coupled inward-rectifying K+ (GIRK) channels. This research will deepen our understanding of cellular and circuit mechanisms underlying adverse effects of opioid withdrawal.

Disclosures: **V. Friedman:** None. **L. Mu:** None. **X. Liu:** None. **H. Yu:** None. **Q. Liu:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Program #/Poster #: PSTR366.12/SS15

Topic: G.09. Drugs of Abuse and Addiction

Support: Work supported by the intramural funds of NIDA.

Title: Mu-opioid-galanin$_1$ receptor heteromers moderate the thalamo-striatal regulation of striatal acetylcholine release

Authors: *C. QUIROZ[1], E. GALAJ[1], A. BERNAL[1], B. SICILIANO[2], D. LUSTBERG[2], C. LILES[2], W. REA[1], D. WEINSHENKER[2], S. FERRE[1];
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Abstract: Previous studies have demonstrated the existence of heteromers of μ-opioid receptors MORs) and galanin Gal1 receptors (Gal1Rs) in the ventral tegmental area, which play a main role in the dopaminergic effects of opioids. It has also been previously demonstrated that MOR-Gal1R heteromers have a specific low sensitivity for methadone, which can explain its less significant dopaminergic effects and abuse liability as compared with morphine and other opioids. Since galanin and MOR agonists have been reported to modify the basal striatal levels of acetylcholine, we evaluated the possibility that those effects were mediated by MORs and Gal1R co-localized and possibly forming heteromers in the striatal cholinergic interneurons (CINs). In fact, using in vivo microdialysis in rats with a modified probe that allows the slow delivery of peptides, we found that in the dorsal striatum the perfusion of morphine (10 μM), but not methadone (up to 100 μM) decreased the striatal extracellular levels of acetylcholine. In addition, the striatal infusion of the Gal1R agonist M40 did not modify acetylcholine levels, but significantly attenuated the effect of morphine. Unexpectedly, in situ hybridization experiments in rats indicated that CINs in the dorsal striatum do not express MORs or Gal1Rs, while CINs in the ventral striatum showed a significant expression of MORs, but again without Gal1Rs. MOR expression could nevertheless be demonstrated in other striatal neuronal elements, while Gal1R expression was very low everywhere in the rat dorsal and ventral striatum. We then evaluated the possible co-expression of both receptors in the origin of two main excitatory inputs to the CINs, the cholinergic neurons of the pedunculopontine nucleus (PPN) and the laterodorsal tegmental nuclei (ldTgN), and the glutamatergic neurons of the midline and intralaminar thalamic nuclei. In the PPN and ldTgN, both receptors were expressed, but not co-expressed, in non-cholinergic cells. On the other hand, a significant co-expression of both receptors was demonstrated in the glutamatergic cells of all explored midline and intralaminar thalamic nuclei. The thalamic results were confirmed with immunohistochemical experiments in Gal1R-mCherry knock-in mice, which could also demonstrate co-localization of both receptors in the striatal neuropil, compatible with their presence in thalamostriatal terminals. Optogenetic-microdialysis experiments are now in progress to demonstrate a functional role of striatal MOR-Gal1R heteromers localized in the thalamostriatal terminals in the regulation of striatal acetylcholine release.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: DE-AC52-07NA27344/20-ERD-009

Title: Transcriptomic analysis reveals altered hippocampal neuronal function and glial cell responses following sub-chronic exposure to fentanyl

Authors: A. SEBASTIAN[1], ***D. LAM**[1], C. BOGGURI[1], M. MENDEZ[1], S. K. G. PETERS[1], S. MCCLOY[1], N. LEON[1], N. R. HUM[1], C. A. VALDEZ[1], N. O. FISCHER[2], G. LOOTS[1], H. A. ENRIGHT[1];
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Abstract: In the past two decades, fentanyl has been responsible for a steady increase in lethal drug overdose cases. Fentanyl, like morphine, binds to mu-opioid receptors (MOR) in many brain regions, and this binding event can lead to addiction, tolerance, impairment of cognitive functions, and inhibit nociception, arousal, and respiration. In recent years, high-throughput omics studies have queried the gene expression changes in morphine exposed brain tissue and cells. However, very limited data is available on neurological consequences of fentanyl use. Using single-cell RNA sequencing (scRNA-seq), we profiled the gene expression in all individual cells purified from the hippocampus of rats having been sub-chronically (i.e., 5 days) exposed to fentanyl (50 µg/kg, daily). In-depth characterization of cells revealed that sub-chronic fentanyl exposure altered the proportion of cell types in this brain region; the proportion of microglia/macrophages, endothelial cells and fibroblasts were increased while oligodendrocytes, oligodendrocyte precursor cells and ependymal cells were decreased. We also identified cell-type specific transcriptomic changes in response to fentanyl exposure. Fentanyl exposure significantly induced the expression of heat shock proteins (*Hspa1a* and *Hspa1b)* in microglia. Inflammatory cytokines (e.g., *Ccl3, Ccl4, Tnf, Il33*) that were downregulated in microglia were upregulated in antigen-presenting-macrophages following fentanyl exposure. While the proportion of astrocytes was not affected by fentanyl, a shift in its phenotype was observed in the fentanyl versus saline group, wherein a higher proportion of $Scrg1^{hi}$ astrocytes was detected in the exposed tissue while saline group had more $Nnat^{hi}$ astrocytes. We detected a small neuronal population using scRNA-seq, but flow cytometry data suggests that neurons were sensitive to the processing steps for scRNA-seq. Thus, using age- and treatment-matched tissue, we purified hippocampal neurons and profiled fentanyl-induced changes using bulk RNA-seq. In rat neurons, fentanyl downregulated the expression of 314 genes associated with biological processes such as synaptic signaling, cognition, behavior, learning and memory. Additional bulk RNA-seq experiments revealed that 44 of these 314 genes (e.g., *Rph3a, Unc13c*, *Dnmt3a* and *Ntng2)* were also down-regulated in fentanyl-treated (40 µM) cell cultures containing human induced pluripotent stem cell-derived neurons and primary astrocytes, suggesting a similar response between species. In summary, this study identified several genes associated with neuronal function and glial responses altered by fentanyl exposure.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant F31 DA057050
NIH grant R01 DA053070

Title: Calcium imaging of central amygdala activity after escalation of fentanyl self-administration

Authors: *S. MALONE[1], P. I. ORTINSKI[2], M. T. BARDO[1];
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Abstract: Our previous research found that rats with a behavioral history of escalated fentanyl intake across 6 hr sessions showed greater drug-primed reinstatement than rats without escalated intake across 1 hr sessions. The central amygdala (CeA) integrates valence and salience information with internal and external sensory cues and has direct connections to reward-relevant brain areas such as the ventral tegmental area and nucleus accumbens. This study determined whether escalation of fentanyl self-administration produces differences in basal and fentanyl-exposed CeA neuronal activity, as measured by *ex vivo* calcium (Ca^{2+}) imaging, following acute withdrawal. In preparation for Ca^{2+} imaging, adult male (n=9) and female (n=9) Sprague-Dawley rats were microinjected bilaterally with AAVhSyn-GCamp6f virus into the CeA. Rats were then trained to self-administer i.v. fentanyl (2.5 µg/kg/infusion) or saline across 7 daily 1-hr sessions, followed by 21 6-hr sessions. On the day after the final session, withdrawal signs were measured immediately prior to euthanasia and brain slices were prepared for Ca^{2+} imaging. CeA video recordings were first taken in the presence of artificial cerebrospinal fluid (ACSF), followed by 0.1 or 1 µM fentanyl. Amplitude, duration, and frequency of Ca^{2+} transients were measured in each cell and cell-weighted, video averages of transient values were used in analyses. All data were processed using Matlab and analyzed using multilevel modeling via SAS statistical software. During initial 1-hr acquisition sessions and when sessions were increased to 6-hr, fentanyl rats escalated their intake, whereas saline rats did not. During acute withdrawal, fentanyl rats expressed fewer exploratory behaviors such as line crosses, digs, and rears and expressed more wet dog shakes. For Ca^{2+} imaging, fentanyl exposure reduced Ca^{2+} transient frequency, duration, and log(amplitude) for both saline and fentanyl rats. Furthermore, while no basal differences were apparent between saline and fentanyl rat frequency, duration, and log(amplitude), rats with a behavioral history of fentanyl escalation demonstrated tolerance to the frequency and duration suppressing effects of fentanyl exposure. These results suggest that

prior fentanyl self-administration exposure did not alter baseline CeA activity but decreased CeA reactivity to fentanyl. Further work is needed to determine whether these tolerance-like changes in CeA calcium activity persevere throughout extended abstinence.

Disclosures: S. Malone: None. **P.I. Ortinski:** None. **M.T. Bardo:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: Investigator-Initiated Studies Program of Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

Title: Dual orexin receptor antagonism improves sleep/wake and reward-seeking alterations in the recovery period produced by multi-day morphine exposure

Authors: *J. T. MCKENNA[1,2], P. RAMESH BABU[1,2], M. MACIVER[1], F. KATSUKI[1], J. G. MCCOY[1,3], J. M. MCNALLY[1], R. E. STRECKER[1,2];
[1]Psychiatry, VA Boston Healthcare System/Harvard Med. Sch., West Roxbury, MA; [2]Boston VA Res. Inst., Boston, MA; [3]Stonehill Col., Easton, MA

Abstract: Opiate withdrawal symptoms include sleep disturbance and hyperarousal, which may contribute to increased relapse probability. Due to the limited efficacy of treatments, investigations of alternative pharmacotherapies are warranted. We exposed mice to an ascending exposure of morphine sulfate and evaluated if withdrawal-like symptoms in recovery were reduced with dual orexin receptor antagonist (DORA) treatment. Male C57BL/6J mice were surgically implanted with EEG/EMG electrodes for sleep/wake recording. 1 week later, baseline (BL) measures were obtained. Animals were then exposed to an ascending dosing schedule of morphine (10, 20, 30, 40 and 50 mg/kg 2x a day at 8AM & 4PM), followed by one final 50 mg/kg dose on day 6. Starting 24 hrs later, DORA-22 (Merck & Co., Inc.; 100mg/kg) or control solution (20% TPGS) was administered (gavage) every morning (8AM) for 4 days. Sleep/wake states in the light and dark period differed comparing BL to morphine exposure day 5 (M5) and recovery days 2 (R2) and 5 (R5) (two-way ANOVA; N=7). Post-hoc comparisons (Fisher's LSD) detected a significant increase of wake in the light period (9AM-7PM) at M5 (137.1% compared to BL), which remained elevated through R2 (20.8%), and NREM sleep was decreased (M5, 69.8%). REM sleep was also decreased at M5 (84.8%), R2 (27,3%), and R5 (30.3%). In the dark period (7PM-7AM), wake was decreased (M5, 34.4%; R2, 19.5%; R5, 20%), as increases in NREM (M5, 54.9%; R2, 27.3%; R5, 27.9%) and REM sleep (M5, 64.3%; R2, 82.1%; R5, 89.3%) were noted. DORA-22 treatment during the recovery period promoted NREM sleep in R2 (N=6; hrs 1-5 following compound administration; wake TPGS 39.3±3.0% vs. DORA-22 22.9±2.5%; NREM TPGS 56.3±2.8% vs. DORA-22 71.9±2.7%) and R5 (hours 1-5; wake TPGS

33.7±2.2% vs. DORA-22 24.9±1.6%; NREM TPGS 59.7±1.9% vs. DORA-22 66.8±1.8%). DORA-22 treatment reduced exploration in the open field maze (N=7, recovery day 6 (R6); TPGS 19.3±7.0% vs. DORA-22 3.9±1.3% of total time in center zone; TPGS 24.9±3.2% vs. DORA-22 11.9±1.5% of total distance in center zone). Preliminary data (N=4) indicates that animals spent 54% less time attending to a novel animal in a social interaction task (R6), suggesting decreased sociability. Morphine exposure promoted circadian dysregulation of sleep-wake behavior which continued up to 5 days of the recovery period. DORA-22 improved the sleep/wake profile and decreased the increased exploration observed, suggesting diminished reward seeking. These findings indicate that DORA-22 may be a useful compound to improve sleep/wake and behavioral alterations seen after opiate exposure.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.16/SS19

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA057767
R01 HL163965

Title: The role of the Locus Coeruleus in context-dependent respiratory tolerance to opioids

Authors: *C. C. SZUJEWSKI[1], M. WARDEN[2], S. SULLERE[2], A. AYYALARAJU[3], A. J. GARCIA, III[4];
[1]Neurobio., [2]Univ. of Chicago, Chicago, IL; [3]Ohio State Univ., Columbus, OH; [4]Emergency Med., The Univ. of Chicago, Chicago, IL

Abstract: The anticipation of reward represents a brain state that is shaped by neuromodulation and is relevant to behaviors and outcomes linked with opioid use disorder. The Locus Coeruleus (LC) is the primary source of noradrenergic neuromodulation throughout the brain. LC activity shapes both cue-reward-related behaviors and breathing responses to deviations in blood gases. During opioid induced respiratory depression (OIRD), blood gas deviations increase the

likelihood of mortality. This study aims to investigate how LC activity impacts ORID following repeat opioid use (ROU). We developed a murine model of context-based repeat fentanyl use where breathing was measured by unrestrained whole-body plethysmography. This protocol consisted of two phases. Phase 1 (duration five days): fentanyl ($0.7 mg \bullet kg^{-1} \bullet day^{-1}$; *i.p.*) was administered in a distinctive context (fentanyl-paired context, FP) and saline in a different salient context (saline-paired context, SP). Phase 2 (duration two days): fentanyl induced respiratory depression was assessed in the FP and SP. In a subset of these experiments (n=6), fiber photometry was simultaneously performed with plethysmography to characterize activity from LC neurons virally expressing GCaMP8s with OIRD. 83% of mice (n=15/18) showed a smaller OIRD during both days in Phase 2 when compared to the OIRD on Day 1 of Phase 1. Furthermore, 67% of these mice (n=12/15) showed a decreased ORID in the FP as compared to the SP (P=0.038). During Phase 2 the frequency of LC activity was greater prior to fentanyl administration in the FP when compared to the SP (n=6/6, P=0.02). During OIRD, the frequency of LC activity in the FP was greater than the SP (n=5/6; P=0.09). Additionally, in the FP, LC activity preceded high-frequency respiratory events during OIRD. However, this activity pattern was not evident during OIRD on Day 1 of Phase 1 or in the SP. Furthermore, optogenetic activation of LC neurons in naïve mice blunted ORID (n=5). These results show that context-dependent tolerance to the respiratory side effects of fentanyl emerges following ROU and indicate that changes in LC activity patterns prior and during OIRD contribute to such tolerance. These findings support the hypothesis that LC activity plays a key interoceptive role emerging from learned associations with fentanyl use that can produce tolerance to OIRD. A failure to establish such associations and the corresponding activity of the LC may increase vulnerability to opioid overdose among chronic opioid users. This work was supported by R01 DA057767 and R01 HL163965.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: Arizona State University Startup Funds

Title: Effects of spontaneous oxycodone withdrawal-induced anhedonia on social behavior and markers of neuroplasticity in mice

Authors: *O. M. LAW, J. C. GEWIRTZ, J. L. VERPEUT;
Psychology, Arizona State Univ., Tempe, AZ

Abstract: Opioid-related deaths in the U.S. have quadrupled in the past 20 years, with increased use of prescription opioids playing a significant role. Opioids produce both somatic and affective withdrawal symptoms. The latter (e.g., anxiety, anhedonia) are seen even after one or limited exposures to the drug (acute dependence). In this project, we are characterizing the likely presence of anhedonia in C57Bl/6J mice following a series of injections of oxycodone (5 mg/kg/day) and consequent effects on social behavior, with a view to spatially mapping underlying changes in cortical gene expression associated with this withdrawal state. Previous research has examined social behavioral changes seen with opioid withdrawal such as reduced contact between mouse pairs (Goeldner et al., 2011), and opioid addiction also negatively impacts social relationships in humans (Christie et al., 2021). We hypothesized that spontaneous withdrawal from oxycodone would result in withdrawal induced anhedonia (WIA), characterized by decreased sucrose preference, decreased locomotion, and increased avoidance of conspecifics. WIA was measured using a 10% sucrose preference test at baseline and during withdrawal. Preliminary results found no significant differences in sucrose preference between oxycodone and control animals during withdrawal. To examine social behavior during withdrawal, groups of mice were allowed to interact in an open field for 30 minutes. Behavior will be measured using the machine learning algorithm, SLEAP (Social Leap Estimates Animal Poses) and post-sacrifice, markers of neural plasticity will be analyzed.Additionally, newer sequencing methods, such as multiplexed error-robust in situ hybridization (MERFISH), allow mapping of single-cell changes in gene expression with high spatial resolution (Chen et al., 2015). Therefore, we hypothesized that opioid withdrawal would lead to an upregulation of genes implicated in neuroplastic processes such as synaptogenesis, as such genes were also found to be upregulated during morphine withdrawal (Liu et al., 2021), specifically in the infralimbic cortex, medial prefrontal cortex, anterior cingulate cortex, and nucleus accumbens. Altogether, this experiment will give us greater insight into the combinatory behavioral and genomic effects of spontaneous oxycodone withdrawal and thus provide additional considerations for identifying interventions and treatment plans for opioid use disorders.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH grant DA047443

Title: Glutmatergic and GABAergic μ-opioid receptor VTA neurons differentially modulate motivational and physiological consequences of fentanyl use

Authors: *E. D. PRÉVOST, L. WARD, D. CAPES, M. HEILBRON, A. JANI, D. J. MCGOVERN, D. H. ROOT;
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Abstract: The ventral tegmental area (VTA) is a major site of opioid reward processing. Canonically, μ-opioid receptor (MOR) activation inhibits GABAergic VTA interneurons, thereby disinhibiting dopamine release from VTA neurons projecting to the nucleus accumbens (NAc). However, recent studies have identified a population of glutamatergic MOR-expressing VTA neurons that also modulate dopamine release from NAc-projecting VTA neurons. In opposition to the canonical model of GABA-MOR-gated disinhibition of dopamine release, the VTA glutamate-MOR circuit decreases NAc dopamine release through MOR-gated reduction in excitatory drive to dopaminergic VTA neurons. Here, we investigated the role of VTA GABA-MOR and glutamate-MOR subpopulations in the rewarding, aversive, and physiological consequences of opioid use. Oprm1::Cre mice received VTA infusions of a Cre-dependent vector encoding small interfering RNA to silence glutamate transmission (via the vesicular glutamate transporter type 2), GABA transmission (via the vesicular GABA transporter), or a control (scrambled RNA sequence). Mice were then tested on fentanyl-conditioned place preference, precipitated withdrawal-conditioned place aversion, and fentanyl-induced hyperlocomotion. Preliminary data suggest that silencing GABA transmission from VTA MOR neurons results in reduced fentanyl place preference and increased fentanyl-induced hyperlocomotion compared to glutamate silencing and control groups. Further results will be discussed at the meeting.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA048055
NIH Grant DA049139

Title: Neurophysiological characterization of a corticolimbic circuit across heroin self-administration, extinction, and reinstatement in rats

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Abstract: Evidence indicates that the rat infralimbic cortex (IL) is important for the inhibition and extinction of drug seeking, although its role in the extinction of opioid seeking is unclear. It is likely, however, that the IL interacts with other regions known to regulate drug seeking during this process. Evidence from both human and rodent work indicates that the anterior insular cortex (aIC) and basolateral amygdala (BLA) mediate drug craving and drug-seeking behaviors. Yet, how the IL, aIC, and BLA interact with each other on a neurophysiological level across opioid-seeking behaviors is unknown. Therefore, we used in vivo electrophysiology to simultaneously record from this network during heroin self-administration, extinction, and reinstatement to explore the neural interactions among these regions during such behavior. Four adult male Sprague-Dawley rats learned to self-administer heroin intravenously on a high-to-low dose tapered FR1 schedule, decreasing heroin dosage every two sessions until arriving at 0.014 mg/kg/infusion. Rats then proceeded to 2-hour trial-based sessions, wherein a lever producing a heroin infusion and light and tone cues was only available during specific signaled trials. This created epochs of heroin-seeking behavior, or inhibition thereof, around which electrophysiological data could be analyzed. After at least 10 days of self-administration, rats were fitted with a custom-designed three-site, 96-channel microdrive array (9 tetrodes/site), with tetrodes lowered into the IL, aIC, and BLA. Rats proceeded with recorded self-administration, followed by at least 5 days of recorded extinction sessions wherein lever presses during a trial had no consequence, and then a recorded reinstatement session wherein a priming injection of heroin was given beforehand and lever presses still had no consequence. Single-unit and local field potential (LFP) data was collected from each tetrode and analyzed for each region during the last self-administration, early and late extinction, and heroin-primed reinstatement sessions. Single-unit findings indicate that subpopulations of IL, aIC, and BLA neurons are responsive to lever presses and trial-onset signals and that these response profiles change across extinction sessions. LFP findings indicate that theta-band oscillations in these regions are also sensitive to changes in heroin-seeking contingencies. Ongoing work is investigating this network in female rats. Overall, these findings suggest that IL-aIC-BLA circuitry comprises a dynamic network during heroin-seeking behaviors.

Disclosures: **M.S. McGregor:** None. **S.J. Farley:** None. **J. Kim:** None. **R.T. LaLumiere:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.20/SS23

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant DA08259

Title: Sex and chronic stress alter the distribution of corticotropin releasing factor receptors within rat hippocampus following oxycodone conditioned place preference

Authors: *D. N. SILBERSTEIN[1], Z. BAIG[2], D. WELT[1], F. YU[1], J. D. GRAY[3], Y. ZHOU[3], B. R. RUBIN[1], T. A. MILNER[1];
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Abstract: Corticotropin-releasing factor receptor 1 (CRFR1) has a role in stress-induced hippocampal plasticity that can affect learning and memory processes including those important for addiction. Prior work (Mcalinn et al., Neurosci. 2018) revealed sex differences in the distribution of CRFR1 within CA3 pyramidal cells and hilar interneurons in rats that could differentially affect associative learning processes in females and males, especially in response to stress. Our subsequent studies (Chalangal et al PBB 2022) showed that both female and male unstressed rats acquire oxycodone (Oxy) conditioned place preference (CPP), whereas only CIS (chronic intermittent stress) females acquire Oxy CPP. We found Oxy CPP induces sex-dependent redistributions of opioid receptors in hippocampal circuits in a way that facilitates opioid-associative learning processes, especially in females. Here, we examine the effect of Oxy CPP in unstressed and CIS adult female and male rats on the subcellular distribution of CRFR1 in CA3 pyramidal cells and dentate gyrus (DG) hilar interneurons using immuno-electron microscopy. **CA3 pyramidal cells:** After Oxy CPP, unstressed Oxy males compared to Oxy females have more CRFR1 on the plasmalemma of CA3 dendrites ($p = 0.04$), suggesting increased binding capacity for CRF. However, Oxy CIS females vs saline (Sal) CIS females ($p = 0.04$) and Oxy CIS males ($p = 0.006$) have greater total CRFR1 density in small CA3 dendrites indicating greater reserve CRFR1 pools. **DG interneurons:** In unstressed females, dendrite CRFR1 density is greater in the cytoplasm ($p = 0.04$) and in total ($p = 0.03$) for the Oxy vs Sal groups. While unstressed Oxy males compared to Sal males similarly show increased total CRFR1 density ($p = 0.0008$), they also have increased CRFR1 density ($p = 0.005$) near the plasmalemma of dendrites. Thus, Oxy increases reserve pools of CRFR1 in both sexes but the pools in males are more readily available for insertion into the plasmalemma of interneuron dendrites. In contrast, following CIS, Oxy decreased CRFR1s in the cytoplasm of dendrites compared to Sal females ($p = 0.01$). Moreover, CRFR1 decreased near the plasmalemma ($p = 0.03$) in small ($< 1\mu m$) dendrites for CIS Oxy females compared to CIS Sal females. Thus, in CIS females Oxy CPP decreases pools of CRFR1, especially those available for insertion. There were no changes in CRFR1 density in dendritic compartments of CIS males (which do not acquire Oxy CPP) in either group. In conclusion, these sex-differences in the distribution and trafficking of CRFR1 in CA3 pyramidal cells and DG interneurons may contribute to the sex differences in Oxy associative learning in baseline conditions and following CIS.

Disclosures: D.N. Silberstein: None. **Z. Baig:** None. **D. Welt:** None. **F. Yu:** None. **J.D. Gray:** None. **Y. Zhou:** None. **B.R. Rubin:** None. **T.A. Milner:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: NICHD Z01-HD001205-27 Intramural Research Award
Center for Compulsive Behaviors Intramural Fellowship

Title: Opioid suppression of inhibition across regions, species, and development

Authors: *A. P. CACCAVANO, A. VLACHOS, S. KIMMEL, J. H. KIM, V. MAHADEVAN, G. VARGISH, X. YUAN, S. HUNT, E. LONDON, R. CHITTAJALLU, K. A. PELKEY, C. J. MCBAIN;
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Abstract: The opioid system in the central nervous system plays a critical role in pain sensation, stress, and mood, and is a key target for drugs of abuse. At the cellular level, significant gaps remain in our understanding of how opioids modulate different neuronal subpopulations. Within the hippocampus, opioids suppress inhibitory parvalbumin-expressing (PV) cells, thus disinhibiting excitatory pyramidal cells (PCs). However, prior research has largely been restricted to the adult rodent hippocampus, and it is uncertain if this disinhibitory motif is conserved in other regions, species, or across development. This study utilized optogenetics to selectively activate PV cells and record light-evoked synaptic currents in downstream PCs. Consistent with prior studies, light-evoked responses exhibited robust opioid sensitivity in the hippocampus. However, across neocortical regions, this opioid sensitivity was highly variable, indicating a potential specialization of hippocampal PV cells. Moreover, this dissociation in function translated to both virally-transfected rhesus macaques and resected human tissue. In mice undergoing morphine withdrawal, this acute opioid-mediated modulation was occluded, with implications for the activity of the microcircuit and hippocampal-dependent learning and memory. Finally, while parvalbumin cells have traditionally been challenging to study in early development due to the delayed onset of parvalbumin expression, using a novel strategy to target Tac1 expressing cells (with high colocalization to parvalbumin), this disinhibitory motif was observed in early postnatal development, just as the inhibitory circuits were being established. Together, these studies provide insight into the effects of this widely-used class of drug on a neuronal subpopulation essential for excitatory-inhibitory balance in the brain.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIDA-IRP, NIH

Title: Xylazine effects on opioid-induced brain hypoxia

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Abstract: Xylazine has emerged in recent years as an adulterant in an increasing number of opioid-positive overdose deaths in the United States. Although its exact role in opioid-induced overdose deaths is largely unknown, xylazine is known to depress vital functions and cause hypotension, bradycardia, hypothermia, and respiratory depression. In this study, we examined the brain-specific hypothermic and hypoxic effects of xylazine and its mixtures with fentanyl and heroin in freely moving rats. In the temperature experiment, we found that intravenous xylazine at low, human-relevant doses (0.33, 1.0, 3.0 mg/kg) dose-dependently decreases locomotor activity and induces modest but prolonged brain and body hypothermia. In the electrochemical experiment, we found that xylazine at the same doses dose-dependently decreases nucleus accumbens oxygenation. In contrast to relatively weak and prolonged decreases induced by xylazine, intravenous fentanyl (20 μg/kg) and heroin (600 μg/kg) induce stronger biphasic brain oxygen responses, with the initial rapid and strong decrease, resulting from respiratory depression, followed by a slower, more prolonged increase reflecting a post-hypoxic compensatory phase, with fentanyl acting much quicker than heroin. The xylazine-fentanyl mixture eliminated the hyperoxic phase of oxygen response and prolonged brain hypoxia, suggesting xylazine-induced attenuation of the brain's compensatory mechanisms to counteract brain hypoxia. The xylazine-heroin mixture strongly potentiated the initial oxygen decrease, and the pattern lacked the hyperoxic portion of the biphasic oxygen response, suggesting more robust and prolonged brain hypoxia. These findings suggest that xylazine exacerbates the life-threatening effects of opioids, proposing worsened brain hypoxia as the mechanism contributing to xylazine-positive opioid-overdose deaths.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH MH111604
MSU DFI
NSF GRFP
HHMI Gilliam Fellowship

Title: Role of neuromedin s-expressing ventral tegmental area neurons in morphine behavior

Authors: *C. M. RIVERA QUILES, O. DODSON, M. ALDAY, M. S. MAZEI-ROBISON;
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Abstract: Opioid dependence and addiction constitute a major health and economic burden, but our limited understanding of the underlying neurobiology limits better interventions. Alteration in the activity and output of dopamine (DA) neurons in the ventral tegmental area (VTA) is known to contribute to drug effects, but the mechanisms underlying these changes remain relatively unexplored. We used TRAP to identify gene expression changes in VTA DA neurons following chronic morphine and found that Neuromedin S (NMS) is enriched in VTA DA neurons, and its expression is robustly increased by morphine. However, whether all VTA DA neurons express NMS, and their potential functional impact has yet to be determined. We hypothesize that NMS neurons represent a novel subset of VTA neurons that contribute to morphine-elicited behavior. Specifically in these studies, we hypothesize that activating and inhibiting VTA-NMS neurons will promote and inhibit morphine behaviors, respectively. To test this, adult male and female NMS-Cre mice and wild-type littermates were used. Cre-dependent viral vectors were stereotaxically injected into the VTA to allow for DREADD-mediated activation (Gq) or inhibition (Gi) of VTA-NMS neurons. Behavioral analyses were completed 2 weeks after surgery. Locomotor activity was assessed following saline (d1), saline + Clozapine-N-oxide (CNO, 0.3mg/kg, ip, d2-d3), and morphine (15mg/kg) + CNO (d4-d8). A morphine + CNO challenge was done 1 week following d8. Conditioned place preference (CPP) was also performed. Mice underwent a 20 min. pre-test, followed by conditioning sessions where they received vehicle + saline in the morning and CNO + morphine (0.3mg/kg and 15mg/kg, respectively) in the afternoon for 4 days. Morphine preference was assessed during a 20 min. post-test. Significant differences ($p<0.05$) were determined using a repeated-measures two-way ANOVA for locomotor behavior and unpaired t-tests for CPP. NMS-Gq mice exhibit increased morphine-induced locomotor activity compared to controls. NMS-Gi mice show a significantly decreased locomotor response to a challenge morphine + CNO injection. In CPP assays, NMS-Gq mice displayed a similar morphine-CNO CPP to controls. However, NMS-Gi mice exhibited significantly decreased morphine-CNO CPP compared to controls. Thus, manipulation of VTA-NMS neuronal activity alters morphine-elicited behaviors including locomotion, sensitization, and CPP. Future studies will determine whether VTA-NMS neuronal activity modulates other morphine behaviors. Our data suggest that VTA-NMS neurons represent a subset of neurons that may be functionally relevant for morphine responses.

Disclosures: C.M. Rivera Quiles: None. **O. Dodson:** None. **M. Alday:** None. **M.S. Mazei-Robison:** None.

Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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DA049139
DA048055

Title: Carbonic anhydrase 4 inhibition attenuates synaptic rearrangements evoked by oxycodone withdrawal in nucleus accumbens and drug-seeking behavior.

Authors: *S. C. GUPTA[1], A. GHOBBEH[1], R. J. TAUGHER-HEBL[1], R. FAN[1], R. T. LALUMIERE[2], J. A. WEMMIE[1];
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Abstract: The United States is overwhelmed by an epidemic of opioid use disorder (OUD) and deaths from opioid overdoses. Thus, a better understanding of the insidious effects of these drugs is urgently needed. Accumulating evidence suggests that opioids hijack brain synapses responsible for long-term memories of pleasurable experiences. The result is a sustained and increasing desire for drugs which can overwhelm normal memory systems. We recently identified a novel role for acid-sensing ion channels (ASICS) and carbonic anhydrase 4 (CA4) in pH-mediated signaling at synapses in nucleus accumbens core medium spiny neurons (NAcc-MSNs). Here we investigated whether manipulating this pH-mediated signaling might alter the insidious and long-lasting effects of opioids. We administered oxycodone (3 mg/kg, i.p.) vs. saline for 5 days to wild-type mice or mice lacking CA4 (*Car4*$^{-/-}$). Following 10 days of abstinence, we administered the carbonic anhydrase inhibitor acetazolamide (30 mg/kg, i.p.) vs. saline and subsequently assessed multiple measures of glutamatergic signaling at NAcc-MSN synapses in acute brain slices. These measures included AMPAR/NMDAR ratio, AMPAR rectification index, and sensitivity to the Ca^{2+}-permeable AMPAR blocker NASPM. We found that these measures were increased in wild-type mice following the oxycodone injections and abstinence. Importantly, in wild-type mice acetazolamide injection reversed these findings within 24 hours. In contrast, these effects of oxycodone and acetazolamide were not observed in *Car4*$^{-/-}$ mice, suggesting CA4 was required. We then assessed opioid-seeking behaviors during 10 days of intravenous oxycodone self-administration (0.25 mg/kg/infusion, 6 hrs access per day) and following 30 days of abstinence. We found that wild-type and *Car4*$^{-/-}$ mice self-administered oxycodone similarly. However, after 30 days of abstinence drug-seeking measured by unreinforced lever pressing was significantly reduced by acetazolamide in wild-type mice. *Car4*$^{-/-}$ mice exhibited significantly less drug seeking than wild-types, and again acetazolamide had no effect in these mice. To probe whether these effects of acetazolamide and CA4 on drug-seeking behavior might be mediated through glutamatergic signaling on NAcc-MSNs we again assessed AMPAR/NMDAR ratio in these mice in brain slices. We observed outcomes very similar to those following experimenter administered oxycodone described above. Together these results suggest the exciting possibility that acetazolamide, a non-opioidergic medication already approved at similar doses for use in humans for other illnesses, might attenuate craving and relapse in patients with OUD.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

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Program #/Poster #: PSTR366.25/TT3

Topic: G.09. Drugs of Abuse and Addiction

Support: BBRF Grant 28334
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Title: Neuroinflammation and gut diversity effects due to opioid self-administration

Authors: *K. M. BRUNETTI, S. SHUCHI, L. COLON-PEREZ;
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Abstract: Gut-Brain Axis (GBA) research will potentially expand our understanding of addiction and provide a new paradigm for developing new substance use disorder (SUD) therapeutics. The use of opioids increases the risk of developing SUDs in humans. Opioid use activates μ-opioid receptors expressed in the gastrointestinal tract of rodents and humans, potentially originating neural signals to affect the brain. GBA and neural analysis can elucidate the complex interactions between the brain and gut that lead to pathological drug seeking and consumption and their relation to GBA components (i.e., bacterial populations, gut peptides, and gut signaling). Opioids are usually prescribed to treat pain in humans, but prolonged use can lead users to physical dependence that co-occurs with gastrointestinal changes. In this study, we will share the determining temporal hallmarks of gut alterations in rats self-administering morphine for 15 days and relate it to neuroinflammatory features in the brain. One of our hypotheses is that abuse of drugs, such as morphine, starts by inducing inflammation of the brain and gut taxonomic changes similar to those observed in human opioid users. In this project, we used Sprague Dawley (SD) rats, and both the experimental and control groups were surgically implanted with intravenous (IV) catheters. The control group was exposed to the self-administration box, but only received sucrose instead of morphine to avoid differences in behavior due to instrument learning or exposure to self-administration chambers. The experimental group was trained to self-administer morphine dosed to their body weight of 0.4 mg/kg for 14 days. The experimental timeline was (1) baseline: 7 days after catheter implantation, (2) acute: 24 hours after the second day of self-administration, and (3) chronic: 24 hours after completion of the 12 days of self-administration. Fecal samples were acquired at the three-time points and analyzed with 16s DNA sequencing to determine the relative abundance of microbial species at the time points. After the last day, we collected the brains from all animals and prepared tissue FFPE for immunohistochemistry and spatial transcriptomics analysis. Concurrently we acquired MRI diffusion-weighted scans in a 7.0 T preclinical scanner. Rats were restrained under sedation (isoflurane 5% induction, 2% maintenance) reducing the stressor of noise and restriction while scanning. This project will help us identify whether neuroinflammation markers occur due to large doses of morphine repetitively in rodents.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.26/TT4

Topic: G.09. Drugs of Abuse and Addiction

Title: Cue reactivity of non-dopamine neurons in the midbrain

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Abstract: An influential model of drug addiction proposes that repeated exposure to addictive drugs results in overvaluation of drug cues and long-lasting cue-drug associations, which results in craving and heightened motivation to seek drugs upon exposure to these cues. This model focuses on the role of midbrain dopamine neurons in forming these associations, known as cue reactivity. However, the role of non-dopamine neurons in the midbrain in cue reactivity is not well understood. Specifically, the neurophysiological responses of these neurons to cues predicting opioids vs. natural rewards have not been examined previously. Single-unit recordings were collected from the midbrain of adult Long-Evans rats during Pavlovian presentation of cues associated with the short acting opioid remifentanil vs. oral sucrose. Sorting was performed using Offline Sorter and the data analyzed with Neuroexplorer and Matlab. Non-dopamine neurons were identified using automated hierarchical clustering. Our data identify a subpopulation of non-dopamine neurons that shows significant cue reactivity in response to opioid or sucrose cues and are inhibited by sucrose or remifentanil reward. However, they show an immediate initial phasic activation response to cues which peaks ~20-30 msec after cue onset, followed by more sustained bursting which peaks ~500-1000 msec after cue presentation and lasts >2sec. The immediate phasic response to cues is faster than the phasic response observed in putative dopamine neurons. Additionally, this subpopulation shows enhanced response to opioid compared to sucrose cue within the same neurons, and to sucrose cues in drug-exposed compared to drug naïve animals. Our results demonstrate biphasic cue reactivity in non-dopamine neurons in the midbrain. Next steps involve establishing the identity of these neurons and their role in drug seeking behavior.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Topic: G.09. Drugs of Abuse and Addiction

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Title: Morphological analysis of the different microglia phenotypes in a chronic morphine self-administration model in wistar rats

Authors: *D. A. ELIZARRARÁS[1], D. MEDINA SÁNCHEZ[2], M. SERRANO[2], J. MAYA[3], E. A. GARZA-VILLARREAL[4], C. J. CARRANZA-AGUILAR[5];
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Abstract: Microglia intrinsic sensitivity to the microenvironment results in the exhibition of different activation states and morphologies. Objectively, four predominant morphologies have been identified: ramified, rod-like, activated, and ameboid, each corresponding to distinct functional states of microglia. Studies have shown that chronic administration of morphine induces microglial activation. This activation plays a crucial role in initiating and sustaining neuroinflammation. However it is still uncertain if distinct states of activation occur in varying brain regions while undergoing morphine treatment. This study aims to investigate potential variations in microglial morphology in specific brain regions known to be important in the stage of morphine intoxication: caudate putamen (CPu), globus pallidus (GP), and dentate gyrus (DG). According to our hypothesis, subjecting rats to long-term morphine self-administration will result in the discovery of distinct and unique morphological characteristics of microglia in those specific brain regions. Materials and Methods: wistar rats at the age of p40 were subjected to a chronic 20 days of self-administration model employing a fixed ratio 1 scheme (FR1) within operant conditioning chambers. Rats were divided into two groups: a morphine (MO+, n= 4, 0.01 kg/mg/infusion) and a sham group (MO-, n= 4, saline 0.9%). Upon conclusion of the self-administration phase, all rats were perfused and fixated. Immunofluorescence staining of microglia was performed using IBA1 antibody, and the resulting images were captured utilizing a confocal microscope with z-stack acquisition employing a 40x objective in the targeted regions. Data analysis was carried out using an individual cell Skeleton Analysis protocol (AnalyzeSkeleton (2D/3D)). This protocol provided a comprehensive summary of cell morphology in terms of process endpoints, junctions, and length.
Results: Chronic self-administration of morphine leads to distinct morphological changes in microglia within CPu, DG, and GP regions compared to the sham group. In line with this, the MO+ group exhibited a higher prevalence of ameboid and activated microglial phenotypes when compared to the MO- group. These findings highlight the presence of region-specific microglial phenotypic heterogeneity. Conclusions: Self administration of morphine can trigger neuroadaptive responses, leading to alterations in microglial morphology associated with neuroinflammatory processes.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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Title: Astrocyte signaling in opioid addiction

Authors: *K. MURLANOVA[1], K. NOVOTOTSKAYA-VLASOVA[1], S. HUSEYNOV[1], O. PLETNIKOVA[2], M. J. MORALES[1], D. M. DIETZ[3], M. V. PLETNIKOV[1,4,5];
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Abstract: Addiction research has focused primarily on neural adaptations to drug exposure; however, there is evidence that glial cells also play a role. Astrocytes express opioid receptors, and these glial cells undergo morphological and/or physiological changes after chronic exposure to opioids. The metabolic processes in astrocytes provide neurons with energy, supporting synaptic neurotransmission and plasticity, but whether these processes, including mitochondrial oxidative phosphorylation (OXPHOS), are causally involved in the neurobiology of opioid-induced neuroadaptation and relapse remains unknown.To assess the contribution of astrocytic mu opioid receptors (MORs) to the behavioral effects of opioids, we generated mice with astrocyte-specific knockout of the gene that encodes MOR-1, *Oprm1*, and then measured the responses of the 10-week-old male and female mice to acute and chronic morphine treatments and morphine withdrawal. We also examined the effects of *Oprm1* deficiency on mitochondrial respiration and glycolysis in brain astrocytes in naïve and opioid-dependent mice. We found that astrocyte-specific knockout of *Oprm1* enhanced naloxone-precipitated conditioned place aversion that persisted for 6 weeks. Furthermore, *Oprm1* knockout produced an elevation in OXPHOS that was further enhanced by naloxone-precipitated morphine withdrawal. Our findings suggest that MORs may be linked to OXPHOS in astrocytes which contribute to long-term neuroadaptation produced by exposure to and/or withdrawal from opioids. Current studies focus on transcriptomic signatures of *Oprm1* deficient astrocytes. Future research in this direction may yield new strategies for manipulating endogenous opioid signaling to combat opioid dependence.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

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MUSC U54-DA016511 Pilot (to EMD)
K99-DA058049 (to EMD)

Title: Resolving activity dynamics and noradrenergic modulation of prelimbic cortical neuronal ensembles during heroin seeking

Authors: *E. DONCHECK, R. I. GRANT, E. ROMANOVA, J. BOQUIREN, S. BUCHMAIER, J. PANICCIA, R. CLARKE, L. GREEN, C. BOWEN, E. SANDAGO, K. T. WINSTON, K. VOLLMER, B. SIEMSENS, A. WARD, M. BELL, M. MARTINO, M. D. SCOFIELD, J. OTIS;
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Abstract: Cue-induced drug seeking requires activation of the prelimbic prefrontal cortex (PL) that is dysregulated in substance use disorder. The heterogeneity in PL cell types has made it difficult to unveil the precise PL circuit dynamics which orchestrate drug seeking. To address this, we developed a head-fixed heroin self-administration procedure to allow longitudinal tracking of PL neuronal activity during behavior. To measure the activity of PL projection neurons, we virally labeled these neurons for calcium imaging (AAVdj-CaMKIIa-GCaMP6s) and implanted a GRIN lens dorsal to PL. Subsequent two-photon recordings reveal both frequency and amplitude of calcium events in PL neurons are reduced following acquisition of heroin seeking, effects which persist through extinction, but then resurge during reinstatement (RST) in a manner time-locked to cue presentation. As local noradrenergic signaling rapidly increases PL activity and mediates drug-cue memory retrieval, we hypothesized that inputs from the locus coeruleus (LC) contribute to rescue of PL activity during RST. We found that chemogenetic inhibition of LC-PL axon terminals blocks cue-induced RST, an effect which surprisingly persisted upon a subsequent cue test. These effects were specific to drug cues, as LC-PL inhibition neither persistently suppressed stress- or drug-primed heroin-seeking RST nor affected cued-induced sucrose-seeking RST. Interestingly, we found suppressed cue-induced RST coincides with suppressed excitatory activity in a discrete PL cell cluster which ordinarily decodes the drug-cue. Preliminary studies suggest effects are due to disruption of retrieval, rather than reconsolidation, of drug-cue memories. Ongoing analyses aim to assess sex differences and changes in single-cell activity dynamics throughout heroin self-administration, extinction, and RST. These studies suggest LC-PL axon activation rescues excitatory activity in downstream PL projection neurons for cue-induced RST of heroin seeking.

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Poster

PSTR366. Opioids: Cell Signaling, Circuitry, and Neurophysiology

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR366.30/TT8

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant UG3 DA047714
Medtronic, Inc.

Title: Longitudinal and cue-associated LFP recorded from NAc in patients receiving DBS for opioid use disorder

Authors: *G. K. ADAMS[1], J. J. MAHONEY, III[1], D. G. Y. THOMPSON-LAKE[1], J. MARTON[1], J. SUFFRIDGE[1], R. S. RAIKE[2], V. S. FINOMORE, Jr[3], A. R. REZAI[4]; [1]Rockefeller Neurosci. Inst., West Virginia Univ., Morgantown, WV; [2]Medtronic, Minneapolis, MN; [3]West Virginia University- Rockefeller Neurosci., Morgantown, WV; [4]Dept of Neurolog. Surgery, Rockefeller Neurosci. Instititute, Morgantown, WV

Abstract: Local field potentials (LFP) are slow (<500 Hz) fluctuations in the extracellular voltage of the brain. Clinically, LFP is a potentially valuable biomarker for pathological circuit activity, but the requirement for surgical intervention has mostly limited the use of LFP to intra-operative or peri-operative contexts. However, recent advances in deep brain stimulation (DBS) implants permit longitudinal sampling of LFP from patients receiving DBS therapy. Here, we report LFP from a pilot study of nucleus accumbens (NAc) DBS for the treatment of refractory opioid use disorder (OUD), recorded with the Medtronic PerceptPC DBS stimulator. LFP were sampled longitudinally in each patient spanning a period of over one year of sessions. We observed both systematical longitudinal trends in LFP spectra and immediate responses to visual cues. These findings lay the foundation for a larger, placebo-controlled trial of NAc DBS and LFP, currently being conducted by our research group.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR367.01/TT9

Topic: H.03. Decision Making

Support: NSERC (RGPIN-2018-04295)

Title: Basolateral amygdala regulation of active/inhibitory avoidance and reward seeking

Authors: *G. DALTON, I. DALY, **S. B. FLORESCO**;
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Abstract: Potentially aversive situations often require flexible allocation of action selection to avoid punishment. For example, some situations require a response be made to actively avoid negative outcomes, whereas others may require behavioural suppression to ensure safety. The basolateral amygdala (BLA) plays a key role in facilitating active avoidance, but how it may contribute to avoiding punishment in situations requiring flexible shifts between active vs inhibitory avoidance is unclear. Relatedly, the BLA also promotes active reward seeking in response to discriminative stimuli, but how it regulates inhibition of responses to obtain rewards has not been explored. In the present study, we investigated the contribution of the BLA to flexible instrumental avoidance and reward seeking. Separate groups of male and female Long Evans rats were trained on variations of a Go/No-Go task that required them to discriminate between two auditory cues, informing them to either press or not press a lever to either avoid footshock or obtain food reward. Each trial began with pseudorandom presentation of one of the two cues and lever insertion. Active avoidance/reward seeking trials required rats to press the lever within 15/10s of cue-lever presentation to avoid a shock or receive reward. Inhibitory trails required animals to withhold a press when the other auditory cue was presented to avoid shock or receive reward. In well-trained rats, BLA inactivation markedly reduced successful active avoidance trials in both sexes, without affecting response latencies. In contrast, these treatments slightly increased the number of successful inhibitory avoidance trials and latencies to make inappropriate responses. In a separate group, BLA inactivation also reduced active reward seeking in both sexes, but, in contrast to avoidance, performance on inhibitory trials was unaffected in rats displaying good inhibitory control at baseline. In addition, these treatments also increased response latencies on both successful active and inappropriate inhibitory trials. Collectively these data suggest that the BLA plays a more fundamental role in promoting instrumental action, guided by discriminative stimuli, to avoid aversive outcomes or obtain rewarding ones. In comparison, this nucleus plays a more complex role in mediating response inhibition in a manner dependent on the valence of the context, promoting inappropriate actions in aversive but not appetitive situations.

Disclosures: G. Dalton: None. **I. Daly:** None. **S.B. Floresco:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR367.02/TT10

Topic: E.04. Voluntary Movements

Support: 2E32212
RS-2023-00208692
2019M3E5D2A01058329

Title: A novel cortico-thalamic circuit regulates innate response conflict

Authors: *G. PARK[1,2], Y. PARK[1,3,2], Y. CHO[1], J. KIM[1,3];
[1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [2]These authors contributed equally to this work, Seoul, Korea, Republic of; [3]KIST school, Div. of Bio-Medical Sci. & Technol., Univ. of Sci. and Technol., Seoul, Korea, Republic of

Abstract: Animals must simultaneously balance multiple contingencies such as evading danger, even during basic behaviors such as feeding. The subcortical pathways involved in the delivery of individual innate responses are relatively well-characterized; however, the neural mechanisms of adjusting innate response balance during conflict remain largely unclear. We herein identify a novel frontal cortical output that controls response balance through thalamus in feeding mice exposed to fear-inducing visual signals. Using micro-endoscopy and fiber photometry, we reveal that cortico-thalamic circuit shows highly correlated activity with bias towards behaviour choices. We also developed a new scheme combining multisynaptic tracing with transcriptomic dissection, using it to identify a novel interneuron subtype which may gate response balance during the threat/feeding conflict situation. Optomodulation of these interneuron types in cortex or frontal outputs can both mediate changes in this respect. Collectively, these reveal a cell type specific top-down control in a frontal-thalamic circuit regulating balance of innate behaviors.

Disclosures: **G. Park:** None. **Y. Park:** None. **Y. Cho:** None. **J. Kim:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR367.03/TT11

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Distinct contributions of prelimbic and infralimbic-basolateral amygdala circuits to cue-guided risk/reward decision making

Authors: *M. ZHAO[1], S. RAMAIAH[2], S. B. FLORESCO[2];
[1]Psychology and Ctr. for Brain Hlth., Univ. of British Colombia, Vancouver, BC, Canada;
[2]Psychology and Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Decisions often carry uncertainty, requiring an individual to assess the likelihood of a desired outcome and evaluate whether the potential gain outweighs the risk of failing to obtain it. Such outcome probabilities are commonly informed by extrinsic cues (e.g., predators use distinct physical features of prey to determine the odds of a successful hunt). Previous research has implicated the prelimbic (PL) and infralimbic (IL) subregions of the rodent medial prefrontal cortex and the basolateral amygdala (BLA) in this type of decision-making. The PL and IL are functionally heterogeneous and exhibit distinct patterns of connectivity with the BLA. Here, we pharmacologically disconnected either PL↔BLA or IL↔BLA communication in rats while they performed on a cued probabilistic decision-making task known as the "Blackjack" task. Animals select between two levers: a safe/certain option that always delivered a smaller, 1-pellet reward, or a large/risky option that might deliver a 4-pellet reward. At the start of each trial, different auditory cues inform rats of whether the odds of obtaining the larger reward are good (50%) or poor (12.5%). The optimal strategy is to select the risky lever on good-odds trials, and the certain option on poor-odds trials. Disconnection of prefrontal-amygdala pathways was achieved by a series of intracranial infusions of GABA receptor agonists, baclofen and muscimol. Disrupting either ipsilateral PL↔BLA or IL↔BLA signaling decreased risky choice on good-odds trials. The same effect was observed with disruption of contralateral IL↔BLA communication, but not contralateral PL↔BLA. When examining risky choice on poor-odds trials, disrupting either contralateral PL↔BLA or IL↔BLA signaling increased risky choice. Together, these findings confirm that communication between the BLA and both PL and IL mediates optimal cue-guided risk/reward decision-making, and suggest differential contributions for the different cortical circuits and for ipsilateral vs. contralateral signaling. This might be attributed to differences in downstream and upstream connectivity patterns. BLA projections to the PL and IL are predominantly ipsilateral. In contrast, while IL sends ipsilateral projections to the BLA, PL sends both ipsilateral and contralateral projections downstream. Future research will directly target ascending vs. descending pathways and characterize direction-specific contributions of prefrontal-amygdala circuits in cued probabilistic reward decision-making.

Disclosures: **M. Zhao:** None. **S. Ramaiah:** None. **S.B. Floresco:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR367.04/TT12

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Differential effects of D1 and D2 receptor antagonists in nucleus accumbens core and shell on cue-guided risk-reward decision-making

Authors: *S. SCHOFIELD-LEWIS, T. LUO, S. B. FLORESCO;
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Abstract: To facilitate efficient decision-making, organisms have evolved the ability to integrate environmental cues, and dysfunctions in the mesolimbic dopamine (DA) system have been linked to sub-optimal decision-making. Previous studies investigating dopamine's influence on decision-making have employed probabilistic discounting tasks, where internal probability representations guide effective choices. However, real-world decision-making often involves utilizing external cues for decision guidance. To assess this aspect of decision-making, we developed a rodent assay called the "Blackjack" task, wherein rats use external stimuli to make optimal decisions. Each trial presents one of two auditory cues, followed by the extension of two levers. Rats choose between a small/certain lever (1 pellet with 100% probability) and a large/risky lever (4 pellets with probabilistic odds). The auditory cues indicate whether the large/risky lever offers good (50%) or poor (12.5%) odds of obtaining the 4-pellet reward. Separate groups of rats were trained on a control auditory discrimination task, in which the same auditory cues signaled a 100% reward probability for responses on either the left or right lever, eliminating the probabilistic element present in the Blackjack task. With these tasks, our current study explored how D_1 and D_2 receptor activity in the nucleus accumbens (NAc) core and shell, influences risk/reward decision-making guided by external cues. Separate groups of well-trained rats received intracranial infusions of either D1 or D2 antagonists before testing. Previous research from our group demonstrated that D1 blockade reduces risky choices during probabilistic discounting guided by internal reward history representations. Preliminary findings using the Blackjack task indicate blockade of D_1 (but not D_2) receptors in the NAc core tended to reduce risky choice on good-odds trials, when the risky option had greater utility. Blockade of D_1 receptors in the shell increased decision latencies. In comparison, infusion of either DA antagonist into either NAc subregion had no impact on performance in conditional auditory discrimination. However, significant decreases in locomotion were observed that occurred in the absences of impairments in accuracy. These findings underscore the functional heterogeneity and receptor specificity of the NAc core and shell, and suggest that D_1 receptor activity in this region promotes advantageous risky choices.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.05/TT13

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Amphetamine, punishment, and rewards:monoaminergic modulation of inhibitory impulse control

Authors: *G. LAINO CHIAVEGATTI[1], S. LEE[2], S. B. FLORESCO[2];
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Abstract: Making and withholding responses appropriately to achieve a goal is essential when dealing with threatening stimuli and achieve the best outcome, a critical skill for survival. Situations involving threat often require integrating multiple cues that predict a negative outcome to guide action-selection, or they may be also avoided by withholding behaviour in response to cues. It is well established certain aspects of impulse control are regulated by different monoamines, although the specific manner in which pharmacological manipulations of monoaminergic transmission may alter impulsive choice or action can vary considerably depending on the particular cognitive and emotional processes being taxed. Here we examined how monoaminergic transmission influenced response inhibition involving reward-seeking under risk of punishment in male and female rats. We adopting a behavioural control task that entailed positively reinforcing active responses intermixed with punishment. Rats were well trained on a task wherein a food pellet was delivered in a cup, and on 30/60 trials the rat merely had to approach and retrieve reward. On the other 30 trials, a 12-s visual/auditory warning cue signalled reward retrieval must be withheld until cue termination to avoid punishment. One group of rats received counterbalanced doses of 0.1, 0.3, and 1.0 mg/kg of the psychostimulant and monoamine releaser D-amphetamine (AMPH). These treatments induced an expected increase in overall locomotor activity. However, AMPH also caused a pronounced reduction in impulsive action in both sexes, as rats were more likely to wait 12 s for the warning cue to terminate and then retrieve reward without being shocked. Subsequent experiments used selective reuptake transporter blockers to isolate which monoaminergic system may underlie the effects of AMPH in separate groups of rats. Interestingly, the effects of the Noradrenaline (NA) transporter *vs.* Dopamine (DA) transporter blockers Atomoxetine (0,1, 0.3, 1.0 mg/kg) and GBR-12909 (1.0, 2.0, 5.0 mg/kg), respectively, differed between the sexes. Specifically, Atomoxetine recapitulated the impuslolytic effects of AMPH in females but not males, whereas GBR-12909 reduced impulsive action in a manner similar to AMPH in males but not females. In contrast, the 5-HT transporter blocker Fluoxetine (0,1, 0.3, 1.0 mg/kg), thankfully, did not have any effects on behavioral control. These findings suggest increased monoamine transmission can markedly enhance the ability of cues signalling potential punishment to suppress impulsive reward-seeking under threat, which may be mediated by either NA or DA in a sex-dependent manner.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.06/TT14

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Characterizing the spatiotemporal profile of mesocorticolimbic dopaminergic modulation of risk/reward decision making

Authors: *J. SCHUMACHER, M. ZHAO, D. A. BERCOVICI, M. HALL, S. B. FLORESCO; Psychology and Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Convergent evidence points towards a key role for the mesocorticolimbic dopamine (DA) system in risk/reward decision making. Risk/reward decision making is often operationalized using rodent operant tasks such a probabilistic discounting which requires rats to choose between a small, guaranteed and a large, uncertain reward whose probability varies systematically across blocks of trials. Prior work has shown that nucleus accumbens (NAc) DA, via D1 receptors, promotes preference for the large/uncertain reward whereas DA in the medial prefrontal cortex (mPFC) refines choice via opposing action on D1 and D2 receptors. Microdialysis studies demonstrate that DA efflux in the NAc and mPFC tracked reward uncertainty and overall reward rates, respectively. However, DA release manifests as discrete phasic events and pharmacology and microdialysis lack the temporal resolution to disentangle the effects of DA release on the sub second timescales on which decisions are made. To explore this in more detail, we expressed the inhibitory opsin Arch3.0 in ventral tegmental DA (VTA) DA neurons of male and female rats to induce optogenetic silencing of either DA cell bodies or terminals in the NAc or mPFC. Temporally discrete suppression of DA neurons/terminal activity was induced during different task events, including prior to choice, and during different choice outcomes (large/risky rewards, small/certain rewards or non-rewarded choices). Complementary studies will use florescent DA and calcium indicators to measure DA release and cellular activity in the mPFC, NAc and VTA during these same behavioral epochs. Preliminary data reveal that silencing VTA DA cell bodies during receipt of large/risky reward shifts choice towards the small/certain option and, conversely, silencing VTA cell bodies during small/certain reward delivery shifts choice towards the large/risky option. Silencing DA terminals in the NAc (but not mPFC) after rewarded large/risky choices reduced risky choice when the odds of reward increased across a session, whereas mPFC or NAC terminal inhibition during small/certain reward delivery was without effect. No manipulation (mPFC terminal inhibition, NAc terminal inhibition, VTA cell body inhibition) altered choice when silencing occurred during reward omissions. Together, these findings demonstrate that dopaminergic modulation of decision-making behavior critically varies along temporal and spatial dimensions such that silencing DA has differential effects in different brain regions and different behavioral epochs.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.07/TT16

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Amphetamine, but not atomoxetine, increases impulsive choice on a cue-guided delay discounting task

Authors: *S. RAMAIAH, M. ZHAO, S. B. FLORESCO;
Psychology and Ctr. for Brain Hlth., Univ. of British Columbia, Vancouver, BC, Canada

Abstract: Adaptive cost/benefit decision-making requires dynamic tuning of one's subjective value of an outcome to the cost of obtaining it. Devaluation of rewards when they are delayed is observed in humans and animals and can be studied in rodents using operant-based models of delay discounting. These tasks require an animal to choose between an immediate/small reward, and a larger, but delayed reward. Typical procedures used with rodents entails tasks where the length of the delay typically changes systematically across blocks of trials. An animal samples its options and uses internal representations of changing delay lengths to guide decision making. Selection of the immediate/small reward is interpreted as a measure of choice impulsivity and is regulated by dopaminergic and noradrenergic systems. In real life, however, rather than sequential sampling of delays, delays are often signaled by environmental cues (e.g., queues when deciding where to eat at a festival). It is unknown whether neural systems are differentially engaged under conditions where delays are cued vs. non-cued. Here, we present a novel cue-guided delay discounting task in rats. Instead of delays shifting across blocks of trials, at the start of each trial, an auditory tone is presented to inform rats of whether the delay of obtaining the large reward is short (1s) or long (30s). After ~3 weeks of training, animals developed a stable preference for the large reward on short-delay trials, and the immediate/small reward on long-delay trials. When given high (0.5 or 1mg/kg), but not low (0.125 or 0.25 mg/kg), doses of amphetamine, animals took longer to decide and were more likely to choose the immediate reward when cues signaled a short delay. Interestingly, this increase in impulsive choice was only observed on long-delay trials at the 0.25mg/kg dose. A subsequent experiment examined the effects of the noradrenaline reuptake inhibitor, atomoxetine. Higher (1 or 3mg/kg), but not low (0.3 mg/kg), doses increased decision latencies and trial omissions, but had no effect on selection biases of the immediate vs. delayed reward options. In both experiments, no sex differences were observed. Together, these findings suggest that amphetamine drives an increase in choice impulsivity and causes a general bias towards smaller/immediate rewards when cues signal delay duration, an effect that is likely not mediated by this drugs effects on noradrenergic transmission. More generally, these data highlight that the neurochemical mechanisms underlying impulsive choice may differ depending on whether information about delays to receiving rewards are internally mediated or signaled by external stimuli.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.08/TT17

Topic: H.03. Decision Making

Support: CIHR grant (PJT-162444)

Title: Sex dependent regulation of effort and delay-related decision making by mu and kappa opioid receptors

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Abstract: The endogenous opioid system has emerged as a locus of dysfunction in those suffering from opiate use disorder and as a major target of translational research. Changes in cost/benefit decision making are characteristic of pathological opiate use, however, our understanding of how this system modulates decision-making processes at baseline is incomplete. Particularly, the contributions of different opioid receptor subtypes to multiple types of cost/benefit decision making and how these contributions differ by sex remains incompletely understood. To address this, separate cohorts of male and female rats were trained on effort and delay discounting tasks which required them to choose between a no cost, small reward and a large reward with an associated effort or delay cost. For both tasks the magnitude of the effort or delay cost increased over the course of a session, causing rats to shift their choice preference to the small reward option over time. Rats were well trained until they displayed stable choice patterns, and then received systemic treatment with the selective mu receptor antagonist Naloxone or the selective kappa receptor antagonist Aticaprant (0, 1, 3, 10mg/kg doses used for both drugs). A separate group of rats was tested on a similar reward magnitude discrimination control task where rats choose between a small and large magnitude reward with no associated cost in order to determine if any drug effects on the discounting tasks resulted from a more fundamental perturbation to preference for large vs small rewards. Blockade of mu opioid receptors significantly decreased choice of the high effort/large reward option on the effort discounting task in both sexes, although the magnitude of this effect was greater in females. These effects on choice were accompanied by increases in response latency and omissions. In comparison, kappa receptor blockade also decreased effort related choice, but only females. Treatment with the highest dose of naloxone reduced choice of the high reward option in males and females performing the reward magnitude discrimination task whereas, again, treatment with the highest dose of Aticaprant only reduced choice of the large reward option in females. Notably, however, these perturbations only appeared in the latter half of the session despite the task contingencies remaining stable over the entire session - suggesting altered satiety processing as a potential mechanism for the change in choice preference. Collectively, these data build a foundation for understanding the contribution of opioid receptor activity to cost/benefit decision making.

Disclosures: V. Iancu: None. **J. Schumacher:** None. **S.B. Floresco:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR367.09/TT18

Topic: H.03. Decision Making

Support: Wellcome Trust WT 110157/Z/15/Z

Title: Primate mediodorsal thalamic electrical microstimulation increases heart rate and causes selectivebrain activations during functional magnetic resonance imaging

Authors: *A. MITCHELL[1], E. PREMEREUR[2], B. A. PERRY[3], J. MENDEZ NUNEZ[5], V. PELEKANOS[6], U. SCHÜFFELGEN[4], M. KUSUNOKI[4];
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Abstract: Learning new information efficiently and making flexible and appropriate choices are essential in everyday life. As evidenced from studies in animal models, the mediodorsal thalamus (MD) plays an important role in cognitive functions, such as learning and decision-making. It is proposed to be an active partner working in support of frontal and medial temporal lobe structures. In order to fully understand the role of a brain structure in cognition, it is critical we know not only the structure's function, but also its anatomical connectivity. The latter helps us understand which cortical and subcortical structures interact during cognition, and how information travels throughout the brain. Here, we investigated what brain structures showed activation, at the whole brain level, by combining electrical microstimulation of either the magnocellular (MDmc) or parvocellular (MDpc) subdivisions of the MD and functional magnetic resonance imaging (fMRI) in three anaesthetized male adult rhesus macaques. The neuroimaging scans demonstrated that microstimulation applied to the MD elicited increased as well as decreased fMRI activations in several cortical and subcortical areas including the anterior cingulate cortex, dorsolateral and ventrolateral prefrontal cortex, insular cortex, and orbitofrontal cortex, while subcortical areas included the caudate and VTA, and parts of the midbrain and brainstem. These areas all have known anatomical connections to MD. Some of the strongest activations were observed in the dorsal anterior cingulate cortex. Interestingly, the monkeys' heart rate substantially increased as a consequence of the MD microstimulation, in particular when applied to the MDmc, highlighting the additional contribution of the MD to physiological, as well as cognitive, functions. fMRI combined with microstimulation can be used to investigate connectivity at the whole brain level. Importantly though, more detailed histological investigations remain necessary to investigate the nature of these connections.

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Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.10/TT19

Topic: H.03. Decision Making

Support: NIH F31 MH131342-02

Title: Orbitofrontal-hippocampal representations of cognitive maps during abstract navigation.

Authors: *E. HU[1], J. D. WALLIS[2];
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Abstract: Animals use their knowledge of the environment to make inferences that guide decision-making in dynamic situations. This knowledge may be organized in the brain as a 'cognitive map': a network of associations that defines the relationships within a task or environment. Although significant effort has been dedicated to characterizing the precise nature of cognitive maps in the brain, little is known about how such maps are transformed into decision-relevant variables that inform choices. We hypothesize that this transformation is carried out by the hippocampal-orbitofrontal circuit. Specifically, the hippocampus (HPC) represents a cognitive map that models the structure of an environment and then communicates this map to the orbitofrontal cortex (OFC) to calculate reward predictions and evaluate the best course of action. To investigate this, we performed simultaneous high-density recordings in the HPC and OFC of macaques during a novel abstract navigation task. Subjects were trained to navigate a graphical structure in search of a hidden reward. The structure consisted of a 16-node graph that was spatially arranged as a two-dimensional grid, with each node connected to vertically and horizontally adjacent nodes. Additionally, a single edge connected one pair of corners, serving as a shortcut that introduced a non-spatial element to the task. A new reward location was cued every 5 trials, necessitating a change in the subjects' navigation policy across the underlying graph. Subjects were able to navigate immediately to the new reward location following a reward-shift and successfully exploited the hidden shortcut, suggesting that they use a cognitive map to solve the task. We find that OFC and HPC represent progression along navigational trajectories, with single neurons in both regions encoding the graph-based distance between the subjects' current location and their intended target location.

Disclosures: E. Hu: None. **J.D. Wallis:** None.

Poster

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Program #/Poster #: PSTR367.11/TT20

Topic: H.03. Decision Making

Support: NIH Grant R01-MH121448

Title: Contextual reasoning by hippocampal-prefrontal circuits in primates

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Abstract: The value of a choice option can vary across contexts. Thus, optimal choice depends on flexibly assigning value to choice options in a contextually appropriate manner. Despite the ubiquity of such contextual reasoning outside of the laboratory, how the brain flexibly switches between value-representations in different contexts remains unknown. We addressed this question by performing simultaneous high channel count recordings from the hippocampus (HPC) and orbitofrontal cortex (OFC) from two rhesus monkeys trained to perform a context-dependent valuation task. The task required the animals to flexibly update the values of 8 choice options based on a context-cue that varied from trial-to-trial. Critically, the context-cue preceded and was not present during choice, requiring the animals to simultaneously update the 8 option values prior to seeing them.
We report evidence of a functional double-dissociation across HPC and OFC: HPC neurons (but not OFC) abstractly encoded context during the cue phase whereas OFC strongly represented context during the choice phase. We also found that distinct OFC subcircuits represented value uniquely in one context or another. Analysis of communication through oscillatory coherence revealed a ramping HPC-to-OFC signal in the theta band (4-8Hz) that started as HPC neurons began encoding context and peaked as the contextually-appropriate OFC value subcircuit came online. This suggests that context information, a compressed representation of the relevant valuation schema, is initially encoded in HPC and then broadcast to OFC via theta synchronization in order to select a contextually-appropriate value subcircuit, thus allowing for contextual reasoning in value-based choice.

Disclosures: T.W. Elston: None. **J.D. Wallis:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR367.12/TT21

Topic: H.03. Decision Making

Support: NIMH 3R01 NS116623-01S1

Title: Prefrontal mechanisms of attention and valuation during decision making

Authors: *N. MUNET, J. D. WALLIS;
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Abstract: Deliberative decision making is a dynamic process in which the decision maker must represent the relevant decision variables, compute each option's overall value, and weigh these values to select the best option. Prior work from our lab shows that the orbitofrontal cortex

(OFC) dynamically represents the value of each of the choice alternatives during deliberation, alternating back-and-forth between the chosen and unchosen values. While the time spent in each value state has been shown to predict choice behavior, the functional role of OFC's alternating dynamics remains unclear. One possibility is that fluctuations in value may be driven by shifts in top-down attention between options. Conversely, the dominant value signal may direct attention to the associated option, with shifts in the value state leading to shifts in attention. To test these hypotheses, we recorded from OFC and nearby lateral prefrontal cortex (LPFC), a region central to attentional control, during a novel experimental paradigm to identify and isolate distinct neural signals for attention and valuation at single-trial resolution. At the single-unit and population scale, we find that both LPFC and OFC encode correlates of space and value in a prioritized manner that may underlie attention and decision making. Moreover, we find evidence that these representations generalize across task contexts, suggesting a task-general code for spatial attention. Single-trial decoding analyses further reveal that PFC represents the locations and values of available options dynamically, which may be pertinent to the time course of the decision. I will discuss how these dynamics relate to choice behavior, as well as whether the dynamics of covert attention and valuation signals are correlated in PFC. Such a correlation would be consistent with an interaction between attentional and evaluative processes that may be important for gathering and weighing evidence over the course of a decision.

Disclosures: **N. Munet:** None. **J.D. Wallis:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: H.03. Decision Making

Support: The Sigrid Juselius Foundation grant 326045 (to T.T.)
Academy of Finland grants 330710 (to S.E.L.)

Title: GluK1 Kainate receptors in parvalbumin interneurons modulate the cortico-limbic network to alter social preference of male adult mice.

Authors: *J. RHEE[1], S. OJANEN[2,1], T. TAIRA[2], S. E. LAURI[1,3];
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Abstract: Appropriate social behavior depends on the functioning of the cortico-limbic network. Ventral hippocampus (vHC) afferents that directly innervate parvalbumin (PV) interneurons in the medial prefrontal cortex (mPFC) are implicated in the appropriate expression of social behavior. Kainate receptors containing GluK1 (GluK1 KARs) are strongly expressed in PV+ interneurons and modulate their function via ionotropic and G-protein coupled mechanisms. To examine the role GluK1 KARs play in the functioning of the vHC-mPFC circuit in the context of

social behavior, we performed multi-site neural recordings of head-fixed male adult mice lacking GluK1 selectively in the PV neurons (PV-*Grik1*$^{-/-}$) undergoing social preference test.
At rest, PV-*Grik1*$^{-/-}$ male mice show elevated theta and gamma power in both vHC and mPFC, coherence between vHC and mPFC, as well as phase-amplitude coupling within each region and across the regions in comparison to wild-type male mice.
During exploration and social interaction, theta and gamma power, coherence, and phase-amplitude coupling increase in wild-type male mice compared to at rest. PV-*Grik1*$^{-/-}$ male mice also show increases, but with notable differences.
First, while both wild-type and PV-*Grik1*$^{-/-}$ mice show an increase in theta and gamma in the vHC during social interactions, only PV-*Grik1*$^{-/-}$ mice display elevated theta and gamma power in the mPFC when interacting with a littermate, coinciding with an increased amount of time spent exploring near a littermate by PV-*Grik1*$^{-/-}$ mice. Second, unlike wild-type male mice, PV-*Grik1*$^{-/-}$ male mice show no increase in theta coherence between vHC and mPFC during exploration and social interaction. Third, there was no increase in theta and gamma phase-amplitude coupling within vHC, within mPFC, and across regions in PV-*Grik1*$^{-/-}$ male mice during exploration and social interaction. These results indicate that GluK1 KARs play a central role in altering the way PV interneurons modulate the cortico-limbic network and suggest a potential mechanistic explanation for how theta and gamma powers, coherence, and phase-amplitude coupling may be elevated in the vHC-mPFC circuit to result in altered social behavior.

Disclosures: J. Rhee: None. **S. Ojanen:** None. **T. Taira:** None. **S.E. Lauri:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

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Program #/Poster #: PSTR367.14/TT23

Topic: H.03. Decision Making

Support: NIH Grant U19NS104648
Burroughs Wellcome CASI Award
C.V. Starr
Simons Foundation

Title: Coordinated geometric representations of learned knowledge in hippocampus and frontal cortex

Authors: *M. SCHOTTDORF[1], J. JULIAN[1], J. KAMINSKY[1], C. D. BRODY[2], D. W. TANK[1]; [1]Princeton Univ., Princeton, NJ; [2]HHMI & Princeton Univ., Princeton, NJ

Abstract: Interactions between frontal cortex and hippocampus (HPC) play a key role in decision-making behaviors. Here, we test how these brain areas coordinate their representations of behavioral and cognitive variables of a complex and cognitively demanding task. We use a virtual reality task in which mice navigate in a T-maze and accumulate transiently visible and

stochastic left/right cues to infer the correct reward location. Neural data is recorded acutely with up to 10 Neuropixel shanks simultaneously in bilateral HPC and medial prefrontal cortex (mPFC). To compare neural activity patterns, we analyze this data with state space models, in which each neuron is represented by an activity axis, and population activity constitutes a trajectory in this high-dimensional space. We demonstrate that mPFC neural activity across trials does not fill this space, but lies on a structured, low-dimensional, and non-linear subspace. Population activity can therefore be parameterized by a relatively small number of latent variables that span this neural activity manifold. Similar to previous observations in the HPC, we find that behavioral and cognitive variables, like position in the maze and accumulated visual evidence, are represented as smooth gradients on the mPFC manifold. HPC and mPFC also encoded position and evidence with similar precision and geometry. We demonstrate this similarity between regions by training a decoder on the HPC manifold and decoding behavioral variables from mPFC and vice versa. Despite similar encoding of position and evidence between regions, a communication subspace analysis suggests a differential representation of position and evidence in both areas. The results reported here, together with a growing literature of related findings across brain regions and tasks, contributes to a better understanding of how task-relevant variables are integrated through mPFC-HPC interactions in decision making.

Disclosures: **M. Schottdorf:** None. **J. Julian:** None. **J. Kaminsky:** None. **C.D. Brody:** None. **D.W. Tank:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR367.15/TT24

Topic: H.03. Decision Making

Support: U19NS104648

Title: Coordinated hippocampal-prefrontal theta-paced flickering of place cell maps during decision-making

Authors: *J. JULIAN[1], J. KAMINSKY[1], M. SCHOTTDORF[1], C. BRODY[2], D. TANK[1]; [1]Princeton Neurosci. Inst., Princeton Univ., Princeton, NJ; [2]HHMI & Princeton Univ., Princeton, NJ

Abstract: Synchronization of neural activity in the hippocampus (HPC) and medial prefrontal cortex (mPFC) is crucial for decision-making and contextual memory. Across different contexts, place representations in both HPC and mPFC remap, and such remapping mediates changes in context-dependent behavior. However, it is unknown whether remapping is coordinated between HPC and mPFC, particularly at a subsecond timescale, and whether such coordination plays a role in context-based decision-making. To address these issues, we trained mice to navigate in a virtual-reality T-maze with visual gratings along the walls. Across trials, the spatial frequency of

the grating ranged continuously from low- to high-frequency, and mice were trained to turn left or right at the T-intersection when the spatial frequency was low or high, respectively. Thus, the spatial frequency gratings indicate the context that drives the decision on each trial. Neural data were recorded acutely with up to 10 Neuropixel shanks simultaneously in the bilateral HPC and mPFC. Across trials, place cell maps in HPC and mPFC remapped to encode the current stimulus context, and remapping in both regions was predictive of choice behavior. During a subset of trials, however, both regions exhibited brief periods of rapid 'flickering' between representations of the current stimulus context and other previously experienced contexts, before typically settling on the former (c.f., Jezek, 2011, Nature). Flickering in both regions was paced by ongoing HPC theta oscillations, with complete remapping events occurring from one theta cycle to the next. Such flickering events were not explained by locomotory behavior (e.g., rapid changes in heading angle), and were more likely to occur prior to incorrect decisions. Critically, HPC and mPFC flickered coherently, such that they transiently remapped to a representation of the same previously experienced stimulus context. These observations suggest that HPC and mPFC coordinate context representations on a subsecond timescale, paced by HPC theta oscillations, to guide decision-making behavior.

Disclosures: **J. Julian:** None. **J. Kaminsky:** None. **M. Schottdorf:** None. **C. Brody:** None. **D. Tank:** None.

Poster

PSTR367. Decision-Making: Corticolimbic Circuits

Location: WCC Halls A-C

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Program #/Poster #: PSTR367.16/TT25

Topic: H.03. Decision Making

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Title: Dorsomedial prefrontal cortical and hippocampal interactions in construction of task representations in macaques

Authors: *K. MARCHE[1,2,3], N. TRUDEL[1,2,4,5,3], J. SCHOLL[1,2,6], J. SALLET[1,2,7], F.-X. NEUBERT[1,2], M. C. KLEIN-FLÜGGE[1,2,8], M. F. RUSHWORTH[1,2,8];
[1]Exptl. Psychology, [2]Wellcome Integrative Neuroimaging (WIN), Univ. of Oxford, Oxford, United Kingdom; [3]contributed equally, Oxford, United Kingdom; [4]Ctr. for Computat. Psychiatry and Ageing Res., Max Planck UCL, London, United Kingdom; [5]Wellcome Ctr. for Human Neuroimaging, Univ. Col. London, London, United Kingdom; [6]Lyon Neurosci. Res. Center, INSERM U1028, CNRS UMR5292, PSYR2 Team, [7]Stem Cell and Brain Res. Institute, INSERM U1208, Univ. Lyon 1, Lyon, France; [8]equal contribution, Oxford, United Kingdom

Abstract: Behavioural flexibility is an important skill for coping with changes in the environment. To adapt to an environment, animals can either adjust the frequency of each action they make or construct changing models of their environment. Sometimes it is better to track the

value of each choice, but sometimes it is easier and more efficient to understand the structure of the environment and estimate when it changes. The aim of our study is to assess whether animals switch between both strategies in environments that are more or less structured, and to examine which brain structures support each strategy. We trained macaques (*Macaca mulatta*) to perform a two-armed reversal bandit task in an MRI scanner. In the correlated environment, when the probability of reward for one option was high, the probability for the other option was low, and reversals corresponded to the moment when reward probabilities swapped. In the uncorrelated environment, the probabilities of reward for both options drifted in an unrelated manner, and reversals corresponded to the moment when the relative probabilities crossed. We used environment-appropriate Bayesian models to track each option's reward probability (RewardP), the associated uncertainty in the estimates (RewardU), and probability of option reversal (ReversalP). Monkeys switched more frequently after repetition of unrewarded trials in correlated compared to uncorrelated environments. Switching rate depended on ReversalP in the correlated environment and RewardU for the recently chosen option in the uncorrelated environment. Activity in two neural circuits was time-locked to the decision time. In the correlated environment, activity related to ReversalP was prominent in dorsomedial prefrontal cortex (dmPFC) and hippocampus. Activity related to RewardP was observed in the correlated environment also in dmPFC and dorsomedial thalamus. Activity in dmPFC was related to activity in the hippocampus and thalamus according to the information the monkeys were tracking. Our results suggest that monkeys adopt behavioural strategies that are adequate for a given environment, using knowledge of the structure of the environment to decide when it is time to change choice behaviour. In ongoing work, we are investigating the relationship between dmPFC, hippocampus and thalamus by collecting a new dataset combining ultrasound stimulation with analyses of activity interactions between areas.

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Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.01/TT26

Topic: H.04. Executive Functions

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NIH/NIA RF1AG062831
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NIH/NIA RF1AG043640

Title: Regional diversity in prefrontal excitatory and inhibitory circuit changes in cognitive aging

Authors: C. A. MOJICA[1], **C. HANSEN**[1], Y. ZHOU[1], B. SNYDER[1], H. BHATT[1], D. L. ROSENE[1,2], J. LUEBKE[1,2], T. L. MOORE[1,2], *M. MEDALLA[1,2];
[1]Boston Univ. Sch. of Med., Boston, MA; [2]Ctr. for Systems Neurosci., Boston Univ., Boston, MA

Abstract: Structural alterations in excitatory and inhibitory synapses in the dorsolateral prefrontal cortex (DLPFC) have been shown to be correlated with age-related cognitive decline in the rhesus monkey model of normal aging. However, the functional contributions of distinct excitatory and inhibitory neurons across the various PFC areas in cognition and cognitive aging is unknown in primates. Specifically, age-related synaptic changes in the anterior cingulate cortex (ACC), a key medial PFC region also important for cognition, are largely unknown. Thus, using *in vitro* whole-cell patch clamp recordings, we compared the properties of spontaneous excitatory (EPSC) and inhibitory (IPSC) postsynaptic currents in layer 3 (L3) pyramidal neurons (Pyr) in the ACC area 24 and DLPFC area 46 of rhesus monkeys across the adult lifespan (n=34; ages 6.0 to 21.5 y.o.). Linear regression revealed significant age-related changes in synaptic current properties that are dependent on area. For DLPFC Pyr, we found a significant decline in frequency of both EPSCs ($r^2 = 0.1$, $p < 0.01$) and IPSCs ($r^2 = 0.1$, $p < 0.02$) with age, replicating previous data. In contrast, ACC Pyr exhibited a significant increase in IPSC frequency ($r^2 = 0.15$, $p < 0.01$) and amplitude ($r^2 = 0.03$, $p < 0.03$) with age, but no changes in EPSC frequency. We then evaluated if excitatory and inhibitory neurons in the two areas are differentially activated in cognitive tasks. A subset of monkeys performed the Delayed Recognition Span Test (DRST), a spatial working memory task, 3 hours prior to tissue harvesting to assess activity-dependent expression of cFOS, an immediate early gene marker of neuronal activation. We quantified the number of cFOS+ neurons co-expressed with excitatory (MAP2) versus inhibitory neuron (calbindin, parvalbumin and calretinin) cell markers in serial sections of the different DLPFC and ACC areas. Using stereological counting methods, we observed the highest proportion of cFOS+ neurons in DLPFC areas 9 and 46, consistent with their role in spatial working memory. The majority (~70%) of these cFOS+ neurons were excitatory (MAP2+) and are predominantly found in deep (L5-6) cortical layers. cFOS+ neurons were also identified in ACC area 32 and ventromedial area 14, but in relatively lower proportions than the DLPFC areas. These data reveal regional specificity in age-related excitatory and inhibitory synaptic changes in distinct PFC areas, which are differentially activated during working memory tasks. These findings emphasize the role of excitatory and inhibitory neuronal balance in cognitive tasks and how this balance is differentially altered in distinct PFC areas during cognitive aging.

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Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.02/TT27

Topic: H.04. Executive Functions

Support: NIH/NIMH R01MH116008
NIH/NINDS R01NS122969

Title: Organization of layer-specific DLPFC area 46 and PMd area 6 projection neurons targeting LPFC area 9 in rhesus monkeys

Authors: *H. BHATT, T. L. MOORE, D. L. ROSENE, J. I. LUEBKE, C. CHANDRASEKARAN, M. MEDALLA;
Dept. of Anat. and Neurobio., Boston Univ. Chobanian and Avedisian Sch. of Med., Boston, MA

Abstract: The communication between the Dorsolateral Prefrontal Cortex (DLPFC) and the Dorsal Premotor cortex (PMd) is important for cognitive processing and decision-making. DLPFC areas 46 and 9 integrate and sustain information in working memory and relay information to PMd, which in turn, connects with the motor cortex for action planning. While the PMd thus acts as a "bridge" between the DLPFC and motor cortex necessary for decision-making, little is known about the properties of pathways within and between DLPFC and PMd in primates. Using retrograde tract-tracing, immunolabeling, and microscopy in rhesus monkeys (M. mulatta), the current study assesses the density and laminar organization of tracer labeled projection neurons in DLPFC area 46 (d46 & v46) and PMd area 6 (6DR & 6DC), directed to the DLPFC area 9 (A46→A9 vs A6→A9). Laminar patterns of connections are relevant since they are integral to flow of information and layer specific neurochemical modulation within neocortex. Here we show that the density of retrogradely labeled somata of A46→A9 projection neurons is greater than those in A6→A9. Projection neurons in both pathways have columnar bilaminar patterns, with ~60% originating from the upper (L2-3) and ~40% deep (L5-6) layers, suggesting predominant 'lateral' processing in both pathways. Further, we found that these layer specific projection neurons receive distinct sources of inhibition, which is an important determinant of their output. Specifically, we quantified IHC labeled inhibitory inputs (vesicular GABAergic transporter, VGAT+) onto somata of retrograde tracer labeled neurons, from parvalbumin (PV) inhibitory neurons that provide strong perisomatic inhibition. The majority (~78%) of perisomatic VGAT+ inputs onto A46→A9 and A6→A9 projection neurons are PV+. Further, we found laminar differences in the density (appositions/surface area) of these perisomatic VGAT+/PV+ inputs that were dependent on the area of origin. For A46→A9 projection neurons, the densities of perisomatic VGAT+/PV+ inputs onto upper-layer projection neurons were greater than those onto deep-layer neurons. The opposite pattern was found for A6→A9, where the densities of perisomatic VGAT+/PV+ inputs were greater for deep-layer than for upper-layer projection neurons. Overall, our findings show that while the two pathways exhibited similar columnar bilaminar projection patterns, the laminar inhibitory influences were distinct. These findings suggest differential inhibitory gating of layer-specific DLPFC and PMd interconnections that may be important for selection and temporal dynamics of signals for motor planning and decision-making.

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Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Topic: H.04. Executive Functions

Support: NIH/NIMH K99/R00MH101234
NIH/NIMH R01 MH116008

Title: Laminar and subregional differences in synaptic and morphological properties of pyramidal neurons in anterior cingulate cortex of rhesus monkeys

Authors: *Y. ZHOU[1], M. HSIUNG[2], C. A. MOJICA[1], W. CHANG[1], A. TSOLIAS[1], T. L. MOORE[1], D. L. ROSENE[1], J. I. LUEBKE[1], M. MEDALLA[1];
[1]Anat. and Neurobio., Boston Univ. Chobanian & Avedisian Sch. of Med., Boston, MA; [2]Boston Univ., Boston, MA

Abstract: The anterior cingulate cortex (ACC) is part of the limbic system, as well as, the 'frontal-executive' network. The ACC consists of three anatomically distinct subregions -- rostral medial area 32 (A32), dorsal caudal area 24 (A24), and ventral subgenual area 25 (A25) -- which have distinct roles in cognitive and emotional processing. Previous studies have shown ACC subregional differences in laminar cytoarchitecture and connectivity. However, the properties of synaptic transmission in these ACC subregions have not been elucidated in primates. In this study, we compared the properties of synaptic currents and synaptic structures of pyramidal (PYR) neurons in layers 2-3 (L2-3) and layers 5-6 (L5-6) of ACC A24, A32 and A25 in adult rhesus monkeys of both sexes (*M. mulatta*; n=9; 6-13 years old; 3 females, 6 males). Spontaneous excitatory (EPSCs) and inhibitory (IPSCs) postsynaptic currents were recorded using *in-vitro* whole-cell patch clamp, under voltage clamp for 2 minutes (V_{hold} = -80mV for EPSCs and -40mV for IPSCs). We found significantly higher mean EPSCs frequency in L5-6 PYR than in L2-3 PYR across all ACC subregions ($p<0.05$). We also found significantly lower EPSC amplitude in L5-6 compared to L2-3 PYR, in A24 and A32 but not in A25 ($p<0.05$). In addition to these laminar differences, subregional differences were found for mean EPSCs decay times; specifically, L2-3 and L5-6 PYR neurons in A25 had greater EPSC decay times than those in A32 and A24. To assess the morphological properties of synaptic sites, recorded cells were filled with biocytin during recording and then processed for assessments of spine density and appositions on inhibitory axon terminals immuno-labeled with vesicular GABA transporter (VGAT). We found significant laminar differences in spines and VGAT appositions on PYR neurons, but no significant subregional differences. Specifically, we found a greater density of mushroom spines (head width ≥0.6μm) on basal dendrites, and an overall greater density of VGAT appositions on apical and basal spines of L5-6 PYR neurons compared to L2-3 neurons. Interestingly, L2-3 neurons had greater VGAT appositions on the head of basal spines, but less on the neck, as compared to the L5-6 cells. Our results highlight the lamina-dependent functional and structural properties of synapses on pyramidal neurons across ACC subregions. These data will allow us to build better models of layer-specific pathways that impinge on distinct ACC areas, to predict circuit dynamics in cognition and specific disruption in cognitive-affective disorders.

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Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.04/UU1

Topic: H.04. Executive Functions

Support: R01-AG071230
R01-AG059028

Title: Age-related myelin dystrophies impair action potential propagation in collateralized dlPFC pyramidal cell axons

Authors: ***N. SENGUPTA**[1], M. L. A. MEDALLA[1], J. I. LUEBKE[1], C. M. WEAVER[2]; [1]Boston Univ., Boston, MA; [2]Mathematics and Computer Sci., Franklin & Marshall Col., Lancaster, PA

Abstract: During normal aging several myelin dystrophies occur in pyramidal neuron axons of the dorsolateral prefrontal cortex (dlPFC) of rhesus monkeys, and these correlate with working memory (WM) decline. We recently demonstrated that biologically plausible levels of demyelination and subsequent remyelination of axons can account for WM impairment in computational models. In individual neurons, this manifested as action potential (AP) failure and reduced AP conduction velocity (CV). Since axon collaterals of dlPFC layer 3 pyramidal neurons target both distant and local pyramidal cells as well as interneurons, here we examine how myelin dystrophy affects signal transmission in collateralized axons. Using the NEURON simulation environment, we applied demyelination and remyelination protocols to a cohort of dlPFC pyramidal cell models with collaterals arising from a single main axon, each with the same diameter but half the length, and quantified changes in AP propagation. Some perturbations, such as relocating potassium channels from the juxtaparanodes to the paranodes within the myelinated segments, had little effect. Along a collateral, we found that CV at its terminal end increased as the branch point moved further from the soma. When simulating localized demyelination along the main axon, collaterals that branched before the dystrophic region (more proximal to the soma) successfully propagated somatic APs with negligible slowdown. However, a collateral arising in a dystrophic region of an axon could worsen the CV slowdown significantly relative to its unbranched counterpart, often causing AP failures. This is because the collateral acts as an extra current sink. For example, if 25% of segments before the branch point lost all their myelin wraps, CV slowed by about 13% in the main axon and 9-11% in the collateral, depending upon the distance of the branch point from the dystrophic zone. Under similar perturbation conditions, CV slowdowns of about 51% occurred in both the main axon and the collateral when the collateral arose within the dystrophic zone. Differential amounts of CV recovery could occur in the main axon vs. collateral when a previously demyelinated

region was subsequently remyelinated. For example, when 25% of segments in the main branch were completely demyelinated then remyelinated by restoring 75% of the healthy number of wraps, CV recovered by 79% in the main branch and 53% in the collateral. Overall, this work develops a heuristic framework for quantifying how myelin dystrophy, together with other age-related changes, affects signal transmission in individual, collateralized dlPFC pyramidal cell axons and, in turn, their local microcircuits.

Disclosures: **N. Sengupta:** None. **M.L.A. Medalla:** None. **J.I. Luebke:** None. **C.M. Weaver:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Topic: H.04. Executive Functions

Support: NIH/NIMH Grant R01MH116008
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NIH/NIA Grant RF1AG043640

Title: Laminar distribution of calbindin-positive inhibitory axon terminals in the macaque medial and lateral prefrontal cortex

Authors: *B. SNYDER[1], M. MEDALLA[2];
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Abstract: Inhibitory neurotransmission from GABAergic interneurons (INs) is necessary for shaping the population activity of excitatory pyramidal (PYR) cells in the primate neocortex. Changes in this inhibitory signaling, including GABAA receptor composition and inhibitory apposition number and location, are associated with cognitive and mood disorders. Our previous work has shown diversity in the somatic inhibition across distinct areas of the macaque prefrontal cortex (PFC). However, the properties of dendrite-targeting INs, including calbindin-expressing (CB+) INs, that have important roles in setting laminar circuit dynamics are unknown. Thus, we studied the laminar distribution of putative CB+ inhibitory inputs onto PYR neuron dendrites within functionally-distinct PFC regions important for cognition, along the mediolateral surface, using immunohistochemistry and confocal microscopy. We labeled inhibitory inputs with CB together with the presynaptic marker, VGAT; postsynaptic GABAA receptor subunit, alpha1; and the PYR neuron dendritic marker, MAP2. Cortical columns within medial PFC A25, A32, A24c, mediodorsal PFC A9, and dorsolateral PFC A46 were imaged at high resolution from the pia to white matter, and colocalization of markers were quantified across the normalized cortical depth, reported as percentile bins. In all areas, we found that the highest density of VGAT+/CB+ puncta were in the upper layers (L1-3, 0-50% from pia surface). [MM1] Inter-areal comparisons within each normalized cortical depth showed that the

proportion of VGAT+ puncta colocalizing with CB+ was higher in middle layers of A25 compared to A46 (L3-5, 40-70% from pia) and A9 (L3-4, 50-60% from pia) (t-test, $p < 0.05$). We then quantified the overlap of VGAT+/CB+ puncta with MAP2+ dendrites to estimate the extent of dendritic inhibitory inputs. We found that the proportion of VGAT+ puncta colocalized with MAP2+ and CB+ was higher in the middle layers of A25 compared to A46 (L3-4, 40-60% from pia), A9 (L3-4, 50-60% from pia), and A24c (L3-5, 40-70% from pia) ($p < 0.05$). Interestingly, the proportion of VGAT+/MAP2+/CB+ puncta that also colocalized with GABAA receptor alpha1 subunit was higher in the upper layers (L2/3, 20-30% from pia) of A9 versus A25 and A32 ($p < 0.01$) and in middle layers of A9 versus A24c ($p < 0.05$). Together, these findings reveal regional differences in the laminar distribution of putative dendritic inhibitory inputs arising from CB+ INs in diverse mediolateral PFC areas. These data will allow us to incorporate the effects of dendritic inhibition on regional PFC local circuit dynamics during cognition, and how they can be disrupted in cognitive and mood disorders.

Disclosures: B. Snyder: None. **M. Medalla:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR368.06/UU3

Topic: H.04. Executive Functions

Support: NIMH R01MH117961

Title: Differences in encoding of anxiety signals and functional properties for layer II/III prefrontal neurons projecting to the striatum and amygdala

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Abstract: The rodent medial prefrontal cortex (mPFC) contributes to diverse behavioral functions related to cognition, emotion, and social interaction. The mPFC receives a wide array of afferent projections and sends efferent information to many cortical and subcortical structures. Pyramidal neurons that project to cortical structures, often referred to as intratelencephaic neurons (IT) have been well-characterized and evolving work has demonstrated that, despite sharing similar morphology and electrophysiology, IT neurons have diverse transcriptomic markers that correspond to different projection targets. Furthermore, IT neurons with different classes of targets may receive different sources and strengths of afferent input. In this context, it remains unclear whether and how neighboring IT neurons which project to different downstream targets may differentially process incoming information and exhibit distinct patterns of behavioral encoding.To better understand how IT neurons with distinct projection targets may

differentially contribute to the flow of information across mPFC circuits, we performed microendoscopic calcium imaging of layer 2/3 mPFC neurons projecting to either the basolateral amygdala (BLA) or dorsal striatum (DS). Mice explored the elevated zero and elevated plus mazes, as well as a maze consisting of closed arms only, in order to compare the activity of single cells as a function of context and arm type (open vs. closed). The same mice were then exposed to new and familiar adolescent mice as well as objects. To obtain cellular and molecular correlates of differential encoding in BLA- and DS-projecting IT neurons, we made whole cell patch clamp electrophysiological recordings from these cells in brain slices to compare their responses to input from the ventral hippocampus (vHPC). Finally, we performed patch seq on these neurons to identify possible underlying transcriptomic differences.

Disclosures: K.C. Donohue: None. **N.A. Frost:** None. **G.L. Short:** None. **L. Hagopian:** None. **V.S. Sohal:** None.

Poster

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR368.07/UU4

Topic: H.04. Executive Functions

Support: NIMH Grant R01MH121342

Title: Local field potential biomarkers of prefrontal PV neuron gamma synchrony during mouse rule shifting behavior

Authors: *A. LI[1,2], V. S. SOHAL[2], A. JACKSON[2], A. PHENSY DOS SANTOS[2];
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Abstract: Cognitive flexibility involves the ability to shift between tasks or different rules within a task. Psychiatric conditions such as schizophrenia are associated with deficits in cognitive flexibility. EEG studies in human subjects have implicated neural oscillations in the gamma-frequency range (~40 Hz) in multiple cognitive functions, including tasks involving cognitive flexibility. Previous work from our laboratory has used optogenetics and genetically-encoded voltage indicators to show that disruptions in gamma-frequency synchronization between parvalbumin-expressing (PV) inhibitory neurons in the left and right medial prefrontal cortex (mPFC) can cause deficits in cognitive flexibility in mice. Here, we used a mouse rule shifting task, which captures some elements of human cognitive flexibility paradigms such as the Wisconsin Card Sorting Task, to explore possible changes in local field potential (LFP) activity that track changes in cognitive flexibility and interhemispheric PV neuron gamma synchrony. We focused on increases in mPFC gamma synchrony that occur after rule shifting errors, as we have previously found that these increases in gamma synchrony are associated with successful task performance. To identify, optimize, and validate LFP-based biomarkers that reflect this

gamma synchrony and could potentially be translatable to clinical settings, we combined LFP recording with optogenetic inhibition, trans-membrane electrical measurements performed optically (TEMPO), and/or pharmacological manipulations. First, we simultaneously measured TEMPO signals and recorded LFPs from the left and right mPFC. In these recordings, we computed various metrics of gamma-frequency synchronization from LFP signals, and will correlate these with changes in TEMPO-based measurements of interhemispheric PV neuron gamma synchrony. Preceding work has found that the increases in interhemispheric gamma synchrony in mPFC PV neurons occurring after rule shift errors can be disrupted by optogenetic inhibition of callosal terminals originating from prefrontal PV neurons. Thus, we will also determine whether putative LFP-derived markers of changes in gamma synchrony are sensitive to inhibition of callosal PV terminals. Finally, we will quantify how LFP-derived markers of gamma synchrony are affected by clonazepam and psilocybin. Using TEMPO, we have previously shown that clonazepam can increase gamma synchrony, and will determine whether LFP-derived markers are also sensitive to these changes. Through these experiments, we hope to define LFP biomarkers that are relevant to cognitive flexibility and prefrontal PV neuron gamma synchrony.

Disclosures: **A. Li:** None. **V.S. Sohal:** None. **A. Jackson:** None. **A. Phensy dos Santos:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.08/UU5

Topic: H.04. Executive Functions

Support: National Science and Technology Innovation STI2030-Major Project (2021ZD0204103 to H.L.)
National Natural Science Foundation of China (31930052 to H.L.)

Title: Neural geometry encodes chunk structure of speech sequences in working memory

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Abstract: Items in working memory (WM) is organized into chunks, but how the items and chunks are neurally encoded remains elusive. Here human listeners temporarily retained a sequence of 9 syllables (i.e., items), which are organized into a sequence of trisyllabic words (i.e., chunks), while their electroencephalography (EEG) or magnetoencephalography (MEG) activities were recorded. A single syllable was presented during retention to probe each syllable's neural representation in WM, and subjects were asked to report the global and local ranks (EEG experiments) or continuous ranks (MEG experiments) of the cuing syllable during retrieval. First, neural decoding of rank information reveals that global ranks exhibit stronger and earlier excitation profiles that are further related to global precedence effects in behavior. Most

crucially, by employing time-resolved representational similarity analysis (RSA) combined with computational modeling, we demonstrate that the neural geometry of the 9-syllable sequence follows a two-dimensional hierarchical structure, one dimension for global rank and the other for local rank, instead of a single-dimensional chain structure. Importantly, the hierarchical neural geometry still holds even when subjects only retain the continuous rank information of the syllable sequence and thereby global and local ranks are completely task-irrelevant. Overall, by proposing a novel approach to seek the neural basis of abstract hierarchical structures during WM, our findings demonstrate that multiple neural representational dimensions exist in memory systems that separately encode items and chunks.

Disclosures: Y. Fan: None. **M. Wang:** None. **N. Ding:** None. **H. Luo:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR368.09/UU6

Topic: H.04. Executive Functions

Title: Decreasing compensatory prefrontal cortex activity during walking in people with Parkinson's disease using an ankle exoskeleton

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Abstract: Over half of individuals with Parkinson's disease (PD) develop freezing of gait (FOG), one of the most debilitating features of PD and one of the major reasons for falls and reduced quality of life. People with PD and FOG (PD+FOG) require a higher attentional demand to walk than those without FOG. The exaggerated attention required during poor walking may be characterized by a shift in locomotor control from healthy automaticity to compensatory executive control, largely located in the prefrontal cortex (PFC). Functional near-infrared spectroscopy (fNIRS) can provide direct evidence of the important role of the PFC during walking. Robot-assisted gait training may improve automaticity in people with PD+FOG by a decrease in abnormal reliance on attention with a concurrent reduction in PFC during gait. Robot-assisted gait training with an ankle exoskeleton provides individualized assistance, which can promote a more natural and rhythmic gait pattern. Although a case study showed that there were some improvements in walking economy and balance using an ankle exoskeleton, this study was performed only on one participant. Thus, the effects of exoskeleton on PFC activity need to be investigated. The aim of our study was to investigate if an ankle exoskeleton can reduce compensatory executive control (PFC activity) during overground walking in people with PD+FOG. Five people with PD+FOG were included in this preliminary study (mean ± SD; age 65.8 ± 7.52 yrs, 4 M). Participants performed 2-minute dual-task walking tests (walk back and forth along a 9-meter hallway) in three conditions: without the exoskeleton (baseline), with the exoskeleton, and again without the exoskeleton (retention). The ankle exoskeleton device

(Biomotum, Flagstaff, AZ) is a lightweight, portable powered ankle assist device that can increase independence, mobility, and deliver gait training to people with impaired plantarflexor function such as people with PD+FOG. PFC activity was simultaneously measured using a mobile, fNIRS system (Octamon, Artinis). Participants had a moderate decrease in PFC activity (effect size = 0.5) during walking with the exoskeleton (0.01 ± 0.37) compared to baseline (0.24 ± 0.52). A moderate retention effect (effect size = 0.56) of the exoskeleton assistance was observed in PFC activity levels (-0.031 ± 0.44) compared to baseline (0.24 ± 0.52). Our results suggest that ankle exoskeleton assistance may decrease the compensatory executive control (PFC activity) during dual-task walking in people with PD+FOG. These findings suggest that exoskeleton training may restore gait automaticity in people with PD+FOG.

Disclosures: P. Antonellis: None. **C. Batista:** None. **W. Liu:** None. **M. Mancini:** None. **L. King:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.10/UU7

Topic: H.04. Executive Functions

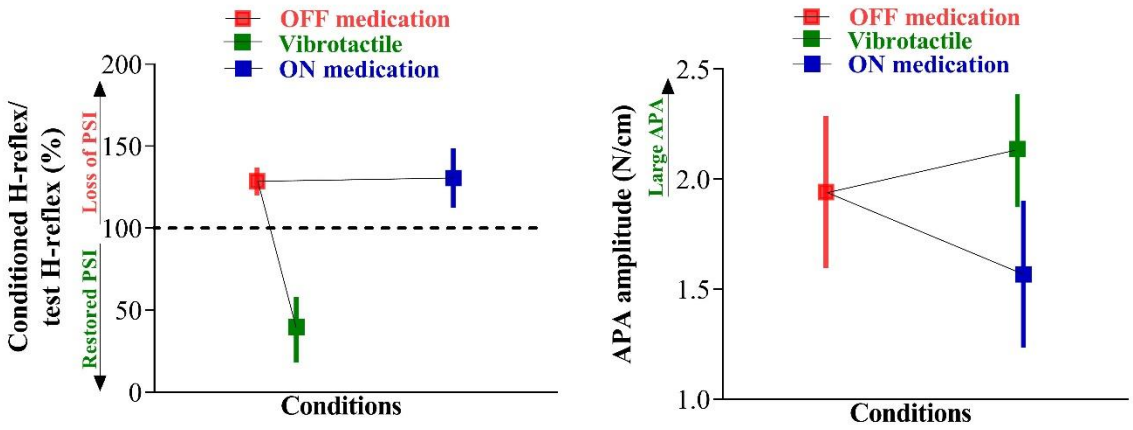
Support: OHSU-OFDIR fellowship
OHSU-PCO pilot grant

Title: Vibration to restore presynaptic inhibition and alleviate start hesitation in people with Parkinson's disease and freezing of gait: a preliminary data

Authors: *C. BATISTA[1], W. LIU[2], D. COELHO[5], J. G. NUTT[3], F. B. HORAK[4], M. MANCINI[6];
[1]OHSU, portland, OR; [2]Neurol., Oregon Hlth. & Sci. Univ., Portland, OR; [3]Oregon Hlth. & Sci. Univ., portland, OR; [4]Oregon Hlth. & Sci. Univ., Portland, OR; [5]Biomed. Engineering, Federal Univ. of ABC, Sao Bernardo do Campo, Sao Bernardo, Brazil; [6]Oregon Hlth. and Sci. Univ., Portland, OR

Abstract: Freezing of gait (FOG) is one of the most debilitating features of Parkinson's disease (PD). FOG may be due to a lack of central inhibition, as people with PD and FOG (PD+FOG) have difficulty inhibiting postural preparation before initiating stepping. We demonstrated that presynaptic inhibition (PSI) in the spinal cord, is crucial for coordinating postural preparation with step initiation. PD+FOG have a loss of PSI during the anticipatory postural adjustments-APAs for step initiation, whereas those without FOG and healthy controls demonstrate PSI during APAs. Also, the loss of PSI was associated with FOG severity. PSI is responsive to vibration in healthy people, and recently, we found that vibrotactile feedback may alleviate FOG severity. Here, we hypothesize that vibrotactile feedback could restore PSI and compensate for the loss of central inhibition to overcome step initiation failure in PD+FOG. This is a cross-over

study (one visit) conducted at the OHSU. So far, we have collected 4 PD+FOG (without deep brain stimulation, with start hesitation, and good response to levodopa) with moderate PD in 3 conditions: OFF medication (without vibrotactile), vibrotactile feedback, and ON medication. Participants performed 20 step-initiation trials while quantifying APAs on a force platform. Ten trials with test H-reflexes and 10 trials with conditioned H-reflexes, randomly, to measure PSI. Vibrotactile feedback consisted of 200-300 Hz vibration to the wrist when the ipsilateral foot is weight-bearing. Our preliminary data show that the 4 PD+FOG presented loss of PSI during stepping trials in OFF medication (128.5±16.8%), which persisted in ON medication (130.5±36.2%), but got restored during vibrotactile feedback (45.6±13.1%). APA amplitude got larger during vibrotactile feedback (2.1±0.2%) compared to OFF (1.9±0.6 N/cm) and ON medication conditions (1.5±0.7 N/cm). These preliminary data suggest that vibrotactile feedback may improve APA during step initiation via restored PSI, which is responsible for modulating sensorimotor integration in the spinal cord.



Disclosures: **C. Batista:** None. **W. Liu:** None. **D. Coelho:** None. **J.G. Nutt:** None. **F.B. Horak:** None. **M. Mancini:** None.

Poster

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Program #/Poster #: PSTR368.11/UU8

Topic: H.04. Executive Functions

Support: NIH Grant R0 1HD100383

Title: Association between pre-frontal cortex activity and instrumented gait domains in people with Parkinson's disease

Authors: *A. RAGOTHAMAN[1], W. LIU[2], C. SILVA-BATISTA[2], P. CARLSON-KUHTA[2], G. HARKER[2], J. ELLISON[2], F. B. HORAK[2], M. MANCINI[2];
[1]Neurol., Oregon Hlth. and Sci. Univ., Portland, OR; [2]Oregon Hlth. & Sci. Univ., Oregon Hlth. & Sci. Univ., Portland, OR

Abstract: BACKGROUND AND AIMS: Walking impairments, such as reduced gait stability, speed and stride length are common in people with Parkinson's disease (PD). In addition, walking impairments are characterized by a shift in locomotor control from healthy automaticity to compensatory executive control, largely located in the prefrontal cortex (PFC). Increased gait variability, observed in people with PD, is an indirect measure of reduced gait automaticity. It is thought that an increase in PFC activity may indicate a higher attentional demand to walk and thus, loss of automaticity. However, it is not known if specific domains of walking (pace, temporal, variability, asymmetry, stability, spatial) are related to changes in PFC activity while walking. Here, we hypothesized that an increase in the PFC activity while walking may relate to increase in variability aspects of gait.
METHODS: In this ongoing study, 33 people with PD (Mean: age 67.9, disease duration 9.6 years, MDS-UPDRS III 33.7) were included and tested while On Levodopa (~ 1 hour from last dose). Objective gait metrics were measured during 2-minute single- (ST) and dual-task (DT) walking using inertial sensors (Opals, APDM Wearable Technologies) worn on head, sternum, lumbar, bilateral wrists and feet. PFC activity was measured using a continuous wave, portable fNIRS system (OctaMon, Artinis Medical systems) with 50 Hz sampling frequency. The walking tasks included 20 s quiet stance, followed by 120 s walking and turning, and end with 10 s quiet stance. We derived the following metrics for gait: mean and variability of gait speed (pace), cadence (temporal), arm velocity asymmetry (asymmetry), double support time (stability) and foot-strike angle (spatial). Pearson correlation was computed for association between fNIRS and gait metrics.
RESULTS: During ST walk, increased variability in foot-strike angle was significantly associated with increased PFC oxygenated blood level ($r = 0.53$, $p = 0.002$). Decreased asymmetry in arm swing velocity during DT was associated with decreased PFC oxygenated blood level ($r = -0.42$, $p = 0.02$).
CONCLUSION: We found that variability and asymmetry domains of gait are significantly associated with pre-frontal cortex activity. Our preliminary results suggest that loss of gait automaticity in people with PD may be explained by a relationship between a compensatory increase in executive control (PFC activity) and increased gait variability and asymmetry during walking. With the availability of more data towards the end of the study we may be able to confirm the findings in a larger sample size.

Disclosures: A. Ragothaman: None. **W. Liu:** None. **C. Silva-Batista:** None. **P. Carlson-Kuhta:** None. **G. Harker:** None. **J. Ellison:** None. **F.B. Horak:** A. Employment/Salary (full or part-time):; Clario. **M. Mancini:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR368.12/UU9

Topic: H.04. Executive Functions

Support: R01 DA049147

Title: Self-control in the Supplementary Eye Field

Authors: *K. LEE, J. G. ELSEY, J. HWANG, E. EMERIC, V. STUPHORN;
Johns Hopkins Univ., Baltimore, MD

Abstract: Humans are often tempted by small, but immediate rewards (such as a fattening but tasty snack) that seem more attractive than a larger, delayed reward (such as losing weight), even when such choices are clearly against one's own best interest. Resisting this temptation and remaining committed to a long-term goal requires self-control, the ability to inhibit self-defeating behavior. A lack of self-control is associated with conduct disorders, drug addiction, and obesity, and it predicts early-onset criminal conduct. However, its neuronal mechanism is not well understood. Here, we report evidence that neurons in the Supplementary Eye Field (SEF), a region thought to contribute to executive control, encodes self-control of oculomotor behavior. We designed a novel, modified version of the intertemporal choice task that can explicitly reveal and distinguish states of high and low self-control. Monkeys were required to initially choose between a larger delayed (L) reward and a smaller immediate (S) reward. Normally, the unchosen option disappeared after the choice and the monkeys had to wait for the chosen reward, but sometimes the initially unchosen reward remained available, acting as a temptation to switch choices. In trials where the monkey initially chose the larger delayed option, the behavioral response to temptation (i.e., switching or staying) revealed the monkey's current level of self-control (low and high, respectively). Behavior from four male monkeys (macaca mulatta) revealed that temptation systematically shifted the monkeys' choice from the long-term reward maximizing optimal (L) reward to the immediate yet inferior (S) reward.
Recording from 204 SEF neurons of two monkeys, we found that SEF encodes distinct levels of self-control. These self-control signals were resent during all stages of the trial, even before any choice targets or a later temptation were presented. Moreover, fluctuations in SEF activity were predictive of the monkeys' behavioral response to temptation, suggesting SEF is critical for self-control behaviors. A partially overlapping population of neurons was also predictive of the monkeys' initial choice of L or S rewards, revealing a common neuronal mechanism underlying the ability to make prudent choices that are more beneficial in the long run, and the ability to adhere to those choices by exercising self-control and resisting temptation.
Our findings suggest that SEF is part of a neuronal circuit that underlies the capacity for self-control, which is crucial for maintaining and achieving long-term goals.

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Poster

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Program #/Poster #: PSTR368.13/UU10

Topic: H.04. Executive Functions

Support: R01DA049147

Title: The role of frontal eye field in response inhibition and self-control

Authors: *J. ELSEY[1], V. STUPHORN[2];
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Abstract: In daily life, we must often interrupt or suppress a behavior in favor of another that is more appropriate in the current circumstances. Two key aspects of such behavioral control are response inhibition and self-control. Response inhibition is the ability to deliberately stop a prepared motor response. Self-control is the ability to inhibit self-defeating behavior in the face of temptation. Clinically, failures of response inhibition and self-control are commonly treated as signs of deficits in behavioral control. Currently, it is unknown whether the neural mechanisms underlying response inhibition and self-control are shared or distinct.
Here, we trained macaque monkeys to perform saccade stop-signal (countermanding) and self-control tasks presented. The monkeys reliably switched between stop-signal and self-control tasks. During the countermanding task, the monkey made a saccade to a peripheral target. On a subset of trials, a visual stop signal was presented after a variable stop-signal delay, requiring the inhibition of the ongoing saccade preparation. During the self-control task, the monkey made a saccade to indicate their choice between a smaller, sooner (S) and a larger, later (L) reward. On a subset of trials, termed temptation trials, the unchosen option remained available, requiring self-control to resist switching from the more costly, but optimal, L option to the suboptimal S option. Critically, in this situation of heightened need for self-control, the monkey was sometimes able to resist temptation and sometimes failed to do so. Temptation trials provide therefore a clear behavioral marker for the level of self-control exerted on a trial-by-trial basis. Frontal eye field (FEF) generates signals sufficient for the generation and inhibition of saccadic eye movements. Further, firing rates from functional cell-types in FEF have been fit to the STOP and GO processes used by the Interactive Race Model (Boucher et al., 2007) to predict whether the animal will successfully inhibit a motor action. Thus, FEF is an excellent candidate region to investigate the extent to which the neural circuitry underlying motor inhibition is shared or dissimilar from the one governing the inhibition of a temptation. Data collection is ongoing, but preliminary data from simultaneous recordings using multi-contact linear probes shows that motor-related activity in FEF is modulated by the animal's level of self-control, suggesting that motor and motivational control may share a common neural circuit.

Disclosures: J. Elsey: None. **V. Stuphorn:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.14/UU11

Topic: H.04. Executive Functions

Support: 5R37MH087027-10

Title: Top-down learning results in more efficient beta (15-30 Hz) networks.

Authors: *M. BROSCHARD[1], J. E. ROY[2], S. BRINCAT[1], E. K. MILLER[3];
[1]MIT, Cambridge, MA; [2]Picower Institue for Learning and Memory, MIT, CAMBRIDGE, MA;
[3]The Picower Inst. for Learning and Memory, MIT, Cambridge, MA

Abstract: Many natural systems, including social relationships and urban power grids, are efficient when they maximize their interactions while minimizing their number of connections. Similarly, the brain achieves neural efficiency by forming "small-world networks" that have 1) highly interconnected subgroups of nodes (i.e., high clustering coefficient) and 2) many direct long-range connections (i.e., low path length).
We examined how neural dynamics in cortex maintain or change their neural efficiency over learning as networks incorporate new information. We applied graph theory to local field potentials and spikes in the prefrontal cortex of macaque monkeys as they learned new categories formed by dot patterns. The level of abstraction (i.e., low, medium, or high) was manipulated by the degree of distortion of exemplars from two category prototypes. Interregional graphs were created by calculating phase locking values between each electrode pair. Connectivity was normalized by the distance between electrodes.
Beta (15-30 Hz) LFP dynamics tended to form higher-efficiency networks than gamma (50-100 Hz) dynamics. Neural efficiency in the dorsolateral prefrontal cortex (dlPFC) beta connectivity was positively correlated with the level of exemplar abstraction. Specifically, the beta dlPFC graphs showed higher clustering and more long-range connections during category learning of exemplars at high levels of distortion. By contrast, neural efficiency was negatively correlated with category distortion level in gamma connectivity in the ventrolateral prefrontal cortex. These graphs showed less clustering and long-range connections for categories with higher distortion. These results suggest that learning of new high-level abstract information forms beta networks that are more efficient (i.e., more clustered and more directly interconnected). This fits with the putative role of beta in top-down cortical processing.

Disclosures: M. Broschard: None. **J.E. Roy:** None. **S. Brincat:** None. **E.K. Miller:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

Location: WCC Halls A-C

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Program #/Poster #: PSTR368.15/UU12

Topic: H.04. Executive Functions

Support: CIHR Postdoctoral Fellowship
NIMH 1R01MH131715-01
ONR N00014-22-1-2453
The JPB Foundation
The Picower Institute for Learning and Memory

Title: Testing the effects of antidepressant and anesthetic dose ketamine on the nonhuman primate corticothalamic circuit

Authors: *A. J. MAJOR, I. C. GARWOOD, M. K. MAHNKE, S. L. BRINCAT, J. E. ROY, E. K. MILLER;
MIT, Cambridge, MA

Abstract: Up to ~20% of patients with major depressive disorder have treatment-resistant depression. Ketamine, historically used as an anesthetic in high doses, has received growing attention as a treatment option for treatment-resistant depression in low doses. Although the molecular effects are well-studied, the macrocircuit neurophysiology of antidepressant dose ketamine remains relatively unexamined.
We tested the effects of low-dose (0.5 mg/kg IV over 40 min) and high-dose (15 mg/kg IM) ketamine in the nonhuman primate corticothalamic circuit within the same session. Acute laminar probes recorded LFPs and spikes in the premotor cortex, parietal 7A, visual area V4, putamen, and ventrolateral thalamus.
Different ketamine doses had different effects in different areas. At high (anesthetic) doses, ketamine increased low frequency (1-4 Hz) and high frequency (> 30 Hz) LFP power across all cortical areas. By contrast, low doses did not increase low frequency power and had mixed effects on different regions. The strongest low-dose effects were in thalamus and premotor cortex. In thalamus, low-dose ketamine increased low beta power (12-20 Hz). Low-dose ketamine increased power in alpha (8-12 Hz), beta (20-35 Hz) gamma (50-80 Hz) bands in premotor cortex. By contrast, premotor cortex alpha power was decreased by high-dose ketamine.
These results illustrate differences in the brain's response to ketamine doses that cause antidepressant effects vs unconsciousness. This work may inform future dosing or circuit targets for major depressive disorder and general anesthesia.

Disclosures: A.J. Major: None. **I.C. Garwood:** None. **M.K. Mahnke:** None. **S.L. Brincat:** None. **J.E. Roy:** None. **E.K. Miller:** None.

Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

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Program #/Poster #: PSTR368.16/UU13

Topic: H.04. Executive Functions

Support: ONR MURI N00014-16-1-2832
The JPB Foundation and The Picower Institute for Learning and Memory

Title: Convergent transformations of "what" and "where" information in primate prefrontal cortex, caudate tail, and hippocampus

Authors: *P. T. WENTZ[1,2], S. L. BRINCAT[1], E. K. MILLER[1];
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Abstract: Intelligent behavior relies on the ability to integrate multiple pieces of information. While several cortical and subcortical areas are known to be involved in this process, their contributions have rarely been studied simultaneously. Thus, we recorded multi-unit activity (MUA) from the lateral prefrontal cortex (PFC), hippocampus (HPC), and caudate tail (CD) of non-human primates performing a multi-level association task. One of four object cues was presented in one of four visual quadrants. When shown in the upper-right or lower-left quadrant, two of the cues instructed a leftward saccade and the other two cues a rightward saccade. This mapping was reversed for the other two quadrants such that diagonal quadrants shared the same object-saccade associations. Therefore, subjects needed to integrate information on the cue's location and its identity to produce a meaningful response. To investigate neural information content over time, we decoded different task variables from the MUA signal using a Linear Discriminant classifier. We also examined differences in representational geometry by regressing each area's Representational Dissimilarity structure on task-relevant condition groupings. Information about cue identity and location appeared in subcortical structures (CD and HPC subregion CA3) before the PFC. Surprisingly, only the CD categorized the cues according to their task-relevant identities, not the PFC or HPC. Moreover, the CD and HPC did not carry information on the saccade direction instructed by the cue. By contrast, PFC activity was quickly dominated by information about the instructed response. This information appeared shortly after the cue-location and -identity information. Only the PFC robustly carried all task-related representations over a memory delay before the saccade. These results illustrate different roles for the PFC, CD, and HPC. The CD and HPC are first to process information about what (cue identity) and where (its location). The PFC then quickly transforms this information into the appropriate response.

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Poster

PSTR368. Prefrontal Mechanisms of Executive Functions I

Location: WCC Halls A-C

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Program #/Poster #: PSTR368.17/UU14

Topic: H.04. Executive Functions

Support: Swiss National Science Foundation (SNSF) Grant 214404
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Title: Two stages of hierarchical cognitive control are coordinated by delta and theta neural oscillations in distinct frontoparietal networks

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Abstract: Cognitive control allows behavior to be guided according to environmental context and internal goals. In laboratory experiments, we operationalize task-related contexts as 'abstract rules' and manipulate the number of rules and stimulus-action associations that must be maintained as 'set-size'. Previous fMRI studies on cognitive control found increased activation in both the lateral frontal-parietal network (FPN) and the dorsal attention network (DAN) and EEG analyses revealed increased amplitude of neural oscillations in the delta/theta-band (0.5-7 Hz) in frontal electrodes. Some studies proposed that the peak frequency of FPN neural oscillations shift depending on task demands. However, other studies found a distinction between delta and theta oscillations for the control of abstract rules and the set-size, respectively, suggesting that these control signals may arise from distinct networks. We tested these alternative predictions using EEG (N=31) and fMRI data (N=30) collected from two groups of healthy human participants, during performance of a hierarchical control task that manipulated level of abstraction of task rules and their set-size (Badre and D'Esposito, 2007; Riddle et al., 2020). We performed source-space power and multivariate connectivity analysis of the EEG data. We then used a multivariate connectivity analysis of the fMRI data to investigate whether the pattern of EEG corticocortical connectivity was similar to that of fMRI, and whether subcortical regions in dorsal striatum or thalamus contributed to the observed task-driven connectivity changes. Taken together, our results show that control of abstract rules engages delta oscillations in the FPN and control of stimulus-action associations engages theta oscillations in the DAN. We show that a subcortical structure in the dorsal striatum, the caudate nucleus, mediates the communication between lateral prefrontal cortex and inferior parietal cortex in the FPN, whereas the DAN is mediated by the putamen. Finally, we discovered a serialization of these control signals such that control over abstract rules unfolded first in the stimulus-locked analysis and control over stimulus-action associations proceeded this signal in response-locked analysis. Our findings support a model by which parallel and distributed association networks operate through dynamic, multiplexed neural processes.

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Poster

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Program #/Poster #: PSTR368.18/UU15

Topic: H.04. Executive Functions

Title: Cortical traveling wave's influence on singe cell selectivity

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Abstract: Recent studies suggest that a common form of neural communication is through traveling waves (TWs) -- patterns of neural oscillations that propagate continuously within and between local brain areas. However, the interactions between cortical traveling waves with local spikes are not well understood. With recording the single unit activity and field potentials in the prefrontal and parietal cortex of animals, we studied the interaction of spiking activity and potential TWs, during a probabilistic reward saccade task. Our analysis verified oscillations are spatially organized in the form of TWs in both regions, propagating in two opposite anatomical directions at 10-35 Hz (the dominant LFP frequency) with biologically plausible speeds of 0.1-0.3 cm/sec. We observed the majority of neurons in frontoparietal areas are coupled with the phase of ongoing beta TWs, mainly releasing a higher firing rate at the peak phase of the beta oscillation cycle amplitude. Surprisingly not only individual neurons responded to the phase of ongoing oscillations but also the direction of ongoing waves. TW direction correlated with the firing probability of a significant population of neurons in both regions. We also reported a prefrontal population of neurons that experienced drift in their selectivity to reward mnemonics, depending on the propagation direction of traveling waves. Following these results, we speculate a more fundamental role for cortical traveling waves, potentially as a mechanism to implement and transmit intra-regional influences required for multiple cognitive processes like attention.

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Poster

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Program #/Poster #: PSTR368.19/UU16

Topic: H.04. Executive Functions

Support: CIHR (Petrides)
CIHR (Tremblay)

Title: Non-necessary neural activity in the primate cortex

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Abstract: When neuroscientists record neural activity from the brain, they often conclude that neural responses to behavioral task variables suggests a functional role of the brain area(s) studied. However, it remains unknown how reliably such correlations between brain and

behavior indicate a true functional role. To answer this question, we chronically recorded neural activity in the prefrontal cortex of the same monkeys during the performance of four different cognitive tasks. The tasks were carefully selected such that only one of them causally depends on the brain area recorded - performance on the other three tasks is not affected by bilateral ablation of this area. Using the most common analyses methods, we found that the prevalence and strength of neural tuning was just as high across all four tasks, including for tasks that do not depend on this brain area. This suggests that neuroscientists are likely to capture non-necessary neural activity and misattribute a functional role to the brain area(s) studied when relying on neural recordings alone.

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Poster

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Topic: H.04. Executive Functions

Support: NIH Grant R01MH132386

Title: Representational geometry adapts inductive bias in a neural kernel model

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Abstract: Neural representational geometry plays a crucial role in learning. It shapes the inductive bias of an agent, affecting the efficiency of learning a task through generalization. It can also change during learning, allowing the agent to adapt to the statistics of the environment. Recent theoretical works have shown that learning dynamics and inductive bias of an artificial neural network can be described by its neural tangent kernel, which determines generalization across conditions. In certain regimes, the kernel can be approximated by the cross-condition covariance of neural activity, which can be considered as a measure of representational similarity, thus linking representational geometry and learning. Here, we developed a kernel learning framework for a categorization task, where the kernel can be decomposed into multiple modes describing generalization across conditions based on shared features or conjunctions. Stronger feature representations allow faster learning of readout along those population axes, and therefore condition-wise learning curves would be distinct for a specific kernel-task combination. We fit this model to data from human subjects that learned multiple task sets. Our analyses revealed that inductive biases of the subjects were more strongly based on linear features than their conjunctions. Between task sets, we varied the specific stimuli but maintained the most relevant features. We observed a differential increase in strengths of task-relevant features,

suggesting that feature representations of subjects adapted to the task statistics and that the adaptation was generalizable to new stimuli. In contrast, standard artificial neural networks do not exhibit such learning behavior and do not adapt their representations continually. We introduce an additional term in the loss function that aligns the kernel with the task, encouraging representations to adapt to task-relevant features in a generalizable way. This regime allows faster learning of readout that drives flexible behavior and slower adaptation of representations to the environment. Our framework provides a new approach for analyzing behavioral and neural data during learning simultaneously and makes specific predictions about changes in feature representations and learning behavior.

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Poster

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Topic: H.04. Executive Functions

Support: AFSOR 20RHCOR04

Title: Vagus Nerve Stimulation Mitigates Hypoxia-Induced Cognitive Impairment in male rats.

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[1]711th Human Performance Wing, Wright Patterson Air Force Base, OH; [2]Oak Ridge Inst. for Sci. and Educ., Oak Ridge, TN; [3]UES, Inc., Dayton, OH; [4]Naval Med. Res. Unit, Wright Patterson Air Force Base, OH; [5]Infocitex, Dayton, OH

Abstract: Hypoxia-induced cognitive deficits pose a significant challenge in Air and Space Force operational environments. Emerging bodies of literature have shown that vagus nerve stimulation (VNS) improves cognition in humans and rodents. Under an approved IACUC protocol, this study aimed to investigate the effect of VNS on hypoxia-induced cognitive impairments using a rat model. Male Sprague-Dawley rats underwent surgical implantation of platinum/iridium electrodes around the left cervical branch of the vagus nerve under anesthesia. The rats were divided into three groups: sham (21% oxygen, n =23), hypoxia only (8% oxygen, n=18), and vns+hypoxia (100 μs biphasic pulses, 30 Hz, 0.8 mA + 8% oxygen, n=13). Behavioral tests including the elevated zero maze (EZM), novel object recognition (NOR), and passive avoidance test (PAT) were conducted. The experimental design involved alternating periods of hypoxia exposure and VNS treatment for a total of 4 hours of hypoxia and 2 hours of VNS, interspersed with behavior training. The rats were humanely euthanized, and different brain regions were collected 24 h after the last vns+hypoxia session for molecular analysis using Immunohistochemistry and Reverse Transcription-Polymerase Chain Reaction. The behavioral

results revealed that hypoxia-induced cognitive impairments were specific to the PAT ($p = 0.004$ for the sham, n=21 and hypoxia only group, n=18), as no significant deficits were observed in the EZM or NOR tests ($p>0.05$ for sham, n=18-22 and hypoxia only, n= 10-13). Interestingly, VNS treatment demonstrated an ability to significantly increase memory performance in the PAT ($p= 0.035$ for hypoxia only, n=18 and vns+hypoxia group, n=13), mitigating the observed deficits from hypoxia. The findings from this study suggest that VNS may hold promise as a therapeutic approach for addressing hypoxia-induced cognitive impairments. Further investigation is required to understand the underlying mechanisms by which VNS exerts its cognitive enhancing effects and to explore its translational potential in human subjects.

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Poster

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Topic: H.04. Executive Functions

Support: NIH Grant 5R01HL091541-25

Title: Novel Object Recognition as Feasible and Reproducible Test of Cognition in a Canine Model of Hypothermic Circulatory Arrest

Authors: *I. D. CHINEDOZI[1], Y. KITASE[2], T. HECK[6], H. RANDO[1], Z. E. DARBY[1], J. K. KANG[1], S. NAIR[3], N. ATHIPATHY[8], J. SANGALANG[4], J. WANG[1], J. SCAFIDI[9], R. M. KANNAN[5], L. J. MARTIN[4], J. S. LAWTON[1], L. L. JANTZIE[7];
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Abstract: Cerebral injury following hypothermic circulatory arrest (HCA) occurs in up to 30% of patients undergoing complex cardiac surgeries resulting in varying degrees of neurological disability. To assess the neuroprotection offered by emerging therapeutic approaches, neurobehavioral assessment, brain histopathology and blood biomarkers have traditionally been used, combined with neurological evaluation with the Pittsburgh and Finnish neurobehavioral batteries. Though these tests provide an overview of gross neurological function, they are limited by the granularity of the testing parameters. Specifically, they do not evaluate memory and executive function, the areas most impacted by the cerebral ischemia-reperfusion injury of HCA. Our investigations expanded to more precise assessments of cognition and aimed to demonstrate the feasibility of using the Novel Object Recognition (NOR) testing in a canine model of 90 minutes of HCA at 18^{o}C. We hypothesized that NOR testing would be a feasible method for

assessing learning and cognition in a canine model of HCA and correlate with Pittsburgh and Finnish neurological assessment scores. Prior to HCA, canines underwent a familiarization trial after 2 days of habituation. Each animal was placed in the arena with two identical novel objects spaced 40 cm apart for 5 minutes. The interaction time was recorded and scored by a blinded observer. The minimum threshold for interaction with either object during the familiarization stage was set *a priori* at 2s. On post op day 3 (72 hours), the canine was presented with both a familiar and a novel object for 5 minutes. The interaction time with each object was recorded. Novel object order of presentation was counterbalanced with equal placement on the left or right side of the arena to minimize bias. Data was evaluated by paired t-tests (n=13). After habituation, canines exhibited similar levels of interaction with the objects in the test. Specifically, canines did not demonstrate a preference for one object over another during the familiarization stage (38.7±7.0 vs. 38.2±7.7s, n=13, p=0.74). However, during the recognition trial, canines spent more time interacting with the novel object versus the familiar object (22.3±9.1 vs. 6.8±2.3, n=6, p=0.09). Increases in novel object interaction time tracked with improvement in Finnish and Pittsburgh scores throughout the postoperative period. These results demonstrate the feasibility of NOR to detect recognition memory deficits after HCA. Future studies will address differences between treatment groups treated with various neuroprotective therapies, correlation with MRI findings and histopathology, and changes in NOR over time.

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Poster

PSTR369. Effects of Executive Functions on Learning and Memory

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Topic: H.04. Executive Functions

Support: R01MH131559

Title: Multimodal evidence for the mental organization of task rules

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Abstract: Switching between tasks requires replacing the previously active task representation with a new one, an operation that typically results in a switch cost. Understanding how we flexibly switch between tasks requires understanding how tasks are represented in the brain. We hypothesize that task representations are parameterized in a map-like structure, such that distinctions between task representations reflect their conceptual differences: The greater the distinction between the two task representations, the more updating is required. We employed a

novel experimental design that parametrically manipulates the difference between task rules. Behaviorally we observed that switch costs scale with the dissimilarity between tasks. Computationally, we also observed the same scaling effect in the representational dissimilarity of artificial neural networks trained to perform these tasks. Furthermore, we aim to provide neural evidence for the organization of task rules. We expect to see in EEG data (n = 40) mid-theta power scale with the degree of task rule switch, which will provide evidence for the similarity-based organization of task rules in the human brain. Lastly, to ensure that our observed effects were driven by the organization of task rules we conducted two control experiments. The first ruled out that the task rule switch cost was due to shifts of spatial attention and/or saccades instead of task reconfiguration. And the second control experiment confirmed that the task rules were represented separately ruling out the possibility of the observed effects being driven by mental rotation. The findings shed light on the organizational principles of task representations and extend the conventional binary task switch effect (task repeat vs. switch) to a parametric fashion within task switches.

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Poster

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Topic: H.04. Executive Functions

Title: Follow-up of cognitive dysfunctions in post-COVID-19 healthcare personnel in a hospital in southeast Mexico

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Abstract: Cognitive dysfunction is one of the long-term neuropsychiatric disorders that post-COVID-19 patients present after the acute stage of infection. Our aim was to identify cognitive changes in health workers in a hospital in southeast Mexico. Twenty-eight healthcare personnel from a hospital in southeast Mexico, with a history of SARS-COV-2 infection, participated in a first interview in 2021 (Interview I). MoCA and MMSE scales were applied to them from December 2022 to February 2023 (Interview II). Post-COVID-19 healthcare workers presented total scores in MMSE of 30.54 ± 3.75 (Interview I) and 30.14 ± 3.9 (Interview II); in MoCA test, the total score for Interview I and II was 23.25 ± 3.12 and 24.36 ± 3.47, respectively. We noticed that interview scores were below the mild cognitive impairment cut-off point. In addition, we found differences in the delayed recall and orientation evaluated by MoCA ($p \leq 0.003$ and 0.043, respectively). However, with MoCA scale we found differences in age from the first interview

42.75 ± 10.73 and 31.00 ± 6.48 years, p<0.045, for workers without dysfunction, in the second interview that was 41.85 ±1 0.97. Our study indicates that the healthcare personnel of this hospital presented cognitive changes (areas of orientation and delayed recall) that remained after 2 years of SARS-COV-2 infection; therefore, a clinical follow-up to post-COVID-19 healthcare workers is suggested.

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Poster

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Topic: H.04. Executive Functions

Support: BECA CONACYT 778952

Title: Effect of stress and psychological factors on the executive functions of informal primary caregivers of cancer patients

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Abstract: Informal primary caregivers go through a process that is characterized by high levels of stress. At the brain level, it has been studied that it influences the strengthening of emotional responses mediated by the amygdala and that it significantly affects the responses of the prefrontal cortex (PFC). This research aimed to determine the effect of stress and psychological factors on the executive functioning of the PFC in caregivers of cancer patients. Women older than 18 years who provided care to patients receiving treatment at the State Cancer Center (N=41). The instruments were also applied to a control group of women with similar characteristics except for being caregivers (N=22). The Neuropsychological Battery of Executive Functions and Frontal Lobes, the Perceived Stress Scale, the Beck Anxiety and Depression Inventories, the Zarit Caregiver Burden Scale, and the Robinson Caregiver Effort Index were used. One-way analyses of variance (ANOVA) were performed, and from them, it was found that spending 1 to 6 months as a caregiver significantly decreases performance in tasks that involve working memory (WM) and executive functions (EF) ($F_{(2,60)} = 4.00$, $p = 0.02$), in addition to global executive functioning ($F_{(2,60)} = 5.50$, $p = 0.00$). Likewise, those who perceived themselves to be highly stressed obtained lower scores in WM and EF ($F_{(2,60)} = 4.01$, $p = 0.02$), as well as in the global assessment of executive functioning ($F_{(2,60)} = 5.45$, $p = 0.00$). The participants who presented a low level of effort were the ones who had the worst performance in

WM and EF($F_{(2,60)} = 3.98$, $p = 0.02$), and also in the overall EF score ($F_{(2,60)} = 5.45$, $p = 0.00$). In addition, the caregivers who reported no overload were those who obtained the lowest scores in metafunctions ($F_{(2,60)} = 3.99$, $p = 0.02$), as well as in MT and FE ($F_{(2,60)} = 4.04$, $p = 0.02$), and the evaluation of executive functioning in general ($F_{(2,60)} = 5.51$, $p = 0.00$). For the depression variable, it was found that those who presented mild depression obtained lower scores in the FE and WM tests ($F_{(3,59)} = 3.75$, $p = 0.01$), and also in those that measured executive functioning more overall ($F_{(3,59)} = 6.00$, $p = 0.00$). Finally, with respect to anxiety, the group of participants who presented anxiety at a low level resulted in lower performance in WM and EF ($F_{(2,60)} = 4.07$, $p = 0.02$), as well as in the global assessment of executive functioning ($F_{(2,60)} = 5.08$, $p = 0.00$). It is concluded that the stress experienced by caregivers can lead to cognitive deterioration; however, the time spent on the task is relevant, how stressed they perceive themselves, and the emotional discomfort they present when providing care to a cancer patient.

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Poster

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Program #/Poster #: PSTR369.06/UU23

Topic: H.04. Executive Functions

Title: Changes in neural representations of task elements are associated with explicit knowledge of an underlying contingency

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Abstract: Detecting and extracting hidden regularities in the environment is the key tenet of implicit learning. Tracking the emergence of explicit knowledge of these hidden regularities, as well as the effect of this transition on how this knowledge is represented, has historically been a difficult problem. Here, we evaluate the potential of multi-variate decoding of task-set representations from EEG data to track and interrogate emerging explicit representations in an implicit learning task. 31 subjects (Age mean(SD) = 20.7 (3.5), 2 left handed, 21 females) completed a simple task switching paradigm while undergoing EEG recording. Embedded in the task was a probabilistic contingency, in which one of three possible cues specified a certain response most frequently. Following the task, a questionnaire was administered to determine the extent of the subjects' explicit knowledge of the contingency, the results of which were used to classify subjects as "explicit" or "implicit" learners. We then applied multi-variate pattern analysis (MVPA) and representational similarity analysis (RSA) to the whole-scalp EEG data to decode the strength of task-set (cue, target stimulus, and response) and conjunctive (comprising all task-set elements) neural representations. RSA results revealed that on trials with the embedded contingency, explicit learners showed significant ($p<0.05$, FDR corrected)

decodability of the contingent target stimulus before it was presented, indicating a predictive representation of this information. Also, on trials where the probabilistic expectation was violated, explicit learners show a sharp decrease in cue representation following presentation of the violating target stimulus. Overall, RSA results demonstrate notable differences in the task-set representations in those with explicit knowledge. Next, we used a mixed effects model to examine how the strengths of the RSA representations were predictive of same-trial RT. When time-locked to stimulus presentation, analysis revealed that the strength of the conjunctive representation was a stronger predictor of fast RTs in the explicit group. When time-locked to the response, the cue-related representational strength was a stronger predictor of fast RTs in the explicit group. These modelling results indicate that representations of task elements related to the embedded contingency were better predictors of RTs in subjects with explicit knowledge of the contingency. Together, our work demonstrates the utility of multi-variate decoding of whole-scalp EEG data in quantifying neural markers of the emergence of explicit knowledge of hidden contingencies in implicit learning tasks.

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Topic: H.04. Executive Functions

Support: P20GM103430

Title: Cognitive impairments in a mouse model of bipolar disorder

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Abstract: Cognitive deficits are a core symptomatic category of bipolar disorder (BD). They are impaired across mood states, present across the lifespan, and observed in unaffected first-degree relatives. In fact, they are predictive of disease outcome and quality of life. Despite the importance of these cognitive deficits, however, they are understudied and undertreated, partly due to a scarcity of valid rodent models. Our goal is to characterize the cognitive profile of adolescent *Clock*Δ19 mice, a promising pre-clinical BD model that has not yet been validated for cognitive research. *Clock*Δ19 is a circadian rhythm-based model which features BD-like phenotypes such as increased risk-taking behavior, addictive tendencies, hyperactivity, mood cyclicity and disrupted circadian clocks. The *Clock*Δ19 mice display mood cyclicity on a 24-hour basis. Rapid mood cyclicity is more pronounced in adolescent BD populations. In this study, we use three established behavioral assays to compare male and female homozygous *Clock*Δ19 mice (+/+), to heterozygous (+/-) and wildtype (-/-) mice. The attentional set-shifting task (AST) measures cognitive flexibility, a type of executive functioning often impaired in BD,

while the novel object recognition (NOR) and novel object location (NOL) tasks assess two forms of recognition memory. In the AST, we do not observe strong group differences, although *Clock*Δ19 mice (+/+) do appear to be more motivated in this rewarded task. In the NOR, preliminary results (n=4 per genotype) suggest that *Clock*Δ19 (+/+) mice are impaired compared to both (-/-) and (+/-) mice, which may be more pronounced in males compared to females. In NOL, there is a trend showing that *Clock*Δ19 (+/+) may be impaired compared to (-/-) but not (+/-) mice. Overall, it appears that *Clock*Δ19 mice do display cognitive impairments, although it is not yet clear how well they mimic the human condition. In the coming months, our results will provide clarification for the sex-specific role of *clock* genes in cognition and in BD. They will also serve to further characterize, and potentially validate the *Clock*Δ19 transgenic mouse model for use in translational studies of cognitive behaviors in BD.

Disclosures: S.C. Soares: None. **S.K. Dhillon:** None. **V.R. Heimer-Mcginn:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.08/UU25

Topic: H.04. Executive Functions

Support: NIA Grant R01 AG064247-04
NIA Grant T32 AG076407

Title: Hippocampal subfield volumes in periadolescent children: association with spatial working memory, learning of paired associates, and age

Authors: *A. HELLER[1], C. PHIPPS[1], M. RAMIREZ[1], J. SEXTON[1], A. ZATKALIK[1], A. WILHELM[1], A. MAERLENDER[3], V. PHATAK[1], D. MURMAN[1], D. WARREN[2]; [2]Neurolog. Sci., [1]Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Univ. of Nebraska Lincoln, Lincoln, NE

Abstract: Periadolescence is a neurodevelopmental period that can be characterized by cognitive development and changes to the brain structure. The hippocampus, a structure necessary for normal memory, undergoes significant developmental change during this period. Specifically, volume changes in several hippocampal subfields have been shown to follow unique trajectories during development, and subfield volumes have also been associated with memory abilities. Spatial working memory (SWM), which involves flexible storage and retrieval of (visuo)spatial information, and paired-associate learning (PAL), or the ability to bind two pieces of information for later recall, are memory abilities supported by the hippocampus. However, it is not clear whether periadolescent developmental changes in SWM and PAL are associated with differences in hippocampal subfield volume. The current study aims to investigate the relationship between hippocampal subfield volume, age, and PAL/SWM task performance in a group of healthy periadolescent children aged 8 - 13 years (N=52). Data was collected as part of the Polygenic

Risk for Alzheimer's disease in Nebraska Kids (PRANK) study. Participants completed the Cambridge Neuropsychological Test Automated Battery (CANTAB) implementation of a SWM and PAL task, in addition to an MRI study to assess brain structure. We used the Automatic Segmentation of Hippocampal Subfields (ASHS) software toolbox to segment the hippocampus into the CA1, CA2-3, and the Dentate Gyrus (DG), and calculated correlations between subfield volumes and age, SWM, and PAL task performance. We did not observe statistically significant correlations between left or right CA1, CA2-3, or DG with either PAL or SWM task performance. However, we did observe a significant correlation between the left CA2-3 and age, $r(52) = .28$, $p = .04$. Also, performance on the SWM task improved with age, $r(52) = .39$, $p < .01$. This study is one of the first to investigate hippocampal subfield development with a specific focus on periadolescent children. The significant association between CA2-3 volume and age may reflect a developmental trajectory associated with changes in subfield volume, and longitudinal studies could test that prediction. Our future efforts will aim to understand how these putative changes influence hippocampal-dependent memory abilities. Characterizing the relationship between hippocampal subfield changes and memory during this critical neurodevelopmental period may lead to insights on healthy brain development throughout adolescence, as well as prevention of diseases associated with hippocampal pathology and dysfunction, such as Alzheimer's disease.

Disclosures: **A. Heller:** None. **C. Phipps:** None. **M. Ramirez:** None. **J. Sexton:** None. **A. Zatkalik:** None. **A. Wilhelm:** None. **A. Maerlender:** None. **V. Phatak:** None. **D. Murman:** None. **D. Warren:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.09/UU26

Topic: H.04. Executive Functions

Support: NIA R01AG064247

Title: Hippocampal resting-state functional connectivity and relational memory differences in periadolescent children: Preliminary findings from the PRANK study

Authors: *A. F. WILHELM[1,2], C. J. PHIPPS[2], M. K. RAMIREZ[2], J. SEXTON[2], A. HELLER[2], A. ZATKALIK[2], A. MAERLENDER[3], V. PHATAK[2], D. MURMAN[2], D. WARREN[2]; [1]Neurolog. Sci., UNMC, Omaha, NE; [2]Univ. of Nebraska Med. Ctr., Omaha, NE; [3]Univ. of Nebraska Lincoln, Lincoln, NE

Abstract: Relational memory performance develops through childhood and adolescence, and this trajectory of cognitive maturation has been associated with structural and functional changes in the hippocampus. The hippocampus is known to be involved in declarative-relational memory, and changes in relational memory performance during development could result from differences

in hippocampal function or connectivity. Such age-related changes in adolescence remain underspecified. Investigating the covariance of hippocampal resting-state functional connectivity (Hc-RSFC) with different memory tasks in a periadolescent sample would enhance the field's understanding of cognitive and brain development. This project utilized preliminary data from the NIA-funded Polygenic Risk of Alzheimer's Disease in Nebraska Kids (PRANK) study, a study that examines how brain and cognitive development is affected by polygenic risk of Alzheimer's disease (AD). Here, we report preliminary cross-sectional findings from the PRANK study, examining how age-related differences in Hc-RSFC covary with relational memory performance on tests of memory for children.
A sample of healthy children (N=115), ages 8-13 years, was drawn from the ongoing PRANK study. Participants completed a series of cognitive and behavioral tests, and an MRI scan, used to measure Hc-RSFC. For the current project, we focused on the Child and Adolescent Memory Profile (ChAMP) instrument. ChAMP measures properties of memory including hippocampal-dependent relational ability. Meanwhile, resting-state functional MRI data from the same participants was analyzed using a seed-based approach with the bilateral hippocampus as the seed region of interest. The Hc-RSFC data were then analyzed to determine the covariance with relational memory performance from ChAMP. The covariance analysis revealed patterns of Hc-RSFC that covaried with ChAMP performance that appeared to be independent of age. We observed statistically significant positive covariance of Hc-RSFC with ChAMP objects performance in prefrontal regions of the frontoparietal network. We also observed statistically significant positive covariance of Hc-RSFC with ChAMP places performance in parietal and frontal regions of the cinguo-opercular network. This project presents preliminary findings of the ongoing PRANK study. We showed that Hc-RSFC covaries with hippocampal-dependent relational memory performance as measured with two components of the ChAMP assessment. Future work from the PRANK study will give more insight on the influence of AD polygenic risk factors on the development of the hippocampus and its functional connectivity.

Disclosures: A.F. Wilhelm: None. **C.J. Phipps:** None. **M.K. Ramirez:** None. **J. Sexton:** None. **A. Heller:** None. **A. Zatkalik:** None. **A. Maerlender:** None. **V. Phatak:** None. **D. Murman:** None. **D. Warren:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.10/UU27

Topic: H.04. Executive Functions

Support: NIA Grant R01 AG064247

Title: Age-related differences of hippocampal recruitment during relational memory: preliminary findings from the PRANK study

Authors: *M. RAMIREZ[1], J. A. ROSE[1], J. N. SEXTON[1], A. M. HELLER[1], C. J. PHIPPS[1], L. BEHM[2], A. ZATKALIK[1], A. WILHELM[1], K. NICKOLAS[1], A. C. MAERLENDER[3], V. PHATAK[1], D. L. MURMAN[1], D. E. WARREN[1];
[1]Dept. of Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE; [2]Yale Univ., New Haven, CT; [3]Univ. of Nebraska - Lincoln, Lincoln, NE

Abstract: Typical adolescent brain development is part of a lifespan trajectory towards functional maturity. We hypothesized that younger children would show increased activation of the posterior hippocampus (Hc) relative to older children, potentially mirroring age-related changes that have been observed later in the lifespan. The present study aimed to investigate age-related change of hippocampal functional organization in periadolescents. Using data from the Polygenic Risk of Alzheimer's disease in Nebraska Kids (PRANK) study, we assessed hippocampal-dependent relational SM (RSM) using task-based fMRI. Participants (N =111) completed a SM paradigm: they studied pairs of items while whole-brain fMRI-BOLD data were collected. After imaging, participants underwent a memory test for the studied materials in which they performed a recognition task that included two types of stimuli: studied match pairs (targets) and non-studied mismatch pairs (lures). Data were analyzed using a univariate approach that contrasted fMRI-BOLD activation for successful with non-successful SM for the pair stimuli. Next, we further contrasted hippocampal activity (posterior vs. anterior and left vs. right - associated with successful SM) between younger (N = 64, ages 8-10 years) and older children (N = 47, ages 11-13 years). In this preliminary analysis, there was a statistically significant difference such that successful RSM encoding in anterior Hc was greater than posterior Hc (p &lt 0.005). We did not observe statistically significant differences in Hc activation (posterior vs. anterior and left vs. right) between age groups on RSM encoding. Removing Hc laterality as a variable, we observed no significant difference between age groups. These preliminary findings are suggestive of a pattern of regionally specific activation of the hippocampus during successful encoding of relational information. That is, children in our periadolescent sample demonstrated increased anterior Hc activation relative to posterior Hc activation. We did not observe age-related differences that might be consistent with a maturational trend in this regional specificity. However, future efforts to study this question might benefit from longitudinal designs capable of measuring within-subjects changes with age. Additionally, covariance of these patterns with lifespan AD risk factors including polygenic AD risk, may prove informative.

Disclosures: M. Ramirez: None. **J.A. Rose:** None. **J.N. Sexton:** None. **A.M. Heller:** None. **C.J. Phipps:** None. **L. Behm:** None. **A. Zatkalik:** None. **A. Wilhelm:** None. **K. Nickolas:** None. **A.C. Maerlender:** None. **V. Phatak:** None. **D.L. Murman:** None. **D.E. Warren:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

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Program #/Poster #: PSTR369.11/UU28

Topic: H.04. Executive Functions

Support: NIA Grant R01 AG064247
Medical Student Summer Research Program - University of Nebraska Medical Center

Title: Associations between salience and frontoparietal intranetwork connectivity and executive function tasks in periadolescent children.

Authors: *S. MIRAJKAR[1], D. E. WARREN[2];
[1]Col. of Med., [2]Neurolog. Sci., Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Brain network organization varies between individuals, and individual differences in intrinsic brain networks have been linked to differences in cognitive ability. Individual differences in brain connectivity may be especially evident in brain networks that support executive functions such as cognitive control and attention such as the frontoparietal and salience networks. The frontoparietal network is theorized to play a role in cognitive control, which refers to the selection of thoughts and behaviors in context of task demands. Specifically, the frontoparietal network is necessary to flexibly coordinate behavior in a goal-driven manner. Meanwhile, the salience network has been hypothesized to modulate the relationship between the default mode network, which contributes to self-oriented cognition, and the frontoparietal network. Prior work suggests that the maturation of executive functions during development parallels the integration of the salience networks with the frontoparietal network. Additionally, increased frontoparietal and salience intranetwork connectivity has been associated with development from periadolescence to adulthood. However, associations between frontoparietal or salience intranetwork connectivity and executive functions abilities have not been tested in periadolescent children. Here we tested whether intranetwork connectivity of frontoparietal and salience networks was correlated with executive function measures in periadolescent children. Participants in the NIA-funded Polygenic Risk of Alzheimer's disease in Nebraska Kids (PRANK) study (N = 94) completed the NIH toolbox cognition battery as well as a functional MRI (fMRI) scan including resting-state data collection. Intranetwork connectivity was measured using the Human Connectome Project's connectome workbench software. Results of the data analysis indicated a positive correlation between scores on fluid cognition measures and frontoparietal intranetwork connectivity, $r(73) = .29$, $p = .01$. A positive correlation was also observed with flanker task scores and frontoparietal intranetwork connectivity, $r(73) = .25$, $p = .03$. Finally, we observed a positive correlation with salience intranetwork connectivity and age-adjusted dimensional card sorting task scores, $r(91) = .22$, $p = .03$. Our preliminary findings show an association of executive function measures with intranetwork connectivity of two key intrinsic functional networks, and they suggest that frontoparietal and salience intranetwork connectivity may reflect increased efficiency of cognitive control during development.

Disclosures: S. Mirajkar: None. **D.E. Warren:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.12/VV1

Topic: H.04. Executive Functions

Title: Impact of Cognitive Training on University Students' Cognitive Performance, A Comparative Analysis of Initial and Final Assessments.

Authors: *N. Y. CORTÉS[1], A. LARA-MORALES[1], C. R. VUELVAS-OLMOS[2], L. G. MARMOLEJO-MURILLO[1];
[1]Univ. of Guanajuato, Guanajuato, Mexico; [2]Univ. of Colima, Colima, Mexico

Abstract: Introduction: The cognitive abilities of university students play a crucial role in their academic success and overall cognitive functioning. With the increasing demands of higher education and the complex cognitive tasks involved, there is a growing interest in understanding and enhancing cognitive performance among university students. Cognitive training has emerged as a potential intervention to improve cognitive abilities and optimize academic outcomes. **Aim:** To investigate the impact of cognitive training on university students' cognitive performance. **Methods:** Quantitative research, with n= 28 university students. There were three phases: 1) Cognitive functions were assessed using the CogniFit-General Cognitive Assessment, 2) Cognitive training was carried out for three months using a mobile application. 3) Phase 1 was carried out again. **Results:** Participants exhibited significantly higher scores (comparing the initial evaluation with the final evaluation) among general reasoning, general memory, working memory, contextual memory, non-verbal memory, short-term memory, general attention, spatial perception, and estimation. **Conclusion:** The improvements observed in general reasoning, memory (both general and specific types), attention, spatial perception, and estimation. Theses finding suggest that cognitive training has the potential to modulate and enhance cognitive abilities, also improving academic success among university students. Each participant exhibited unique patterns of improvement; therefore, it is likely that personalized cognitive training programs may be more effective in improve cognitive abilities.

Disclosures: N.Y. Cortés: None. **A. Lara-Morales:** None. **C.R. Vuelvas-Olmos:** None. **L.G. Marmolejo-Murillo:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.13/VV2

Topic: H.04. Executive Functions

Title: Comparative Analysis of Cognitive Functions in Women Experiencing Intimate Partner Violence with and without Posttraumatic Stress Disorder

Authors: *C. VUELVAS-OLMOS[1], N. Y. CORTÉS-ÁLVAREZ[2], A. LARA-MORALES[2], L. G. MARMOLEJO-MURILLO[3];

[1]Univ. of Colima, Colima, Mexico; [2]Univ. of Guanajuato, Guanajuato, Mexico; [3]Univ. of Guanajuato, Leon, Mexico

Abstract: Comparative Analysis of Cognitive Functions in Women Experiencing Intimate Partner Violence with and without Posttraumatic Stress Disorder

AuthorsC.R. Vuelvas-Olmos[1], N.Y. Cortés-Alvarez[2], A. Lara-Morales[1]; L.G. Marmolejo-Murillo[3]. [1]University of Colima, Colima, Mexico, [2]Department of Nursing and Obstetrics, Division of Natural and Exact Sciences, University of Guanajuato, Guanajuato, Mexico, [3]Department of Medicine and Nutrition, Division of Health Sciences, University of Guanajuato, Mexico

DisclosuresC.R. Vuelvas-Olmos: None, **N.Y Cortés-Alvarez:** None, A**. Lara-Morales**: None, **L.G. Marmolejo-Murillo**: None

AbstractIntroduction: The neuropsychological functioning of women who experience abuse and suffer from posttraumatic stress disorder (PTSD) is emerging as an important area of research. However, there are few studies that specifically address cognitive functioning in this population. Furthermore, PTSD is associated with reductions in hippocampal volume, medial frontal activity, and amygdala function, which can influence cognitive processes. **Aim**: To compare the cognitive functions in women experiencing intimate partner violence with and without posttraumatic stress disorder. **Methods:** We included women divided into three groups: 1) victims of physical abuse by an intimate partner with PTSD, 2) victims of physical abuse by an intimate partner without PTSD and 3) women without history physical abuse. In case of victims of physical abuse: who attended a Violence Care Center and who had been released from their abusive relationship for at least 4 weeks (but not more than 2 years) before their participation in the study. The Posttraumatic Stress Disorder Checklist for DSM-5 and the Impact of Event Scale-Revised were applied. The participants were divided into two groups: and without PTSD, both groups were applied. Cognifit® Neuropsychological Battery. **Results:** The analysis showed that the General function score was significant higher between women without history physical abuse compared to other groups. Also, victims of physical abuse by an intimate partner with PTSD evidenced lower significant score among attention, cognitive flexibility, reasoning, coordination, memory, and perception compared to victims of physical abuse by an intimate partner without PTSD. **Conclusion:** Therefore, this study highlights the cognitive impairments experienced by women who have been victims of physical abuse, with and without PTSD. The findings underscore the importance of addressing cognitive deficits in therapeutic interventions and support services for this population.

Disclosures: C. Vuelvas-Olmos: None. **N.Y. Cortés-Álvarez:** None. **A. Lara-Morales:** None. **L.G. Marmolejo-Murillo:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.14/VV3

Topic: H.04. Executive Functions

Support: CFREF VISTA

Title: No impact of frequent use or acute use of cannabis on cognitive-and visuomotor function.

Authors: *D. HENRIQUES[1], K. KOLPASHNIKOVA[2], H. A. CLAYTON[1], A. AL-BAYATI[2], R. CORTEZ[2], S. DESAI[3], B. M. 'T HART[2];
[2]Ctr. for Vision Res., [3]Arts Media Performance and Design, [1]York Univ., Toronto, ON, Canada

Abstract: Since the legalization of recreational use of cannabis took effect in Canada, many questions have been brought forward regarding its immediate and sustained effect on daily performances. To investigate the effect of cannabis on various cognitive functions, we created an online battery of well-established, cognitive and motor tasks which we have run on over 700 high-functioning young adults (over the age of 19, average age of 22). Here, we will describe results of reported frequency of cannabis use, as well as the immediate effect of cannabis on seven common tasks used to investigate attention (visual search), working-memory (N-back), impulsivity (No-Go), executive function (Task switching), motor acuity and visuomotor mapping (Mirror reversed reaches). Our first set of findings indicate that frequent users (N>100), infrequent users (N>100), and non-users(N>200) performed similarly on all the tasks (no evidence of differences using Bayesian statistics). These results suggest that frequent cannabis-use in young adults is not associated with visual-cognitive and motor impairments. Surprisingly, even when 'high' with their typical, personal dose of cannabis, we again find users (N>30) show no substantial impairment on these same tasks. Our results suggests that cannabis shows no effect on cognitive and motor function in high-functioning young adults.

Disclosures: D. Henriques: None. **K. Kolpashnikova:** None. **H.A. Clayton:** None. **A. Al-Bayati:** None. **R. Cortez:** None. **S. Desai:** None. **B.M. 't Hart:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.15/VV4

Topic: H.04. Executive Functions

Support: Psychology Research Opportunity Programs
CSORDA Pilot Grant

Title: Effects of repeated opioid oral self-administration on cognition/learning and behavior in a rat model.

Authors: *V. CHANCHYKOV[1], T. LUGO[1], P. J. KENNEDY[1], M. S. FANSELOW[1], C. ZUVIA[3], G. R. POE[2];
[1]Dept. of Psychology, [2]Dept. of Integrative Biol. and Physiol., UCLA, Los Angeles, CA; [3]Dept. of Molecular, Cell, and Systems Biol., Univ. of California, Riverside, Riverside, CA

Abstract: Background: Chronic opioid use leads to numerous devastating side effects, ranging in severity from drowsiness and nausea to dependence and overdose. A significant part of the problem lies in the lack of understanding about the effects opioids have on brain regions that mediate higher cognitive processes, such as the hippocampus. Most studies of animal models of opioid use disorder directly inject the drug. While this paradigm is useful in informing us about the pharmacological effects of opioid exposure, spontaneous free consumption behaviors likely also affect learning. Methods: Long Evans rats (n = 4) were given the choice to orally consume oxycodone via a two-bottle choice paradigm for 24 hours over 14 days, where one bottle contained water with oxycodone (0.1 mg/mL), and another only water. The effects of oxycodone on learning and memory were assessed using behavioral tests conducted before and after the 14 days of oxycodone consumption. Object Location Memory (OLM) and Novel Object Recognition (NOR) tasks were analyzed. The OLM test was conducted to assess the rats' use of hippocampal-learning strategies, where rats were required to distinguish between the object that was moved to a novel place and the object that was left in a familiar place. The NOR test assesses nonspatial memory, discriminating between a familiar and a novel object. Results: Rats consumed oxycodone orally without the use of adulterants such as sugar. Oxycodone consumption and withdrawal worsened OLM, showing impairment in hippocampal learning strategy use. In contrast, rats performed better in the NOR test after drug consumption, indicating that hippocampus-independent memory remained intact. Conclusion and future directions: The findings suggest that chronic opioid use deteriorates hippocampus-based learning and memory and reliance on intact competing learning strategies. We will next turn to sleep interventions that improve hippocampus-dependent learning to develop targeted interventions that mitigate the negative effects of chronic opioid use on learning and memory processes.

Disclosures: V. Chanchykov: None. **T. Lugo:** None. **P.J. Kennedy:** None. **M.S. Fanselow:** None. **C. Zuvia:** None. **G.R. Poe:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.16/VV5

Topic: H.04. Executive Functions

Title: Long-term effects of chronic opioid exposure on sleep architecture and cognition

Authors: *T. LUGO[1], V. CHANCHYKOV[1], P. J. KENNEDY[1], M. S. FANSELOW[1], G. R. POE[2];
[1]Psychology, [2]Dept. of Integrative Biol. and Physiol., UCLA Chapter, Los Angeles, CA

Abstract: Background: Recent studies have shown that locus coeruleus (LC) inactivity during REM sleep is important for the consolidation of hippocampus dependent memories. The LC is active during stress response and enables rapid learning. The LC is tonically active at all times except REM sleep when it is actively inhibited. LC activity bursts reliably awaken animals from

sleep. Endogenous and exogenous opiates quell LC activity and long-term opiate exposure (>3 days) downregulates opiate receptors in LC neurons. Opiate withdrawal thus renders the LC hyperactive. Such LC hyperactivity in drug dependent individuals may induce abnormal LC activity during REM sleep and could explain the sleep disturbances and memory deficits seen in drug-dependent individuals. We tested the learning and memory deficits of substance use with oxycodone administration and two learning systems, hippocampal and procedural learning, in male and female rats. **Methods:** Long-Evans (n = 8) rats were tested on a hippocampus-dependent test (object location memory (OLM)), and a nonspatial memory test, (novel object recognition (NOR)). A marble burying task assessed anxiety-like behavior and a procedural T-maze task assessed striatal learning, for 15 trials/day across 3 days. All behavioral tasks were conducted before and after 7 days of oxycodone i.p injections (3 mg/kg/d) with the exception of the T-maze task which was conducted only after oxycodone withdrawal. **Results:** In the OLM task, exploration of the object placed in the novel location significantly decreased after oxycodone withdrawal, indicating an impairment in rats' hippocampal-learning strategy use. Exploration of the novel object remained significantly higher than exploration of the familiar object; thus performance in the NOR task remained unaffected. Rats also buried more marbles after withdrawal, indicating increased anxiety-like behavior. Oxycodone-treated females improved more on day 2 of the T-maze task compared to males, suggesting a faster pivot to procedural-learning strategies in females. **Conclusion:** Previous studies indicate that REM sleep deprivation impairs performance in the OLM task but not in the NOR task. It is possible that sleep disturbances associated with opioid use withdrawal may explain why we see poor performance in hippocampal but not striatal memory. Ongoing experiments will address the role of opioids on locus coeruleus activity and sleep disturbance, and those effects on different learning and memory systems.

Disclosures: T. Lugo: None. **V. Chanchykov:** None. **P.J. Kennedy:** None. **M.S. Fanselow:** None. **G.R. Poe:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.17/Web Only

Topic: H.04. Executive Functions

Title: Transdiagnostic assessment of neurocognitive impairment in youth

Authors: *E. ZOUPOU[1], T. M. MOORE[1], C. J. SCOTT[1,2], M. E. CALKINS[1,3], R. E. GUR[1,3], R. C. GUR[1,3];
[1]Psychiatry, Univ. of Pennsylvania, Philadelphia, PA; [2]Corporal Michael J. Crescenz VA Med. Ctr., Philadelphia, PA; [3]Children's Hosp. of Philadelphia, Philadelphia, PA

Abstract: Background: Research has shown that neurocognitive dysfunction occurs across psychiatric disorders, such as schizophrenia (Reichenberg, 2010) and mood (Merikangas et al.,

2017), and transdiagnostic symptom dimensions (Chavez-Baldini et al., 2023; Zhu et al., 2019). However, the relationships among broad sets of cognitive domains and symptom dimensions are yet to be elucidated. This study aims to evaluate relationships among domains of cognition and dimensions of psychopathology in a large youth cohort.**Method**: The sample (N = 9,350; age 8-21 years) was drawn from the Philadelphia Neurodevelopmental Cohort (Calkins et al., 2015). Factor analyses on data from a structured clinical interview (Calkins et al., 2015) resulted in 6 symptom domains: anxiety/fear, dysphoria, obsessive-compulsive (OC), attention deficit hyperactivity (ADH), externalizing, psychosis, and an overall psychopathology (P) factor score. Cognitive data from the Penn Computerized Neurocognitive Battery (Gur et al., 2010) were aggregated into 5 domains based on previous work (Moore et al., 2015): executive function, memory, complex cognition, social cognition, sensorimotor speed. Cognition was modeled using a correlated-traits confirmatory factor analysis (CFA) of scores; psychopathology was modeled using CFA with a bifactor configuration. Effects of interest were the relationships between clinical and cognitive factors. All analyses covaried for age, sex, socioeconomic status.**Results**: Cognition and psychopathology domains showed both factor-specific and overall significant associations and model fit was acceptable (CFI = .91; TLI = .90; RMSEA = .024; SRMR = .054). Most clinical factors were negatively associated with complex cognition ($p < .01$) but dysphoria and OC showed positive associations ($p < .05$). Performance in all cognitive domains was negatively associated with anxiety ($p < .001$), ADH ($p < .001$) and psychosis ($p < .05$). Overall P showed a negative association with complex cognition ($p < .001$) but not with other cognitive domains.**Conclusion**: Consistent with prior research (White et al., 2017) all clinical dimensions were associated with reduced neurocognitive functioning in at least one domain. However some symptom domains like Dysphoria were associated with better performance. The P factor was only associated with complex cognition after accounting for other psychopathology dimensions. Notably, previous studies have shown a positive association between the anxious-misery clinical dimension and cognition. Our study extends these findings by parsing this relationship showing that dysphoric mood is associated with better complex cognition performance.

Disclosures: E. Zoupou: None. **T.M. Moore:** None. **C.J. Scott:** None. **M.E. Calkins:** None. **R.E. Gur:** None. **R.C. Gur:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.18/VV6

Topic: H.04. Executive Functions

Title: Deficits in executive functioning in young women with high adiposity is associated with dietary intake of micro and macronutrients

Authors: *M. SOLIS-ORTIZ;
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Abstract: Deficits in cognitive functions dependent on the prefrontal cortex related to the dietary intake of nutrients and adiposity in young individuals are not well known. The objective of this study was to examine the effects of high adiposity on executive functioning and their relationship with dietary intake of macro and micronutrients in young women. Ninety five young women were categorized based on body fat percentage. The study included 42 women with normal adiposity and 53 women with high adiposity. Executive functions, sustained attention, selective attention, category formation, cognitive flexibility, and verbal fluency test scores were obtained to assess executive functioning. Dietary intake of macro and micronutrients was measured using three 24 h recalls and correlated with the test scores. The high adiposity group was characterized by deficits in executive function, category formation and cognitive flexibility, poor sustained and selective attention, and less verbal fluency. Executive functions were negatively correlated with saturated fat and positively correlated with cholesterol and carbohydrates. Category formation was negatively correlated with saturated fat and vitamin E. Sustained attention was positively correlated with lipids, carbohydrates, and cholesterol. Long reaction times in the selective attention test were positively correlated with unsaturated fat and negatively correlated with vitamin C. Cognitive flexibility test scores were negatively correlated with vitamin E. Cholesterol, vitamin C, and vitamin E were predictors of executive functioning in the high adiposity group. These findings suggest that impairments in executive functioning may predispose young women to overconsumption of unhealthy nutrients that consequently induces obesity.

Disclosures: M. Solis-Ortiz: None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR369.19/VV7

Topic: H.04. Executive Functions

Support: NIMH Grant R01MH122613

Title: Connectivity perdictive modeling of thalamocortical diaschisis

Authors: *X. CHEN[1], J. JIANG[1], J. E. BRUSS[2], A. D. BOES[2], K. HWANG[1];
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Abstract: The thalamus serves as a crucial hub in the central nervous system, connecting various cortical regions through thalamocortical connectivity. It is hypothesized that localized thalamic lesions can indirectly affect cortical function through these pathways, a phenomenon known as thalamocortical diaschisis. Studies suggest thalamic lesions can result in global neuropsychological deficit associated with hypometabolism of glucose or oxygen in ipsilateral cerebral cortex (Baron, 1989; Baron et al., 1992). Our study aims to develop a predictive,

quantitative model of thalamocortical diaschisis, using cognitive control as the cognitive system for prediction. We assessed cognitive control using the Trail-making task and calculated the difference in time taken to complete the Trail B and Trail A tasks. We implemented a connectome-based predictive analysis to evaluate how thalamocortical disconnection patterns could predict the impact of thalamic lesions on cognitive control. The key rationale of our model being that focal thalamic lesions disrupt thalamocortical connectivity to yield similar behavioral impairments as observed in patients with lesions of the connected cortical areas. To test this, we first used normative connectome data from healthy adults to generate functional connectivity maps of cortical regions (N=360). We then estimated the dysconnectivity from brain lesions by summating the functional connectivity weights within each lesion volume. The model first learned patterns of disconnection between cortical regions that maximally predict cognitive control performance (N=861). We then tested the hypothesis that thalamic lesions strongly connected to this 'cognitive control' cortical network should produce similar behavior deficits (N=21). We multiplied the learned weights of the cortical 'cognitive control' network with each thalamic patient's thalamocortical dysconnectivity pattern to predict their cognitive control performance. Our results showed a prediction accuracy of 0.41 (AIC = -10.14, 95% CI [-11.40, -8.87]), which significantly outperformed null models that assume no systematic relationship between the thalamus and cortical regions (ΔAIC = -63.16, $p = 1.93 \times 10^{-14}$). In conclusion, this approach of using network connectivity from cortical lesions, which are more common, to predict deficits from thalamic lesions, which are rarer, provides a novel framework for inferring the functional neuroanatomy of the thalamus that can be applied to other cognitive domains in the future.

Disclosures: **X. Chen:** None. **J. Jiang:** None. **J.E. Bruss:** None. **A.D. Boes:** None. **K. Hwang:** None.

Poster

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Topic: H.04. Executive Functions

Support: DFG grant awarded to the Graduiertenkolleg 1957 "Adipocyte-Brain Crosstalk."

Title: Interplay between the amount and distribution of white adipose tissue and brain structure

Authors: *L. OKUDZHAVA[1], M. HELDMANN[1], E. FISCHI-GOMEZ[2], G. GIRARD[2], J. MACHANN[3], J.-P. THIRAN[2], T. MÜNTE[1];
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Abstract: Obesity represents a significant public health concern and is associated with structural alterations in the brain. Previous research indicates that an elevated body mass index (BMI) is linked to changes in white matter (WM) connectivity in regions involved in inhibitory control, reward and emotion processing, and sensory integration. However, due to the indirect nature of BMI as a measure of adiposity, a more comprehensive evaluation of body composition is required. Additionally, investigating how different fat depots communicate with the brain is crucial, given the strong association between visceral and liver fat and metabolic dysfunctions. This study aims to enhance our understanding of the relationship between obesity and WM connectivity by directly assessing white adipose tissue amount and distribution. The study includes 63 metabolically healthy males aged 24 to 61 years across normal-weight, overweight, and obese categories. Whole-body magnetic resonance imaging (MRI) was performed to evaluate total, visceral, and subcutaneous fat, while MR liver spectroscopy measured liver fat content. Diffusion-weighted brain imaging was used to assess WM connectivity. Connectome-based predictive modeling was employed to investigate the relationship between WM connectivity and quantified fat measures. Notably, BMI showed a positive association with WM connectivity in the frontal pole, amygdala, middle frontal gyrus, medial orbitofrontal cortex, insula, and rostral anterior cingulate ($p=0.005$). Additionally, increased connectivity was observed in a network related to total body fat, including the frontal pole, middle frontal gyrus, medial orbitofrontal cortex, insula, accumbens, and rostral anterior cingulate ($p=0.001$). No significant networks were found in relation to visceral, subcutaneous, or liver fat. These findings suggest that elevated BMI and total body fat are linked to changes in WM connectivity in regions critical for cognitive processes, emotional regulation, and reward perception. Impaired neural communication between these brain regions may affect cognitive and affective functions in individuals with obesity, potentially contributing to weight gain. The limited sensitivity of brain connectivity measures to regional fat distributions suggests the potential for other brain measures, such as functional connectivity or gray matter volumetry, to capture the impact of these fat depots on brain alterations. Further research is needed to delineate the relationship between brain structure and body composition and develop targeted interventions for addressing obesity and its neurological implications.

Disclosures: L. Okudzhava: None. **M. Heldmann:** None. **E. Fischi-Gomez:** None. **G. Girard:** None. **J. Machann:** None. **J. Thiran:** None. **T. Münte:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

Location: WCC Halls A-C

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Program #/Poster #: PSTR369.21/VV8

Topic: H.04. Executive Functions

Support: NIH Grant R01DA021421

Title: Length of abstinence and neurocognitive functioning. A comparative analysis in opiate and stimulant users

Authors: *E. PSEDERSKA[1], K. BOZGUNOV[2], D. NEDELCHEV[2], G. VASILEV[2], J. VASSILEVA[3];
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Abstract: Chronic drug use is associated with impairments in cognitive functioning, most notably in the domains of decision-making and response inhibition. Opiates and stimulants have unique effects and modulate distinct neurotransmitter systems, leading to substance-specific neuroadaptations within brain regions crucial for these functions. Although abstinence is widely recognized as essential for cognitive recovery due to the brain's neuroplasticity, the extent of the brain's recovery of function with abstinence remains unclear. Studying the relationship between neurocognitive performance and length of abstinence in opiate (OU) and stimulant users (SU) offers insights into the neurocognitive consequences of addictions to different classes of drugs and the potential for recovery over time. We examined 45 OU and 53 SU in early abstinence [0-12 months], 68 OU and 70 SU in protracted abstinence [>12 months], and 68 control participants with the Iowa Gambling Task (IGT) to measure decision-making under ambiguity, the Cambridge Gambling Task (CGT) to measure decision-making under risk, the Monetary Choice Questionnaire (MCQ) to examine delay discounting, and the Stop Signal Task (SST) to index response inhibition. All substance-using participants were "pure" (e.g., mono-dependent) opiate or stimulant users. Significant group differences were found in decision-making under ambiguity (IGT), with control participants demonstrating better decision-making compared to both early abstinence groups ($p = .005$). In contrast, only OU in early abstinence exhibited disadvantageous decision-making under risk (CGT) ($p = .012$), along with increased delay discounting (MCQ) ($p = .025$). With regards to response inhibition, both groups of stimulant users, regardless of length of abstinence, were characterized by reduced response inhibition efficiency compared to control participants and OU in early and protracted abstinence. Regression analyses, controlling for demographic variables and fluid intelligence, revealed that length of abstinence significantly predicted quality of decision-making under risk specifically for OU ($\beta = .285$, $p = .005$). Overall, our findings underscore the substance-specific nature of neurocognitive impairments and suggest that neurocognitive function may not fully recover even with protracted abstinence, which requires the development of personalized relapse prevention and cognitive remediation programs targeting the key neurocognitive functions impaired by chronic opiate and stimulant use.

Disclosures: E. Psederska: None. **K. Bozgunov:** None. **D. Nedelchev:** None. **G. Vasilev:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Bulgarian Addictions Institute. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bulgarian Addictions Institute. **J. Vassileva:** None.

Poster

PSTR369. Effects of Executive Functions on Learning and Memory

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Topic: H.04. Executive Functions

Support: NIH P01 HL040962
NIH R01 HL 1089850

Title: Opposing engagement of cerebellar and basal ganglia networks with shifts of cortical network topology

Authors: *K. NESTOR[1], J. RASERO[2], R. BETZEL[3], A. GERLACH[4], P. J. GIANAROS[5], T. D. VERSTYNEN[2];
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Abstract: The brain is a dynamical network with a highly flexible topology, shifting from integrated to segregated network states depending on task demands (Shine & Poldrack, 2018). Integrated topology means that regions are highly interconnected, facilitating information flow across communities, while segregated topologies isolate information flow to largely within communities. The basal ganglia and cerebellum are hypothesized to be driving forces in shifting cortical topology from integrated to segregated states respectively, leading to pliable cortical network topologies that allow for flexibility in information processing (Shine, 2021). To test this hypothesis we used dynamic functional connectivity on fMRI data while humans (N = 242) performed block designed adaptive Stroop and MSIT tasks (Gianaros et al., 2017). We applied an edge time series analysis (Zamani Esfahlani et al., 2021), using k = 268 parcels of functional regions (Shen et al., 2013) and measured cortical topology using the modularity index, which provides a quantitative value of segregation of cortical networks. Eigenvector centrality was used to determine the engagement of subcortical regions basal ganglia and cerebellum over time. Using a general linear model (GLM), we observed that cortical topology was highly flexible, shifting between integrated and segregated states as participants shifted from task to rest states, as well as a slight decrease in modularity during the more complex task blocks. There was decreased modularity in incongruent blocks ($\beta = 0.072$; 95% CI = 0.028, 0.116), indicating more integration, and increased modularity during congruent blocks ($\beta = 0.128$; 95% CI = 0.081, 0.175), indicating more segregation. Additionally, we determined a significant difference between cortical modularity within the task conditions ($p = 0.014$). Engagement of the subcortical regions were time locked within task blocks: basal ganglia engagement increased in early block periods, while cerebellar engagement increased at the end of task blocks. A cross correlation analysis between the cortical modularity and subcortical eigenvector centrality values showed that only the basal ganglia engagement preceded shifts in cortical modularity, consistent with a control effect. Cerebellar engagement was coincident with increases in task modularity. Our results give partial support to the Shine (2021) hypothesis, consistent with the idea that the basal ganglia drives a decrease in modularity, but ultimately indicate a more nuanced association between subcortical regions and cortical topology.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.01/Web Only

Topic: H.06. Social Cognition

Support: MEXT KAKENHI 22H05220

Title: Joint Simon effects were correlated with the scores of neuroticism

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Abstract: The Joint Simon Effects (JSE) is a psychological phenomenon used to indicate the cognition perception of the presence near an individual. It can be measured through a joint go- and no-go task designed for two individuals, commonly called the Joint Simon task. An incongruent Go signal on the partner's side resulted in delayed reaction times (RTs) compared to when the signal was on the participants' side (congruent condition), known as the JSE. The JSE is associated with various social emotions experienced with a partner, but the underlying mechanisms that modulate the JSE through social emotions are not precise. This study utilized a dataset comprising the JSE and personality traits associated with social emotions to explore the psychological mechanisms of JSE modulation. A total of 30 Japanese participants (15 women; aged 18-49 years) with no history of neuromuscular disorders participated in the experiments. Written informed consent was obtained before the experiments. The local research ethics committee approved the study protocol per the Declaration of Helsinki. During experiments, participants were asked to sit on the chair on the right side facing the table. We designed the single and joint Go- or No-Go tasks. In the single condition, only a participant sits on the chair and performs the Go- or No-Go task using the right response button. In the joint condition, an experimenter was present and responded on behalf of the participant during the No-Go trials using a left button. A total of 256 Go, and No-Go cues for participants were presented on the right (congruent) and left (incongruent) to a center fixation cross. For personality assessments, we also obtained the questionnaire of the Japanese version of ten item personal traits inventory (TIPI-J) from all participants. Finally, we calculated Spearman's correlation coefficients between five traits and JSE. Consequently, the JSE in both conditions was significantly and negatively correlated with the neuroticism score (*rho* = -0.279, Joint condition; -0.361, Single condition). Our finding suggested neuroticism, an essential personality trait for regulating emotion, should share common neural mechanisms with the Joint Simon effect. Interestingly, previous reports demonstrated both the magnitude of the Joint Simon Effect and neuroticism were associated with the regional volumes in the medial prefrontal cortex (mPFC). Thus, the Joint Simon effects have the potential to serve as a shared psychological marker for various communicative disorders related to mPFC functions, including social anxiety.

Disclosures: S. Irie: None. **A. Tachibana:** None. **A. Matsuo:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR370.02/VV10

Topic: H.06. Social Cognition

Support: Grant-in-Aid for Young Scientists: 20K20155

Title: Spatio-temporal dynamics of calculation-related high-gamma modulation; an electrocorticography study

Authors: *Y. NAKAI, S. OE, H. NISHIBAYASHI, N. NAKAO;
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Abstract: During mathematical operations, the inferior parietal lobe, precentral gyrus, superior parietal lobe, supramarginal gyrus, and middle temporal gyrus on the dominant side were activated in the previous fMRI studies. We determined the spatio-temporal dynamics of calculation-related neural activity by intracranially measuring high-gamma modulation during visual and auditory arithmetic tasks. We studied three patients (168 artefact-free non-epileptic electrodes) with intractable focal epilepsy who underwent chronic extraoperative electrocorticography recording as part of presurgical evaluation. For each electrode, anatomical labelling was performed by using FreeSurfer on individual MRI data. Time-frequency analysis was performed to determine cortical activation and deactivation at given moments. Augmentation and attenuation of high-gamma activity at 70-110Hz was treated as summary measures. We measured high-gamma activity when the patients performed the arithmetic tasks involving addition and subtraction with and without carry and borrow using visual and auditory presentations, and when they overtly answered the questions. Immediately after the auditory presentation of the first number stimuli, high-gamma augmentation was elicited in the superior-temporal and pre-central gyri. High-gamma activity was augmented in the supra-marginal gyrus 900 ms after the auditory presentation of the second number stimuli. High-gamma augmentation involved in the rostral- and caudal-middle frontal gyri 300ms after the end of auditory presentation. We found that greater high-gamma activity was augmented in the supra-marginal gyrus during the auditory task with carry than the one without carry or the visual task with carry. Also, high-gamma activity was augmented in pre-central gyrus during the task with carry rather than the one without carry. Our preliminary results provided the different dynamic cortical activity with an excellent temporal resolution during visual and auditory arithmetic tasks. The result of the time-frequency analysis may support that the supra-marginal gyrus plays an executive role during the calculation task and the neural activation in the pre-central gyrus reflects the working memory function.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR370.03/VV11

Topic: H.06. Social Cognition

Title: Intra and interpersonal neural connectivity correlates of dynamic context-related changes in infant explore-exploit behaviour

Authors: *P. SANTHANAKRISHNAN[1], P. CHOCKALINGAM[1], A. V. DEVARAJAN[1], S. GEORGIEVA[1], L. SANTAMARIA[1], V. LEONG[1,2];
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Abstract: Introduction: The 'A-not-B' error is a robust and well - replicated phenomenon where repeatedly retrieving an object from a location (A) biases infants to search the same location despite observing the object being hidden at another new location (B). This perseverative error can also be framed in terms of a cognitive flexibility - stability trade off: repeated exposure to location A results in greater activation and resistance for its working memory representation, but antagonises that of location B, hence facilitating stable (exploitative) behaviour at the expense of flexible switching (exploratory) behaviour. In this study, we examine the effects of social influences, specifically mother - infant interactions on modulating the flexibility-stability trade off in infants. **Methods:** (N = 49) mother-infant dyads (mean age of infants: 16.2 months) completed a naturalistic A-not-B task where mothers delivered the stimulus (hiding of the toy) in an interactive responsive manner to their infants whilst their neural activity was concurrently acquired via dyadic-EEG. Intrabrain and interbrain connectivity in the 6 - 9 Hz (infant Alpha) and the 3 - 5 Hz (infant Theta) ranges was computed using the weighted phase lag index (wPLI), comparing no-switch (i.e., A-A) and switch (i.e., A-B) trial contexts. **Results:** Preliminary analyses suggest that in no-switch contexts where the target location does not change, *intra*infant Alpha neural connectivity is associated with successful object retrievals ($p = 0.014$). By contrast in switch contexts where there is a change in target location, mother-infant Theta *inter*personal neural connectivity is associated with infant searching at the previous target location ($p = 0.048$). We plan to further define the neurocomputational mechanisms underlying this effect using a computational neural network model with attractor dynamics to fit mother-infant behavioural data. **Conclusion:** Here, we assess how moment-to-moment changes in infant explore-exploit behaviour, i.e., flexibility-stability trade off, are reflected in neural connectivity dynamics. We find that intra- and interpersonal connectivity capture complementary situational aspects of infant behaviour in no-switch and switch contexts respectively. Specifically, social influences (indexed through mother-infant connectivity) may modulate infant explore-exploit behaviour more strongly in contexts of novelty or change (i.e., switch trials).

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.04/VV12

Topic: H.06. Social Cognition

Support: 1R01MH119430-01 (PI JH)

Title: Neural responses to eye-to-eye contact predict ados scores of individuals with asd using support vector machine learning

Authors: *X. ZHANG[1], **J. NOAH**[2], J. HIRSCH[4,3,5,6];
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Abstract: Introduction: Social deficits in autism spectrum disorder (ASD) have been linked to the reluctance of eye contacts. The brain activity related to the eye contact is hypothesized to reveal neural mechanisms associated with ASD. We have recorded functional near infrared spectroscopy fNIRS, during eye contact task for 17 adults with ASD and 19 Typically Developed, TD, adults to investigate the patterns and magnitude of brain activity related to ASD diagnosis as well as the severity of ASD as measured by the Autistic Diagnostic Schedule. 2nd Edition, ADOS, scores. We adopted an SVM approach with performance inflation controlled. **Methods: 1.** Task: the subject and a lab partner were seated face to face approximately 140 cm apart. A tone cued both subjects to either look at each others eyes or look at an LED light diverted by 10° to the left or the right [1]. **2.** Signal: Both the oxy and deoxy hemoglobin signals were recorded with a Shimadzu fNIRS system. The derivedbrain activity from both signals, Hbdiff, were used in the GLM analysis and beta values for the channels were projected onto the brain surface. **3.** SVM: The beta value surfaces were converted into 36 principal components (PC). Those PCs were sorted based on the t-tests between the two groups, ASD and TD. The feature selection of SVM determined the number of PCs used in the SVM. Nested cross validation was adopted so that the testing data were not involved in feature selection. One thoushand random fNIRS inputs were used for calculating the p value of the SVM. **Results:** When trained with fNIRS data and the diagnosis, SVM predicted ASD with accuracy of 80.5% and <0.4% p value using the nested leave one out cross validation. Further, a correlation of 0.72 was observed between the individual measured ADOS scores and SVM predicted ADOS scores (a variable not trained on) for the eye-to-eye condition, and was not significant for the video viewing condition. Findings demonstrate that neural responses during eye-to-eye contact predict ADOS scores, a gold standard indicator of symptom severity. **Conclusion:** We show that SVM approaches can be successfully applied to predict ADOS scores for individuals with ASD providing an additional quantitative measure of clinically determined symptom severity. Further,

findings also highlight the potential role of neural systems that are responsive to live eye-to-eye contact as an indicator of ASD.

Disclosures: X. Zhang: None. **J. Noah:** None. **J. Hirsch:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Topic: H.06. Social Cognition

Support: NIH Grant 1R01MH119430-01

Title: A pilot investigation using fNIRS and TMS to detect and disrupt visual processing of live faces

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Abstract: Background. Face-to-face interaction is a primary ecological context for human social behavior. Previous investigations of live face-to-face processing using functional near-infrared spectroscopy (fNIRS) have identified neural activity in lateral and dorsal aspects of the posterior superficial cortex (1-4) including supramarginal (SMG) and angular gyri as well as the superior parietal lobe (SPL). These findings developed a model of Interactive face processing consisting of dissociable pathways for dyadic face interaction. To further investigate this model of how dynamic face information, including eye contact, is processed in these pathways, we applied transcranial magnetic stimulation (TMS) to the SPL in healthy individuals. **Methods.** We recorded fNIRS and eye-tracking data from 4 typically developed adult participants using a Shimadzu LABNIRS and Tobii x3 eye-tracker, respectively. Participants engaged in a live face viewing paradigm, making face-to-face contact with either live human or dynamic robot partners. Timing of viewing epochs was controlled by transparency-modulated 'smart glass' in a blocked design. After initial pre-stimulation behavioral and neural recordings, we applied continuous Theta-Burst Stimulation (cTBS) TMS to the SSAC with 600 pulses at 50 Hz with an inter pulse interval of 200 ms. Following stimulation, participants repeated the same face-viewing paradigm. Raw data were processed using SPM-NIRS, applying band-pass filtering (0.01-0.10 Hz) and removing global components. Event-triggered averages were calculated, and a general linear model (GLM) analysis was performed using standard hemodynamic response function responses provided in SPM8. Eye-tracking data, recorded at 120 Hz, was processed to determine dwell time on face, number of fixations, and individual fixation duration before and after TMS. **Results.** Prior to cTBS, neural responses in the SMG were observed during direct eye-to-eye interaction with human partners, but not when interacting with the robot, consistent

with previous findings. Following cTBS activity in the same region during direct eye contact decreased in all participants. **Conclusion.** We show that cTBS stimulation of the SPL disrupts neural responses during direct eye-to-eye interaction as predicted by the interactive face model. Gaining a better understanding of this pathway could contribute to our understanding of flat affect and eye gaze differences in individuals diagnosed with ASD, SZ, and social anxiety. **Refs.** 1. Hirsch, J, et al., Plos one 2022. 2. Dravida, S, et al., Neurophotonics 2019. 3. Dravida, S, et al., Front. Hum. Neurosci. 2020. 4. Noah, JA, et al., Front. Hum. Neurosci. 2020.

Disclosures: J.A. Noah: None. **X. Zhang:** None. **M. Tiede:** None. **D. Ostry:** None. **J. Hirsch:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Topic: H.06. Social Cognition

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NRF-2019R1A5A2026045

Title: personality and individual variation in brain volume

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Abstract: The individual tendency to think, feel and behave affects the brain over a long period of time. Notably, the five-factor model is one of the strongest personality theories, including agreeableness, openness to experience, conscientiousness, neuroticism, and extraversion. Though these personality traits are known to be concerned on affective, cognitive and behavioral process, each would have different importance on those processes. In this study, we investigated what personality traits affect brain volumes differently and how such effects are related to individual variation in brain volumes. For this purpose, we associated brain volume variation with personality traits using Human Connectome Project S1200 release dataset. We used structural MRI data of 1080 participants from the Human Connectome Project S1200 release dataset (age: 28.78±3.70, male = 495). To measure the regional brain volumes, voxel-based morphometry analysis was performed with SPM12 VBM-DARTEL procedure. The preprocessing procedure included manual reorientation to the anterior commissure, gray matter segmentation, creation of study-specific template, spatial normalization with DARTEL template, modulation to adjust for volume signal change during spatial normalization, and spatial smoothing. After the preprocess, we extracted regional gray matter volume by averaging the values of each brain region defined using the Schaefer 200 atlas. The personalities were assessed by NEO Five-Factor Inventory. To

examine the personality trait effect on variation in the brain volumes, we regressed brain volumetric variation on personality traits, which is known as the multivariate distance matrix regression. The multivariate regression is statistically significant, resulting in four significant personality predictors, agreeableness, openness to experience, conscientiousness, and neuroticism. Neuroticism is the most powerful predictor of those while openness to experience appeared the weakest variable explaining the cortical volumes. Lastly, extraversion is non-significant predictor of the brain structural volume variation. Since personality is a trait difficult to change and is entangled with cognitive, emotional, and behavioral process, we hypothesized the personality effect on brain volumes of healthy adults. Also, there will be the relative importance given that personalities reflect other tendencies. The finding suggests that neuroticism can explain individual variation in 200 cortical volumes more than agreeableness, openness to experience, and conscientiousness.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.07/VV15

Topic: H.06. Social Cognition

Support: National Institute of Mental Health(1R01MH116252)

Title: Neural representations of coarse- and fine-grained social knowledge during social learning

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Abstract: Social interactions in humans hinge on employing and acquiring knowledge about others, for example of their preferences for items. Humans employ relatively effective learning strategies that incorporate coarser or fine-grained social knowledge. They may also shift efficiently between these representations to adapt to task demands. Coarser-grained social representations allow rapid learning and generalization whereas fine-grained representations offer the opportunity to fine-tune predictions.
In a previous fMRI study, we asked adults (N=21) to rate the preferences of three peers for a total of 120 items belonging to different main categories (e.g., activities and foods) and respective subcategories (e.g., activity subcategories: arts and crafts, music, sports etc.). Participants received feedback about the peers' preferences on each trial. We found that participants reduced prediction errors over time, and they relied on a Rescorla-Wagner learning model to update their estimates of items within the same subcategory. Activity in the medial prefrontal cortex (MPFC) scaled with participants' model-derived preference predictions.

In the current study, we investigated whether participants' brain activity reflected coarser subcategory-level or fine-grained item-level relationships. Subcategory and item-level preference relationships were obtained from an online study, in which a larger group of adults (N=264) rated their own preferences for the task items. To test social knowledge representations in neural activity, we conducted a representational similarity analysis (RSA) using both the MPFC as a region of interest (ROI) and a whole-brain searchlight (SL) analysis. We created representational dissimilarity matrices (RDMs) of item-level and subcategory-level neural activity and compared these to the respective behavioral RDMs obtained from the online study.
We did not find evidence of fine-grained item-level neural representations, but we did find significant representations of coarser subcategory structure. Both the ROI and SL analysis revealed significant results. Subcategory structure was reflected in MPFC activity and additionally in the hippocampus, cerebellum and medial occipitotemporal gyrus, regions implicated in learning and object category representation. Overall, we found evidence of a coarser subcategory representation during social learning in adults. In a next step, we will extend our analysis to investigate differences in knowledge representations between adult and adolescent groups and test whether these differences are related to differences in learning task performance.

Disclosures: **W. Liu:** None. **K. Frolichs:** None. **C.W. Korn:** None. **G. Rosenblau:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.08/VV16

Topic: H.06. Social Cognition

Support: NIH Grant R01DC019653

Title: Using deep neural networks to study single-neuronal components of language in humans

Authors: *D. KELLAR[1], J. CAI[2], Y. KFIR[2], I. CAPRARA[2], M. JAMALI[2], A. PAULK[3], S. S. CASH[3], Z. WILLIAMS[2];
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Abstract: Despite a growing understanding of the frontotemporal network of brain areas involved in language, the single-cellular mechanisms by which we communicate information through natural speech remain largely unknown. Here, we took the rare opportunity to record from frontotemporal neurons using Utah arrays, employing deep neural networks (DNNs) alongside population modeling and speech tracking techniques as participants engaged in natural dialogue to begin addressing these questions. By following their action potential activities, we find neurons that reliably tracked core components of the participant's dialogue and reflected unique aspects of their communication such as co-construction and cohesion. We also find cells

whose activities reflected specific points of transition, such as question-answer, invitation-acceptance, and assessment-agreement shifts that defined participant's conversations. By using DNN-based language models, we find that these cell populations provided a hierarchical representation of conversations being held. These models also found that cellular response patterns were tightly coupled to the information being relayed and comprehended when listening or speaking. Together, these findings reveal a detailed cellular representation and arrangement of processes involved in natural dialogue, and highlight the prospective use of DNNs in studying human language at a cellular scale.

Disclosures: **D. Kellar:** None. **J. Cai:** None. **Y. Kfir:** None. **I. Caprara:** None. **M. Jamali:** None. **A. Paulk:** None. **S.S. Cash:** None. **Z. Williams:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.09/Web Only

Topic: H.06. Social Cognition

Support: KAKEN 21B103

Title: The Influence of Remote Tactile Communication on Functional Connectivity in Social Interaction

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Abstract: Recent developments in tactile communication technologies have opened new possibilities for extending remote communication systems. While the use of tactile stimuli in social interaction situations has been discussed, the effects of such stimuli on psychology and, moreover, on brain function during remote communication remain largely unexplored. This study aimed to investigate the effect of a remote tactile communication system on brain connectivity on resting-state functional magnetic resonance imaging (rs-fMRI). Seventy participants (34 females, mean age of 21.1 years) were recruited and divided into a tactile group (n=36) and a control group (n=34). Excluding 25 participants due to failing to identify tactile information correctly, the final analysis included 45 participants. During the social interaction session, participants engaged in a 15-minute conversation with the experimenter using a standardized set of questions. The experimenter indicated agreement with subject's response with a nod and finger bending, and the participants received the experimenter's agreement as tactile feedback to their wrists, seeing finger bending and nodding. Before and after the conversation, the participants undertook the rs-fMRI using a 3.0T MRI scanner (Siemens PRISM), measures of the Inclusion of Other in the Self (IOS), State-Trait Anxiety Inventory (STAI), Self-Assessment

Manikin (SAM), and the self-reports of the attractiveness. Only the self report of emotional connection (EC) to the partner was obtained from the participants after the conversation. A mixed two-way ANOVA (time: pre/post × group: tactile/control) was performed on all measures except for EC, which underwent an independent t-test. The EC score was higher in the tactile group than that in the control group ($p < .05$). No significant differences were found in attractiveness, IOS, STAY, and SAM. The connection for the striatum-orbitofrontal cortex involved in social rewards and encoding touch processing showed neural plastic changes in the tactile group compared to the control group (cluster size p-FDR < .05). Additionally, a significant interaction between striatum and right inferior frontal gyrus was identified (cluster-size p-FDR < .05). These findings suggest that remote tactile information might enhance the emotional connection with the partners and modulate functional connectivity related to social reward. Further research should explore the underlying neural mechanisms and potential applications in remote tactile communication.

Disclosures: T. Matsuhashi: None. **M. Ito:** None. **T. Kuhara:** None. **K. Hosokawa:** None. **K. Inukai:** None. **Y. Tanaka:** None. **C. Hosoda:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

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Program #/Poster #: PSTR370.10/VV17

Topic: H.06. Social Cognition

Support: NINDS Grant NS21135

Title: Direct electrophysiological evidence of context accumulation in human communicative interactions

Authors: *K. J. KHAN[1], S. SADHUKHA[1], R. T. KNIGHT[2], A. STOLK[1];
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Abstract: Communication is often described as a process of exchanging signals, presupposing that individuals already share a common understanding of how to use and interpret them. However, the meanings of our everyday words and gestures can vary dramatically depending on the specific communicative context in which they are used. This context is thought to accumulate and undergo constant revision as the interaction unfolds. However direct evidence of a neural mechanism sensitive to context has remained elusive due in part to (1) limitations in our ability to experimentally isolate the build-up and tracking of context in communication and (2) methodological shortcomings in measuring the underlying neural processes with moment-to-moment precision throughout the duration of an interaction. Here, we sought to address these challenges by conducting electrocorticography (ECoG) and stereoencephalography (SEEG) recordings in neurosurgical patients during experimentally-controlled dyadic interactions to

identify electrophysiological signatures of context accumulation in communication. We find neuronal populations spatially localized to the temporal lobe that track the accumulation of communicative context throughout an interaction, over and above the dynamics of signal production and comprehension. Further, we show that neural activity in these populations is sensitive to the contextual dynamics of an interaction at multiple temporal scales. Specifically, we find that (1) high gamma band (HGB) power in these areas is predictive of contextual buildup across the course of an entire communicative interaction and (2) HGB power in these areas is predictive of contextual demands at the temporal scale of individual communicative signals. Together, these findings characterize a neural infrastructure for tracking and accumulating communicative context, a process fundamental to everyday social interaction. Further, these findings substantiate a theoretical framework that takes meaning to be a complex function of dynamically constructed communicative context rather than a mere property of a signal.

Disclosures: K.J. Khan: None. **S. Sadhukha:** None. **R.T. Knight:** None. **A. Stolk:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.11/VV18

Topic: H.06. Social Cognition

Support: National Institute for Mental Health (R01MH116252)

Title: The use of prior knowledge for social learning in autistic and non-autistic adolescents

Authors: *S. CAHALAN[1], S. BLOCK[3], A. CLAWSON[4], C. KORN[5], G. ROSENBLAU[2]; [1]Psychological and Brain Sci., [2]George Washington Univ., Washington, DC; [3]The George Washington Univ., Washington, DC; [4]Children's Natl. Med. Ctr., Washington, DC; [5]Univ. of Heidelberg, Heidelberg, Germany

Abstract: Difficulties with social interactions is a core diagnostic feature of autism spectrum disorder (ASD). Social learning is a key prerequisite for successful social interactions; thus, identifying how autistic individuals differ in social learning from typically developing (TD) individuals may establish a mechanistic account of social deficits in ASD. A prior study revealed that autistic adolescents differed from their TD counterparts in how they learned about peer preferences. While autistic adolescents solely relied on their self-preferences to infer those of peers, the TD group applied knowledge about TD peers and reduced their prediction errors (PEs)-the difference between task ratings and feedback-over time. Among TDs, PEs scaled with activity in the medial prefrontal cortex (MPFC) - a region involved in mental state attribution. In line with behavioral observations, autistic adolescents did not show PE related brain activity. Extending our previous study, this study probes whether autistic teens rely on themselves or on knowledge about average autistic preferences, that may differ from TD average preferences. We tested this notion in a large online study with young TD adults (N = 194) and autistic adolescents

(N = 217). As predicted, self-preferences differed between TD and autistic groups. Next, we tested whether TD and autistic groups relied on knowledge of their respective group when learning about TD and autistic peers. Participants learned about peers who resembled the average preference profile (i.e., mean profile) or diverged from the average (i.e., odd profile) of both TD and autistic groups. Contrary to our expectation, both TD and autistic groups had the lowest PEs when learning about the average TD adolescent and significantly reduced PEs over time when learning about autistic adolescents. Notably, autistic teens relied on the average autistic preferences to make ratings, while young TD adults relied on knowledge about TD adolescents. In a more controlled neuroimaging study, we are examining the neural mechanisms underlying these social knowledge representations during learning. To this end, TD and autistic teens perform the aforementioned preference learning task in an fMRI scanner. A preliminary fixed effects analysis of 13 adolescents (TD: N = 10, ASD: N = 3) and a separate analysis of only the TD group found that MPFC activity scaled with PE magnitude across conditions. Future analyses in a larger sample will compare PE and social knowledge related brain activity of the mean versus odd conditions between groups to reveal differences in the neural encoding of social learning variables between autistic and TD adolescents.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Topic: H.06. Social Cognition

Support: NIH R01-DC04290
OVPR Early Career Scholars Award

Title: Computational linguistic analyses of social inference in pre-surgical neurological patients

Authors: *J. Y. PETERS[1], C. M. GARCIA[2], A. E. RHONE[2], K. V. NOURSKI[2], C. DEIFELT STREESE[2], A. B. ZAHEER[1], A. D. BOES[3,4], D. TRANEL[1,3,5], B. J. DLOUHY[2,5], M. A. HOWARD, III[2,5], D. KLIEMANN[1,3,4,5];
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Abstract: Interacting with others lies at the core of human behavior and individual well-being. Cognitive neuroscience has provided valuable insights about candidate brain regions and networks involved in social interactions, however, non-invasive neuroimaging typically fails to demonstrate causal mechanisms between brain and behavior. Lesion-deficit approaches can investigate causal links between brain organization and social inference. Moreover, a longitudinal approach comparing data before and after surgical brain tissue resection (e.g., to

treat epilepsy or remove a tumor) can provide especially definitive insights into the mechanisms of brain network reorganization that typically produce social behavior. Also, to adequately assess behavioral and neural changes across time, sensitive measures of social inference are needed. We hypothesize that previously introduced computational linguistic analyses applied to a well-established behavioral task [1, 2] are feasible, extend standard scoring approaches in neurological patients compared to healthy controls, and are suitable for a longitudinal design. Neurological patients (total n = 16, epilepsy: n = 11, focal brain lesions: n = 5, mean age = 29.25 (SD 13.57), n female = 9) watched 12 short film clips (Frith-Happé animations, [2]) prior to brain surgery. We then assessed the capacity to make appropriate inferences about whether geometric shapes were moving with social intention (ToM), goal-directed (GD) or randomly (RD) with standard scoring, Linguistic Inquiry and Word Count (LIWC), and topic modeling (Latent Dirichlet Allocation, LDA), and compared to a previously published healthy comparison sample (n =14) [1]. Neurological patients provided less appropriate descriptions for intentional interactions and overall shorter responses for goal-directed interaction clips (ToM: $U = 178$, GD: $U = 190$, both $p < 0.008$, Bonferroni corrected). Intentionality descriptions were similar for all conditions across groups. A leave-one-out LDA model trained on comparison participants' responses further revealed that patients' descriptions were less typical for both GD ($U = 215$, $p = 2.04e-05$) and ToM ($U = 208.0$, $p = 7.19e-05$). The proportion of words in socially relevant semantic categories in the LIWC, however, was similar between the groups. The results indicate that these linguistic analyses are feasible and have the potential to provide additional insight into subtle social inference changes between groups, and across timepoints within individuals in our ongoing longitudinal data collection.
[1] Abell et al. Cogn Dev 2000 15:1-16. [2] Renteria-Vazquez et al. J Autism Dev Disord 2022 52:569-83.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.13/VV20

Topic: H.06. Social Cognition

Support: R01AG075582
RF1NS128534

Title: Large Language Model Assist Diagnosis of Alzheimer's Disease

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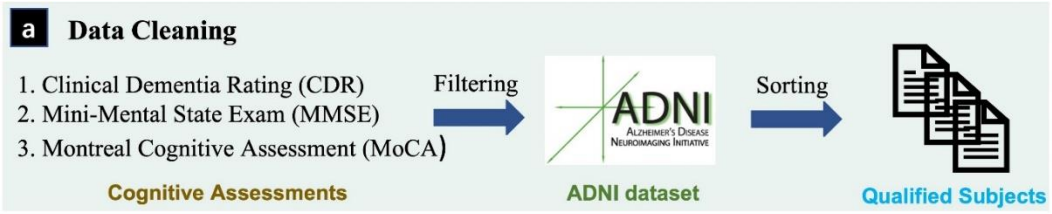
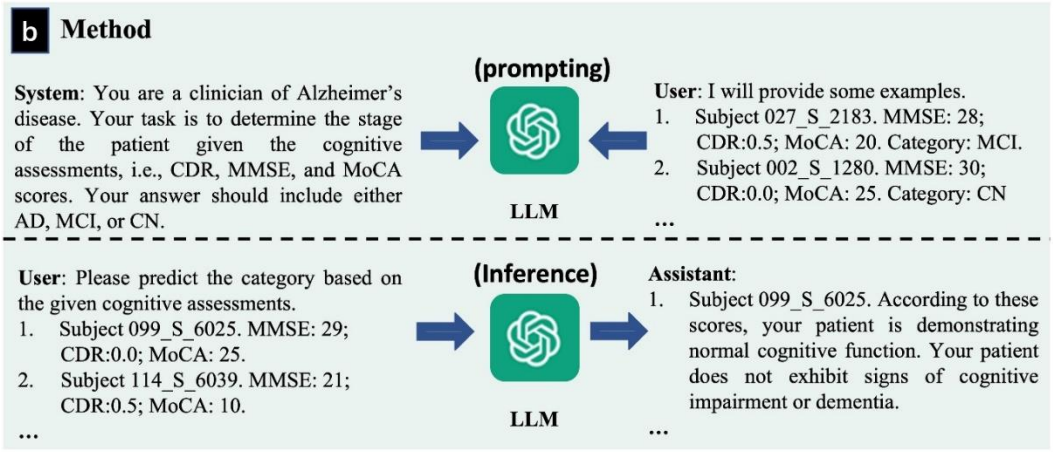
TX; [3]Univ. of Texas at Arlington, Arlington, TX; [4]Dept. of Computer Sci. and Engin., Univ. of Texas At Arlington, Arlington, TX

Abstract: Title: Large Language Model Assist Diagnosis of Alzheimer's Disease

Xiaowei Yu, Lu Zhang, Yanjun Lyu, Chao Cao, Dajiang Zhu;

Early detection and accurate diagnosis of this disease are crucial for effective management and treatment. Recently, large language models (LLM), such as GPT-3.5 (ChatGPT) and GPT-4, have shown great potential in various clinical tasks beyond natural language processing. In this work, we aim to explore the potential of such models in assisting the diagnosis of Alzheimer's disease. We used prompt engineering and in-context learning to leverage the vast knowledge base of LLMs by providing clinical assessment examples. We used multiple neuropsychological measurements, including the mini-mental state exam (MMSE), Montreal cognitive assessment (MoCA), and the clinical dementia rating (CDR) scores from the ADNI dataset to improve the diagnosis accuracy of the LLMs. Using in-context learning, we trained the model to enable the LLMs to learn distinctive cognitive impairments associated with the disease. The method is in Fig. 1. We employed two scenarios: binary classification, i.e., AD or non-AD, and multiple-category classification, i.e., AD, mild cognitive impairment (MCI), and cognitively normal (CN). After data cleaning, the number of available subjects in ADNI who have MoCA, CDR, and MMSE simultaneously is 1068. We used 100 subjects for training, and the rest 968 subjects for inference. GPT-3.5 achieved an accuracy of 94.4% in binary classification and 73.8% in multiple-class classification. While GPT-4 achieved 98.9% and 81.2% accordingly. The promising results, with high accuracy in identifying the clinical status in AD studies, suggest that LLMs have a great potential to provide valuable support to healthcare professionals by offering an additional diagnostic tool that can analyze a wide range of patient information and provide personalized insights. In conclusion, this study presents a novel approach utilizing large language models to assist in the diagnosis of Alzheimer's disease, prompting the usage of LLMs in medical applications.





Disclosures: **S. Yu:** None. **L. Zhang:** None. **Y. Lyu:** None. **C. Cao:** None. **D. Zhu:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Support: This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) and funded by the Korean government (MSIT) [NRF2019M3E5D2A01066265].

Title: Computational Modeling of Gossip Behavior: Integrating Social Values Into Decision-Making

Authors: *J. LEE, Y. SONG, J. JEONG;
KAIST, Daejeon, Korea, Republic of

Abstract: Gossip, a conversation between people about absent others (i.e., targets), is a common form of everyday social interaction. Although gossip is traditionally seen as a harmful behavior that goes against the unity and norms of a community, studies have indicated that gossip also functions as an efficient tool for fostering social connections and maintaining an ordered society. Spreading gossip has advantages (e.g., allowing social control, sharing social information, and strengthening social bonds) and disadvantages (e.g., potential retaliation). These aspects of gossip make it a clear example of value-based decision-making that requires people to estimate the benefits and costs of spreading gossip. In the previous fMRI study, contrast analyses showed that various gossip types, such as *morality*, *daily social affairs*, *close friends*, and *negative events*, were processed in the dedicated brain regions (occipital pole, precuneus, posterior cingulate gyrus, and frontal pole, respectively). Moreover, we also found that the two major decision variables, benefits and costs of gossip, were computed separately in vmPFC and dmPFC while the choice among 4 gossip options (i.e., *spread to a few close friends*, *to acquaintances*, *to anyone*, and *not spread*) was being made. Although the fMRI results enabled us to list the candidates of *gossip variables* that could be used for estimating benefits and costs (i.e., decision variables), the causal structure of how those different variables are integrated into forming a gossip decision is yet to be revealed. Here, we employed computational modeling to examine the structure of gossip decision-making. We developed and compared 80 gossip models with various decision structures to explain 50 participants' gossip behaviors observed in the previous fMRI experiment. Different combinations of the potential gossip variables derived from the fMRI results listed above were used as model inputs. The gossip models were fitted and compared via Bayesian model inversion with variational approximation. Our best-fitted model showed that particular inputs (*morality*, *daily social affairs*, and *close friends*) that reflect the social functions of gossip (*social control*, *social information sharing*, and *social bonding*, respectively) were

necessary to accurately explain participants' gossip decisions. Furthermore, a 3-level hierarchical structure was strongly favored (i.e., 4 gossip options were considered and chosen one after the others at each decision level), indicating that the multi-level design may allow the system to manage such complex decisions. Additional fMRI analyses provided neural evidence for this decision structure.

Disclosures: J. Lee: None. **Y. Song:** None. **J. Jeong:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Program #/Poster #: PSTR370.15/VV22

Topic: H.06. Social Cognition

Support: DFG Emmy Noether Research Group grant 392443797

Title: Cooperation decisions in women with borderline personality disorder (BPD) - evidence from computational models and fMRI data

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Abstract: Patients with borderline personality disorder (BPD) often fail to establish or maintain cooperation and seem to act in a more economically rational or selfish way. It is an open question whether the uncooperative behavior of patients with BPD is based on strategic reward-maximizing considerations or a general distrust of others.
We developed a new multistep social decision-making task to test whether patients with BPD (compared to healthy controls) cooperate less when cooperating is costly to them. In the context of a virtual "foraging" task with the threat of starvation, participants were paired with a confederate and led to believe that their choices would influence their own and the other person's outcome. We used functional magnetic resonance imaging (fMRI) to test whether differences in strategies between individuals with BPD and healthy controls are related to differences in medial prefrontal cortex activity. In an additional behavioral task, we tested whether the decision to cooperate depends on expectations about the other person's cooperative behavior. We preregistered our study (https://osf.io/sd2tm; embargoed for now) and tested 31 women with BPD and 35 age-matched healthy women.
We found that both groups cooperated more when they could help their partner compared to when their partner did not need help. We found no group differences when cooperating was risky (could lead to starvation), but individuals with BPD cooperated less in the less risky task conditions. In addition, participants with BPD (compared to healthy controls) expected less help from their partner when cooperating was risky for their partner.
Preliminary fMRI analyses revealed differences in brain activity in the helping (partner needs

help) vs. the control (partner doesn't need help) conditions in the dorsomedial prefrontal cortex (dmPFC), the posterior cingulate cortex, and bilaterally in the inferior frontal gyrus.
In further analyses, we are testing how reduced helping behavior links to differences in activation in the mPFC and dmPFC. In addition, we are testing heuristic models and optimal policies to test whether individuals with BPD behave less optimal than healthy controls.
Our results suggest that individuals with BPD help less in less risky contexts and expect less help from others. This suggests that the differences between BPD and the general population may stem from different expectations.

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Poster

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Topic: H.06. Social Cognition

Support: JST-MIRAI JPMJMI19B4
JSPS-KAKENHI 22H04855

Title: Neural correlates of trust decision making

Authors: *M. NISHIO[1], N. MIURA[2], R. ISHIBASHI[3], M. SUGIURA[1];
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Abstract: In our daily lives, interpersonal trust is important. Previous studies revealed the brain regions that predict trustworthiness based on facial and vocal cues or through a trust game. Though real decision-making to trust a person in society often depends on a person's characteristics such as ability, benevolence, or the similar sense of values, how our brain reaches this decision based on these three characteristics is still unknown. The present study aimed to clarify the neural foundations of the interpersonal trust decision-making processes and to find brain regions that reflect the level of trust based on these characteristics.We used fMRI to measure brain activity in 36 healthy participants while they performed two tasks: evaluation task and trust one. In both tasks same episodes expressing each characteristic (e.g., ability, benevolence, and similar sense of values) is used. In the evaluation task, participants evaluated the degree of each characteristic corresponding to the episode. In the trust task, they answered how much they would be willing to trust the person based on the episode. We explored the activation during trust decisions (vs. evaluation) and the one representing trust level, both common to the three characteristics and specific to one of them.Our experiment revealed that during trust decisions (vs. evaluation) there was activation in the bilateral temporo-parietal junction (TPJ). The left dorsolateral prefrontal cortex (dlPFC) exhibited common activation across all three characteristics. Furthermore, activation dependent on trust level was revealed in

the dorsomedial prefrontal cortex (dmPFC) while activation based on value similarity was found in the left insula. A major finding is that trust recruits the mentalizing network. The process of trust decision-making might be related to predicting the intent to deceive since the TPJ is related to evaluating people's mental perspective and the dlPFC is associated with combining predictions based on social norms with inferences about the intent to deceive. Trust level specifically based on value similarity might be associated with predicting a trustee's behavior since the insula is related to empathy and the dmPFC plays a role in inferring the traits of others. Trust decision-making is thought to entail the process of attaining insights into a counterpart's information to confirm the absence of deceptive intentions regardless of the personal characteristics and to precisely anticipate behavioral patterns.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

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Topic: H.06. Social Cognition

Support: NRF of Korea, Basic Science Research Program (2020R1A2C2007770)
NRF of Korea, Neurological Disorder Research Program (2020M3E5D9079913)

Title: Distinct Neural Representations of Social Recognition Dimensions in the Human Superior Temporal Sulcus

Authors: *W. KANG[1], M. KWON[1], S.-H. LEE[2];
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[2]Dept. of Psychology, Col. of Social Sci., Seoul Natl. Univ., Seoul, Korea, Republic of

Abstract: Recognizing the social relationships of others is critical for our social interactions. Previous studies in the field of social psychology have proposed conceptual models for interpersonal relationships, which involve two orthogonal axes: affiliation and autonomy. However, whether the brain actually processes social recognition information based on these two axes remains elusive. Therefore, to investigate the neural representations of social recognition dimensions, we conducted an event-related functional magnetic resonance imaging (fMRI) experiment. During the experiment, participants viewed movie clips and assessed social relationships based on the interactions between a designated actor and target character in each clip. In order to identify the orthogonal dimensions of social recognition, we employed principal component analysis (PCA) and identified two components (PC1 and PC2) that can explain approximately 85% of the subjective rating of social relationships. Subsequently, using representational similarity analysis (RSA) across the entire brain, we examined the distribution of information for each principal component (PC). Our findings revealed that PC1 information

was predominantly processed in the right middle and posterior superior temporal sulcus (STS), as well as the right inferior parietal lobule, while PC2 information was significantly represented in the anterior STS. Furthermore, region of interest (ROI) analysis focusing on the STS provided additional support for distinct neural representations of PC1 and PC2 along the anterior-posterior axis in this region. These results suggest distinct neural processing of the two crucial dimensions of social recognition in the human superior temporal sulcus.

Disclosures: W. Kang: None. **M. Kwon:** None. **S. Lee:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.18/VV25

Topic: H.06. Social Cognition

Support: NIH Grant 1R01MH119430-01

Title: Increased neural coupling and ratings of subjective connection during live face-to-face gaze and harmonic music

Authors: *A. A. WATTS[1], A. S. ALLSOP[2], X. ZHANG[2], J. A. NOAH[2], J. HIRSCH[2,3,4,5]; [1]Psychiatry, Yale Univ. Med. Sch., New Haven, CT; [2]Psychiatry, [3]Neurosci., [4]Comparative Med., Yale Univ. Sch. of Med., New Haven, CT; [5]Wu Tsai Inst., Yale Univ., New Haven, CT

Abstract: Introduction: Social connection is critical for human health and survival[1], but little is known about the neural mechanisms underlying the phenomenon[2]. Music has been shown to facilitate pro-social behavior and alleviate symptoms of anxiety and depression[3], but the underlying mechanisms are not known. Harmonic structure is a fundamental element of music that alters human emotion. Here, we investigate the impact of harmonic structure on social connection and cross-brain coherence (i.e., neural synchrony between dyadic partners) during face-to-face gaze. We hypothesize that listening to music with familiar harmonic structure increases feelings of connectedness and enhances cross-brain coherence in the temporoparietal junction (TPJ).
Methods: We collected hemodynamic signals using functional near-infrared spectroscopy (fNIRS). Twenty dyads listened to harmonic and disharmonized (tonal notes randomly scrambled) instrumental music while viewing their partner's face. In the control conditions the partner's face was hidden. A run (~two min each) for each condition consisted of four musical phrases (15 s each). After each run and before beginning the paradigm, participants used a dial to rate the connection to their partner; 1 = no connectedness and 5 = maximum connectedness. Wavelet analysis was performed on fNIRS data to assess cross-brain coherence and behavioral ratings were averaged.
Results: We found increased average connectedness ratings during Face and Harmony-On compared to other conditions and increased cross-brain coherence in signals between the

supramarginal gyrus and premotor cortex of partners during Face and Harmony-On condition compared to Face-Off, Harmony-On condition in the optimal wavelength range[4].
Conclusion: These findings suggest that listening to harmonious music during face-to-face gaze increases cross-brain coherence of neural activity and subjective feeling of connectedness between partners. This corroborates previous findings that face-to-face interaction between human dyads increases neural activity and cross-brain coherence in the right TPJ[5]. This work is the first of its kind to suggest neural mechanisms to be targeted in innovative music therapies employing naturalistic social situations and other multi-modal healing techniques.
[1]Perissinotto, C. M. et al. (2012). *Archives of Internal Medicine*, *172*(14), 1078-1084.[2]Eisenberger, N. I., & Cole, S. W. (2012). *Nature Neuroscience*, *15*(5), 669-674.[3]Lin, S.-T. et al. (2011). *Harvard Review of Psychiatry*, *19*(1), 34-46.[4]Zhang, X. et al. (2020). *Neurophotonics*, *7(1)*, 015010.[5]Noah, J. A. et al. (2020). *Frontiers in Human Neuroscience, 14*.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.19/VV26

Topic: H.06. Social Cognition

Support: UZH

Title: Brain structure relates to ingroup bias

Authors: *P. KANG[1], Y. SUN[2], J. KIM[3], S. SUL[5], H. KIM[4], G. HEIN[6], P. N. TOBLER[1]; [1]Univ. of Zurich, Zurich, Switzerland; [2]East China Normal Univ., Shanghai, China; [4]Dept. of Psychology, [3]Korea Univ., Seoul, Korea, Republic of; [5]Pusan Univ., Pusan, Korea, Republic of; [6]Wurzburg Univ., Wurzburg, Germany

Abstract: Ingroup bias is the tendency to favor ingroup members over outgroup members. It is pervasive in humans and contributes to inter-group conflict based on nationality or race. Although many neuroimaging studies investigated functional brain correlates of ingroup bias, most of them focused on differences in neural activity elicited by ingroup versus outgroup conditions. In contrast, it remained largely unknown whether structural differences underpin ingroup bias. Here, we investigated the grey-matter volume of brain areas associated with ingroup bias. Specifically, we combined two studies, performed with two different types of outgroups in South Korea, and measured ingroup bias with explicit donation decisions and implicit response bias favoring ingroup over outgroup members. More specifically, in both studies, participants (Study 1: n=70, 35 female, age: 25.75±3.86 years; Study 2: n=108, 52 female, age: 23.44±2.24 years) decided whether to incur a monetary cost to donate to charities

benefitting the ingroup (Study 1 and 2: South-Koreans) or outgroup (Study 1: North Koreans; Study 2: Southeast Asians in South Korea) and performed an implicit association task towards ingroup and outgroup members with positive and negative words. We examined whether the association between the volume of cortical and subcortical brain regions and behavior were specific for the type of ingroup bias measure and/or for the type of outgroup or whether a brain region commonly associated with multiple ingroup bias behavior. We found that the grey-matter volume of clusters in putamen ($Z=3.76$, $k=171$, uncorrected $p < 0.001$) and postcentral gyrus ($Z=3.88$, $k=414$, uncorrected $p < 0.001$) was positively correlated with greater donation to charities benefitting the ingroup. In contrast, the volume of clusters in anterior cingulate cortex ($Z=4.74$, $k=580$, uncorrected $p < 0.001$) and inferior temporal lobe ($Z = 3.86$, $k=762$, uncorrected $p < 0.001$) was positively correlated with stronger implicit bias (i.e. D score) favoring the ingroup. Age, total intracranial volume and the experiment types were controlled in all analyses. Note that some of those results were not significant or weaker when testing each group separately; however, it became significant when combined indicating that the association between the behaviors and brain volume might be common regardless of the group settings.

Disclosures: **P. Kang:** None. **Y. Sun:** None. **J. Kim:** None. **S. Sul:** None. **H. Kim:** None. **G. Hein:** None. **P.N. Tobler:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.20/VV27

Topic: H.06. Social Cognition

Title: The significance of embodied safety for social cognition

Authors: *T. TAKANO[1], K. MOGI[2];
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Abstract: According to attachment theory (Bowlby, 1969), physical proximity and contact (e.g., affective touch) have a great impact on the development of social animals. Harlow (1958) showed tactile perception given by “warm” and “soft” blanket had a critical role for physical and mental health in infant rhesus macaque. It is known that physical touch releases oxytocin (Light et al., 2005), which is related to bonding (Williams et al., 1994) and prosocial behavior (Tai et al., 2011), reducing the level of stress hormone cortisol (Heinrichs et al., 2003). Recently, studies focusing on the effects of tactile perception on social cognition have increased. Williams and Bargh (2008) showed that participants holding a hot coffee judge others as warmer persons. There are many studies reporting embodied cognition of warmth on social cognitive processing. In the context of attachment theory (Bowlby, 1969), tactile softness is likely to be crucial as well as tactile warmth for social animals. Participants sitting on a soft chair showed more flexible attitude at a negotiation task, judging the opponent as a less rigid person (Ackerman et al., 2010).

Numazaki et al. (2016) also reported that squeezing a soft ball made participants judge more sex-ambiguous faces as female associated with traits of kindness. Also, tactile perception of teddy bear alleviates negative consequence of social exclusion and elicits participants' prosocial behavior, such as offering more money to a stranger in a dictator game (Tai et al., 2011). Though Tai et al.'s study reported the effect of touching a teddy bear emerged only in social exclusion (not in social inclusion), some studies showed tactile softness influences processing of social cognition. Considering these results, it is possible that tactile softness provides participants psychological safety, making other's impression better (softer). According to attachment theory, when there is a secure base giving infants a sense of safety through physical contact with their caregivers, they can explore novel environment easily. Here we examined two hypotheses: 1) Softness perception may induce a sense of safety, generating social behavior as an indication of openness. 2) Tactile softness may moderate in-group bias. In the present study, we illustrate new aspects of sensory processing affecting embodied social cognition, and discuss how the tactile softness brought by physical touch contributes to building an affectional bond such as attachment not only in childhood but in adulthood, with implications for the neural mechanisms involved.

Disclosures: **T. Takano:** None. **K. Mogi:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.21/VV28

Topic: H.06. Social Cognition

Support: University of Nebraska Collaboration Initiative

Title: Evaluating and assessing social interaction between parents and their children with Autism Spectrum Disorder

Authors: *P. LAI[1], Y. WANG[2], A. ZANGRILLO[4], Y. ZHANG[5], H. YOON[3], C. TONER[2], K. VANDENBOS[2];
[1]Communication Disorders, Univ. of Nebraska, Kearney, Kearney, NE; [2]Dept. of Special Educ. and Communication Disorders, [3]Nebraska Acad. for Methodology, Analytics and Psychometrics, Univ. of Nebraska-Lincoln, Lincoln, NE; [4]Munroe-Meyer Inst., Univ. of Nebraska Med. Ctr., Omaha, NE; [5]Grace Abbott Sch. of Social Work, Univ. of Nebraska at Omaha, Omaha, NE

Abstract: Children with Autism Spectrum Disorder (ASD) have significant impairments in social communication and social interaction that interfere with effective management of personal, professional, and academic skills. The disorder affects an estimated 1 in 44 children in the U.S. The lifetime total cost of assessing and treating ASD in the U.S. was estimated to be more than 7 trillion dollars in 2019 dollars and projected to cost 15 trillion dollars by 2029 if the prevalence rate continues to rise. One area gaining attention in ASD research is the role of parents and their effectiveness in providing interventions and treatments to their child with ASD.

There is a critical need for studies to investigate, for example, the effectiveness of social interaction by exploring variables from both the parents' and the child's perspective. In this ongoing study, we are collecting data from both parents and their child through monitoring real-time brain activity during parent-child interactions. In addition, we are collecting standardized questionnaires and video recordings of parent-child interactions to access the dynamics of social interaction. One of our goals is to investigate brain-behavior relationship across the autism spectrum. Our parental questionnaire data assessing parental anxiety, parental depression, and parents' perception of their child's sociability highlights the vast spectrum of the disorder. For example, on the Beck Anxiety Inventory (BAI), scores range from 5 to 11, and the Beck Depression Inventory (BDI) scores range from 6 to 13. On both Beck measures, parents' mental states are within the minimal to mild range. On the other hand, on the Social Responsiveness Scale, Second Edition (SRS-2), T-scores range from 53 to 78, highlighting severity scores ranging from mild impairment all the way to severe impairment. This range of scores will come into play when functional near-infrared spectroscopy (fNIRS) data are accessed to observe if differences are observed during social interaction. Insights from this study add unique knowledge to our understanding of communication, the breadth of the autism spectrum, as well as the relationship between social interaction and the brain.

Disclosures: **P. Lai:** None. **Y. Wang:** None. **A. Zangrillo:** None. **Y. Zhang:** None. **H. Yoon:** None. **C. Toner:** None. **K. VandenBos:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.22/VV29

Topic:

Support: STI2030-Major Projects 2021ZD0204200

Title: Genetic Contributions to critical dynamics in resting-state brain activity

Authors: *Y. XIN[1,2], Y. CUI[1,3], S. YU[1,3], N. LIU[1,2];
[1]Chinese Acad. of Sci., Beijing, China; [2]Inst. of Biophysics, Chinese Acad. of Sci., Beijing, China; [3]Inst. of Automation, Chinese Acad. of Sci., Beijing, China

Abstract: Accumulating evidence has suggested that a healthy brain may operate near a critical point. This criticality optimizes numerous capabilities of the brain network that are inextricably linked with information processing, including information capacity, transmission, and dynamic range. Studies have shown that cognitive functions strongly correlate with the brain's criticality, and deviation from the critical state may cause severe mental diseases. However, whether the brain's criticality is modulated by genetic factors, and if so, how the criticality is related to other inheritable cognitive traits, remains unclear. The answer to these questions would advance our understanding of the biological underpinning of the brain's criticality and its functional

implications. In the current study, we evaluated the critical dynamics of resting-state functional magnetic resonance imaging (rs-fMRI) signals in 525 subjects (140 monozygotic twins, 76 dizygotic twins, and 309 non-twins) from the Human Connectome Project (HCP) by using both the neuronal avalanche framework and long-range temporal correlation (LRTC). The heritability of the brain's criticality and the genetic correlation between the brain's criticality and intelligence were determined by the classical ACE twin model, which can effectively evaluate the additive genetic (A), common environmental (C), and unique environmental (E) variance of a phenotype. We found that the rs-fMRI signals of the human brain exhibited distinctive characteristics of critical avalanche dynamics and LRTC. In addition, approximately 70% of variances in both the avalanche dynamics and LRTC were attributed to additive genetic factors. Our results further showed that the relationship between the brain's criticality and cognition, especially fluid intelligence, was modulated by shared genetic effects. Our findings suggest that the brain's criticality is highly heritable and has shared genetic influences with cognitive traits. These findings provide novel biological insights into the criticality theory of the brain.

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Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.23/VV30

Topic: H.06. Social Cognition

Support: UC Berkeley internal funds

Title: Representation of self and others in the human cerebral cortex

Authors: *C. TSENG, S. SLIVKOFF, J. L. GALLANT;
UC Berkeley, Berkeley, CA

Abstract: The ability to distinguish the self from others is fundamental to social cognition. Prior neuroimaging studies suggest that the ventromedial prefrontal cortex (vmPFC) represents self knowledge, while the dorsomedial prefrontal cortex (dmPFC) represents other knowledge (Wagner et al., 2012). However, few studies have directly compared self knowledge with other knowledge, and there is little agreement in the brain regions identified in those studies. In addition, most prior studies only compared the self to one type of other (e.g., mother). Thus, it is unclear how the brain represents information about the self and different types of others. Here, we mapped the cortical representations of the self and six types of others in individual participants. To do this, we used functional MRI to record blood oxygen level-dependent (BOLD) responses in three participants while they answered questions about themselves, close friends, family, acquaintances, work colleagues, famous people, and fictional people. The questions were presented one word at a time using rapid serial visual presentation, and participants responded to each question by rating 1 (low/disagree) to 5 (high/agree). Each

participant answered 1120 unique questions spread across 8 ~10-minute scanning runs. To identify voxels that represent the self and each type of other, we fit a linearized encoding model to every voxel in each participant. The linearized encoding model consisted of one feature space that represented the seven types of people, and ten feature spaces that captured other information in the experiment. To avoid overfitting, the model was trained on seven runs of BOLD data, and the estimated model weights were used to predict the eighth test run of BOLD data. This was done for every split of seven training runs and one test run, and the results were averaged across splits. In all participants, we find that self knowledge and other knowledge are represented in distinct patches of voxels in bilateral vmPFC and dmPFC. We find that self knowledge is additionally represented in bilateral anterior cingulate sulcus, left precuneus, bilateral anterior superior frontal gyrus (SFG), and right superior frontal sulcus. We find that other knowledge is also represented in bilateral precuneus, left anterior superior temporal gyrus, right posterior SFG, and a small region posterior to left temporoparietal junction. Furthermore, we find that cortical representations of others are primarily organized by whether one knows the other abstractly (e.g., famous people) or concretely (e.g., family). These results suggest that the brain has distinct representations for the self, and for different types of others.

Disclosures: C. Tseng: None. **S. Slivkoff:** None. **J.L. Gallant:** None.

Poster

PSTR370. Human Behavior, Disorders, and Mechanisms Including Social Cognition

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR370.24/VV31

Topic: H.06. Social Cognition

Support: NIBIB R01EB026549

Title: Read and hear as if you see: neural correlates of social interaction perception are modality-free

Authors: *Z. MIAO[1], H. JUNG[1], P. A. KRAGEL[2], P. SADIL[3], M. A. LINDQUIST[3], T. D. WAGER[1];
[1]Dartmouth Col., Hanover, NH; [2]Emory Univ., Atlanta, GA; [3]Johns Hopkins Univ., Baltimore, MD

Abstract: Social interaction perception, the process of observing others' social interactions, consistently activates several brain regions in healthy adults, including anterior and posterior superior temporal sulcus (aSTS and pSTS) and precuneus. However, almost all previous studies investigating social interaction perception used visual stimuli (principally animations and movies), and it is unclear whether the brain responses generalize to social interactions derived from other types of stimuli and presented in other sensory modalities. In the current study, participants (N = 84) underwent fMRI scanning while they listened to ("Audio") or read ("Text") brief narratives describing a range of Social interactions and Nonsocial scenarios. Social

interaction scenarios explicitly involved interpersonal communication or shared activity (e.g., “he told her to take care”), while Nonsocial scenarios involved only one person or did not involve interactions (e.g., “she jogs while contemplating her situation”, “they were friends for years”). Results showed very similar Social versus Nonsocial activity in Audio and Text conditions (*Pearson’s r* = .70, *p* < .001), and a conjunction analysis revealed significant activity in pSTS, precuneus, temporoparietal junction (TPJ), and dorsal medial prefrontal cortex (mPFC) across both conditions (false discovery rate (FDR) corrected, *q* < .05). Consistent with previous findings, ventral mPFC showed greater activity for Nonsocial scenarios, and the Social vs. Nonsocial contrast map correlated most strongly with a Neurosynth meta-analytic topic map of “Social interaction” among 54 maps tested. Multivariate linear support vector machine (SVM) models accurately classified Social vs. Nonsocial beta maps with high accuracy in both Audio and Text conditions in five-fold leave-whole-subject-out cross-validation (accuracy in within-subject forced-choice classification was 100% ± 0%, *Cohen’s d* = 3.25 for the Audio model, and 98% ± 1.7%, *d* = 1.90 for the Text mode). pSTS, TPJ, left precuneus, and right dorsal mPFC were significant in the prediction of Social vs. Nonsocial in both models (FDR *q* < .05, 5000 bootstrap samples). Importantly, the Audio model transferred to the classification of Social vs. Nonsocial in the Text condition (95.2% accuracy, *d* = 1.52) and the Text model transferred to the Audio condition (96.4% accuracy, *d* = 1.73). Together, those results provided evidence that the neural correlates of perceiving social interaction generalize to linguistic narratives and across sensory modalities, supporting that they reflect higher-order processing of conceptual information embedded in social interactions.

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Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.01/VV32

Topic: H.08. Learning and Memory

Support: T32HD007491
Brain Research Foundation Seed Grant
University of Utah School of Medicine Seed Grant

Title: The Role of Kirrel3-expressing GABA Neurons in Learning & Memory

Authors: *A. B. N. TUNON-ORTIZ[1], A. C. HUGHES[3], A. MAHNKE[1], A. JOHNSON[1], L. A. SCHWARZ[4], M. E. WILLIAMS[2];
[1]Univ. of Utah, Salt Lake City, UT; [2]Univ. of Utah, salt lake city, UT; [3]St. Jude Children's Res. Hosp., [4]Stanford Univ., St. Jude Children's Res. Hosp., Memphis, TN

Abstract: Neuronal inhibition in the hippocampus is critical for shaping patterned activity that underlies learning and memory. This is can be observed in hippocampal area CA3, where the excitatory DG neurons synapse onto CA3 pyramidal neurons via giant excitatory mossy fiber terminals. This strong excitatory drive, coupled with the highly interconnected CA3 neurons, can result in run-away excitation without GABA neuron inhibition. However, little is known about how different types of inhibitory GABA neurons shape activity in the CA3. In this study, we identified a unique group of GABA neurons that share expression of the synaptic cell adhesion molecule Kirrel3 (Kirrel3-GABA neurons). We found that Kirrel3-GABA neurons are a heterogeneous group of dendrite-targeting GABA neurons that inhibit CA3 activity. Activation of Kirrel3-GABA neurons in mice induces a specific decrease in contextual fear memory discrimination. This decrease is surprisingly not observed when parvalbumin-GABA neurons are activated. This work utilizes a new framework for studying GABA neurons through shared synaptic connectivity genes and reveals how they modulate learning and memory

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Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.02/VV33

Topic: H.08. Learning and Memory

Title: Mice with LRRC26-mediated hyperactivity of BK channels in the forebrain exhibited symptoms resembling ADHD

Authors: *G. CHEN, X. GUAN, J. YAN;
Univ. of Texas MD Anderson Cancer Ctr., Houston, TX

Abstract: The large-conductance, Ca^{2+}, and voltage-activated K^+ (BK) channels consist of the pore-forming α (BKα) subunit and regulatory β and γ subunits. BK channels are widely distributed in the central nervous system and play a multifaceted role in brain activity regulation. The γ1 (LRRC26) subunit functions as a potent activator of BK channels, inducing a large shift in the voltage-dependence of the channel activation towards the hyperpolarization direction by ~150 mV. Given their large conductance, activators of BK channels have promising therapeutic potential in the treatment of various neurological diseases. Notably, LRRC26 expression is largely absent in the adult brain. To investigate the impact of activator-induced hyperactivity of BK channels on brain function, we generated conditional LRRC26-transgenic mice in which recombinant human LRRC26 is expressed in the forebrain under the control of the CAMK2A promoter and the tetracycline Tet-Off system. Suppression of LRRC26 expression with doxycycline was necessary to obtain CAMK2A-tTA/TRE3G-LRRC26 mice, indicating a prenatal lethal effect of LRRC26 overexpression. Histological analysis of mice with post-

weaning withdrawal of doxycycline revealed high expression of hLRRC26 in the hippocampus. Through behavioral tests, we observed that LRRC26-induced hyperactivity of BK channels resulted in locomotor hyperactivity and cognitive defects, resembling two core symptoms of attention deficit hyperactivity disorder (ADHD). The hLRRC26 transgenic mice exhibited significantly elevated locomotor activity, with increased moving time and velocity in the open field test, despite displaying shorter strides in the footprint test. Furthermore, these transgenic mice showed impaired learning performance in the water maze, novel object recognition, and rotarod tests compared to the control groups. In summary, the hLRRC26 transgenic mice demonstrated heightened locomotor activity and impaired learning abilities, resembling characteristics of ADHD.

Disclosures: G. Chen: None. **X. Guan:** None. **J. Yan:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.03/VV34

Topic: H.08. Learning and Memory

Support: NICHD Intramural Award given to Chris J. McBain

Title: Selectivity and evolutionary conservation of inhibition in a pattern separation circuit

Authors: *G. VARGISH, X. YUAN, K. A. PELKEY, C. J. MCBAIN;
NICHD, Bethesda, MD

Abstract: The mammalian hippocampus supports spatial information processing and episodic memory encoding. Fundamental to this function is the ability to distinguish perceptually similar places or events, a process known as pattern separation. In the hippocampal circuit, this computation is performed by the dentate gyrus (DG). While granule cells (GCs), the main excitatory cell type in the DG, were canonically thought to enable pattern separation with their sparse activity profile and large cell numbers, recent evidence indicates that mossy cells (MCs), the other DG excitatory cell type, may play a critical role. In contrast to GCs, MCs are highly active and strongly innervate inhibitory interneurons (INs) in the DG. These divergent activity patterns coupled with MCs innervation of DG INs suggests that MCs drive disynaptic inhibition to actively shape GC sparseness, enabling pattern separation. However, the inhibitory circuits that regulate MC excitability and maintain high MC/low GC activity dynamics in the DG remain poorly understood. To address this, we used a combination of optogenetics, electrophysiology and *in vivo* 2-photon Ca^{2+} imaging to dissect inhibitory inputs to MCs and GCs. In mice, we identified a novel DG IN subtype that expresses vesicular glutamate transporter 3 (VGluT3) and has axonal arbors primarily in the hilus, where MCs reside. Whole cell patch clamp recordings revealed that VGluT3+ INs preferentially innervate MCs over GCs while parvalbumin- and somatostatin-expressing IN subtypes were strongly biased to GC innervation. 2-photon Ca^{2+}

imaging in awake, behaving mice corroborated these findings showing that chemogenetic activation of VGluT3+ INs significantly reduced *in vivo* MC activity but not GC activity. To probe the translational relevance of these unique inhibitory innervation patterns, we also evaluated inhibitory inputs to MCs and GCs in non-human primate (NHP) and human DG. Recordings of electrically evoked inhibition in both species, as well as optogenetically-evoked inhibition in NHPs using IN-specific viruses, revealed that inhibitory input to MCs was predominantly comprised of cannabinoid receptor subtype 1 (CB1)+ INs, consistent with prominent VGluT3+ innervation, while GCs were innervated by PV+ and SOM+ INs. These findings establish that MCs and GCs have unique, evolutionarily conserved IN innervation patterns and suggest selective inhibitory circuits may be necessary to maintain DG circuit dynamics and enable pattern separation.

Disclosures: G. Vargish: None. **X. Yuan:** None. **K.A. Pelkey:** None. **C.J. McBain:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.04/VV35

Topic: H.08. Learning and Memory

Support: NRF Grant 2017R1D1A1B05036195
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NRF Grant RS-2023-00245605

Title: The brain damage caused by febrile seizures in early childhood is accompanied by cognitive/emotional sequelae

Authors: *Y. YU, H. IM, G. KIM, Y. LEE, D.-K. PARK, D.-S. KIM;
Soonchunhyang Univ., Cheonan-Si, Chungcheongnam-Do, Korea, Republic of

Abstract: Febrile seizure (FS) is a common type of seizure occurring in human during infancy and childhood. Although an epileptic seizure is associated with psychiatric disorders and comorbid diseases such as depression, anxiety, autism spectrum disorders, sleep disorders, attention deficits, cognitive impairment, and migraine, the causal relationship between FS and psychiatric disorders is poorly understood. Early developmental insults by FS may affect medial temporal lobe functions associated with disorders in emotional recognition and the growth of cognitive functions in children is sensitive with the adverse influence of epilepsy. Therefore, in order to investigate the cognitive/emotional sequelae correlated with hyperthermic damage to brain tissue, we study a comprehensive analysis of these phenotypes following FS. There was distinct difference of characteristics for each behavior results. The exploration activities for locomotion in FS rats were recovered to normal levels at recurrent seizure phase, while locomotor activities were conversely enhanced at 3 weeks. In addition, diverse emotional and cognitive phenotypes also significantly declined in FS animal groups more than in control level,

and its local field potential and field excitatory postsynaptic potential signals were shown deteriorated properties at same time period. Taken together, our findings suggest that FS occurrence in infants is causally related to increased levels of anxiety-related behaviors, depression-like symptoms and cognitive disability in juvenile and adult rodents.

Disclosures: **Y. Yu:** None. **H. Im:** None. **G. Kim:** None. **Y. Lee:** None. **D. Park:** None. **D. Kim:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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Program #/Poster #: PSTR371.05/Web Only

Topic: H.08. Learning and Memory

Support: FAPESP 2016/13136-6
CAPES 88887.502819/2020-00

Title: Implications of diet reduction in adult rats cognition whose mothers were submitted to food restriction during pregnancy and lactation

Authors: *G. DA SILVA[1], A. G. MOREIRA[2], C. M. MACHADO[1], M. G. MARTINS[1], *G. DA SILVA[1], J. A. C. HORTA-JUNIOR[1];
[1]Dept. of Structural and Functional Biol., [2]São Paulo State Univ. - Unesp, Botucatu, Brazil

Abstract: Around 820 million individuals, including pregnant and lactating women, endure hunger. Food restriction during pregnancy and lactation serves as a significant developmental insult that could potentially induce programming (DOHaD), which may impact memory later. Thus, animals may exhibit distinct outcomes when subjected to food restriction in adulthood. The combined effects of both periods remains unknown. This study aimed to assess memory and hippocampus of adult Wistar rats under food reduction combined with programming through pregnancy and lactation dietary restriction. Pregnant Wistar rats (CEUA: 8789260620) were divided into two groups: control programming (CP, N=14), diet ad libitum; and food restriction programming (RP, N=17), 50% restriction diet. Male offspring of each group were subdivided into two subgroups: the control food group, ad libitum diet (CF) throughout life, and the restricted food group (RF), 30% reduction diet from 60 to 100 days old. This resulted in a total of four groups: CP-CF, CP-RF, RP-CF, and RP-RF. Animal behavior was evaluated through the open field (OF) and elevated-plus maze (EPM). Memory was evaluated through novel object recognition and object location tests (NORT and OLT) and Barnes Maze (BM). On the day of euthanasia, their body and brain weight were measured and hair samples were taken for nitrogen and carbon isotopic ratio analysis. The brains underwent immunohistochemistry protocols for GFAP, FOS, DCX and NeuN. In the EPM, NORT and OLT tests, there were no significant differences between the groups. In the OF, animals from RP groups spent significantly more time on self-grooming and showed an increased ambulation in the periphery. The BM results

indicated that all groups exhibited learning, but the RP-CF group displayed a higher number of errors during the memory retention test. Biometric data revealed that PR animals had smaller brains without alterations in the encephalization index. Moreover, there were differences in the carbon and nitrogen isotopic ratios, denoting variations in body constitution among the groups. An increase in FOS expression was found in the inferior dentate gyrus of diet-restricted animals, whether experienced early or later in life. Regarding neurogenesis, the RP-RF group exhibited a lower number of DCX cells. However, there were no differences in GFAP and NeuN expression between groups. In conclusion, RP-RF group shows reduced neurogenesis, increased FOS expression, and achieves better results compared to RP-CF. However, it does not generate changes in anxiety and hippocampal cytoarchitecture.

Disclosures: G. da Silva: None. **A.G. Moreira:** None. **C.M. Machado:** None. **M.G. Martins:** None. **G. da Silva:** None. **J.A.C. Horta-Junior:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.06/VV36

Topic: H.08. Learning and Memory

Support: NSERC Discovery Grant
NSERC Canada Graduate Scholarship - Master's
Killam Predoctoral Fellowship (Level I)
Nova Scotia Graduate Scholarship (Life Sciences)

Title: Seasonal patterns of hippocampal plasticity in wild black-capped chickadees (Poecile atricapillus) are restricted to females

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Abstract: In black-capped chickadees (*Poecile atricapillus*), food-storing behaviour fluctuates seasonally: chickadees store and retrieve more food during the fall and winter months when food sources are scarce, and less during the spring and summer when food is abundant. Supporting this seasonal behaviour are changes in the hippocampus (Hp), a neural structure critical for spatial behaviour and memory. While seasonal change in Hp (including volume, neuron number, and neurogenesis) is well-documented, we cannot easily compare findings across studies due to variation in capture dates and length of time in captivity; captivity is known to alter Hp plasticity (particularly neurogenesis). The interaction between spatial behaviour and the hippocampus may also be influenced by natural seasonal and sex differences in the songbird endocrine profile associated with reproduction, driven primarily by photoperiod (day length). Here, we captured a large sample of both male and female black-capped chickadees (*N*=48, 36 adults) from the wild

at three times of year corresponding to the three distinct photoperiodic conditions: photostimulated (March-April), photorefractory (August-September), and photosensitive (December-January). All birds were sacrificed within ca. 1 hour of capture to mitigate potential captivity effects. We quantified gross Hp neuroanatomical plasticity by staining tissue with cresyl violet and measuring absolute and relative Hp volume, and quantified Hp neurogenesis using doublecortin (DCX) immunohistochemistry. While Hp plasticity in males was minimal, photosensitive females had increased Hp neurogenesis (specifically numbers of DCX+ round cells) compared to photostimulated and photorefractory females. Interestingly, these same female-specific seasonal patterns of neurogenesis were also observed in the neighbouring region, hyperpallium apicale (HA), adding to recent evidence suggesting this structure may also be implicated in spatial processing. While sex differences in brain and behaviour are observed in some avian and non-avian species, this is the first evidence of sex differences in the hippocampus of black-capped chickadees, and suggest there may be winter-specific physiological and behavioural pressures borne only by females which may require specialized and flexible neural infrastructure.

Disclosures: B.M.B. Parks: None. **M. Oulton:** None. **M.R. Bhaskara:** None. **T.B. Franklin:** None. **L.S. Phillmore:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.07/VV37

Topic: H.08. Learning and Memory

Support: NIH K99 EY034700
Helen Hay Whitney Fellowship
New York Stem Cell Foundation - Robertson Neuroscience Investigator Award
Beckman Young Investigator Award
NIH DP2 AG071918

Title: A neural code linking place and gaze in freely moving birds

Authors: *H. L. PAYNE[1], D. ARONOV[2];
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Abstract: Our ability to recall *where* something happened - the spatial component of episodic memory - is thought to depend on hippocampal place cells, which have been found across distantly related vertebrate species, including in rodents, primates, and birds (Payne et al. 2021). However, it is unclear how the brain retrieves memories associated with a remote location without physically revisiting that location in order to activate the corresponding place cells. Both

primates and birds primarily experience their spatial context through high-resolution vision. Can viewing of remote locations reactivate associated spatial representations? Birds mainly direct their gaze with head rather than eye movements, so they are experimentally amenable to both gaze tracking and neural recording during free motion. We therefore asked how remote viewed locations are represented in the avian hippocampus. We engineered a system that uses real-time head tracking combined with offline video-oculography calibration to estimate gaze in freely moving black-capped chickadees (*Poecile atricapillus*), a food-caching bird. We designed a simple foraging task to behaviorally dissociate place and gaze locations. We find that spatial representations in the avian hippocampus are remotely activated by gaze: the same cells that fire when physically visiting a specific location are transiently activated when the bird looks at that location from elsewhere. Additionally, this remote activation is coordinated across the population by quasi-rhythmic gaze saccades. This may be analogous to remote activation of spatial codes during internal neural states such as sharp wave ripples and theta cycles described in rodents. Our results suggest that in a highly visual avian species, viewed location and current location are represented by a shared neural code, and these representations are coordinated by active vision.

Disclosures: H.L. Payne: None. **D. Aronov:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.08/VV38

Topic: H.08. Learning and Memory

Support: DP2-AG071918
5F32MH123015
1K99NS121256

Title: Barcoding of episodic memories in the hippocampus of a food-caching bird

Authors: *S. N. CHETTIH, E. L. MACKEVICIUS, S. HALE, D. ARONOV;
Zuckerman Inst., Columbia Univ., New York, NY

Abstract: Episodic memory, or memory of experienced events, is a critical function of the hippocampus. It is therefore important to understand how hippocampal activity represents specific events in an animal's life. We addressed this question in chickadees - specialist food-caching birds that hide food at scattered locations and use memory to find their caches later in time. We performed high-density neural recordings in the hippocampus of chickadees as they cached and retrieved seeds in a laboratory arena. We found that each caching event was represented by a burst of firing in a unique set of hippocampal neurons. These 'barcode-like' patterns of activity were sparse (<10% of neurons active), uncorrelated even for immediately adjacent caches, and different even for separate caches at the same location. The barcode

representing a specific caching event was transiently reactivated whenever a bird later interacted with the same cache - for example, to retrieve food. Barcodes co-occurred with the conventional activity of place cells, as well as with responses to cached seeds. We propose that barcodes are signatures of episodic memories evoked during memory recall. These patterns assign a unique identifier to each event and may be a mechanism for rapid formation and storage of many non-interfering memories.

Disclosures: **S.N. Chettih:** None. **E.L. Mackevicius:** None. **S. Hale:** None. **D. Aronov:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

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Topic: H.08. Learning and Memory

Support: New York Stem Cell Foundation Robertson Neuroscience Investigator Award
NSF Graduate Research Fellowship Program
Beckman Young Investigator Award
NIH T32 EY013933
NIH Director's New Innovator Award (DP2-AG071918)

Title: Topography of inputs into the hippocampal formation of a food-caching bird

Authors: *M. APPLEGATE[1], K. S. GUTNICHENKO[2], D. ARONOV[2];
[1]Columbia Univ. in the City of New York, New Haven, CT; [2]Columbia Univ., Columbia Univ., New York, NY

Abstract: The mammalian hippocampal formation (HF) is organized into domains associated with different functions. These differences are driven in part by the pattern of input along the hippocampal long axis, such as visual input to the septal hippocampus and amygdalar input to temporal hippocampus. HF is also organized along the transverse axis, with different patterns of neural activity in the hippocampus and the entorhinal cortex. In some birds, such as memory-specialist food-caching birds, a similar functional organization has been observed along both axes. These animals have enlarged hippocampi that are believed to facilitate their ability to find hidden caches in their environment. However, it was unknown what other food-caching specializations might exist in these animal's hippocampal circuits. Specifically, no prior connectivity studies of HF inputs had been performed in food-caching birds.
To address this, we mapped the inputs into HF of a food-caching bird, the black-capped chickadee. Using the retrograde tracer Cholera toxin subunit B, we made injections spaced along the long and transverse HF axes. We first cataloged the inputs to the HF of the food caching bird, finding some intriguing differences from the inputs observed in other avian species. For example, we identified a previously unreported robust projection from the lateral nidopallium.

Next, we asked how input varied along each axis. We compared two locations along the transverse axis: the hippocampus and the dorsolateral hippocampal area (DL), which is analogous to the entorhinal cortex. Similar to mammalian connectivity, we found that pallial regions predominantly targeted DL, while some subcortical regions like the lateral hypothalamus preferentially targeted the hippocampus. We then examined the hippocampal long axis and found that almost all inputs were topographic along this direction. For example, the anterior hippocampus was preferentially innervated by thalamic regions, while posterior hippocampus received more amygdalar input. Some of the topographies we found bear resemblance to those described in the mammalian brain, revealing a remarkable anatomical similarity of phylogenetically distant animals. More generally, our work establishes the pattern of inputs to HF in chickadees. Some of these patterns may be unique to chickadees, laying the groundwork for studying the anatomical basis of these birds' exceptional hippocampal memory.

Disclosures: M. Applegate: None. **K.S. Gutnichenko:** None. **D. Aronov:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

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Program #/Poster #: PSTR371.10/VV40

Topic: H.08. Learning and Memory

Support: NSF GRFP
New York Stem Cell Foundation (NYSCF-R-NI40)

Title: Neural representation of human experimenters in the bat hippocampus

Authors: *M. C. SNYDER[1], K. K. QI[3], M. M. YARTSEV[2];
[2]282 Li Ka Shing Ctr., [1]UC Berkeley, Berkeley, CA; [3]Univ. of California Berkeley, Univ. of California, Berkeley, Berkeley, CA

Abstract: Human experimenters are a ubiquitous feature of nearly all laboratory animal studies. Yet, it remains largely unknown whether and how the presence and behavior of humans in the experimental environment influences neural activity in the animal's brain. Here we addressed this question using Egyptian fruit bats because they quickly adapt to human handling and attend to human behaviors. Importantly, the bats' natural tendency to navigate using spatially structured flight patterns (Liberti et al., 2022) allowed us to isolate the influence of human behavior while rigorously controlling for the bat's behavioral variability. In the first set of experiments, bats flew to different humans present in the room to obtain fruit reward. Every few minutes the humans moved to new locations in order to extend spatial coverage. We wirelessly recorded neural activity from hippocampal area dCA1 while bats performed this reward-guided spatial behavioral task. We found that many hippocampal units activated in specific locations throughout the room, consistent with the positional code in the hippocampus found across various species, including in bats. Intriguingly, we found that most neurons' peak activity

occurred around the locations in the room where humans were standing. Furthermore, a substantial portion of the recorded neurons were modulated by the identity of the human at the landing location. To further investigate the extent to which hippocampal units encode information about the spatial behavior of humans, we performed a complementary experiment in which bats were stationary while humans moved around the room. We found that a subset of hippocampal units carried significant spatial information about the position of the different humans. Combined, these results demonstrate that hippocampal neurons are robustly modulated by the presence, movement, and identity of human experimenters during active navigation as well as during rest. These findings underscore the importance of accounting for human experimenter behavior in animal studies and provide a key demonstration of cross-species neural representation. William A. Liberti III*, Tobias A. Schmid*, Angelo Forli, Madeleine Snyder & Michael M. Yartsev (2022). Nature doi: 10.1038/s41586-022-04560-0

Disclosures: **M.C. Snyder:** None. **K.K. Qi:** None. **M.M. Yartsev:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

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Air Force Office of Scientific Research (FA9550-17-1-0412)
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Vallee Foundation (VS-2020-34)
Office of Naval Research (N00014-21-1-2063)

Title: Towards wireless Neuropixel recordings in freely flying Egyptian fruit bats

Authors: *K. K. QI, T. YOSHIDA, M. M. YARTSEV;
Univ. of California, Berkeley, Berkeley, CA

Abstract: Natural behaviors provide a rich basis for understanding brain function. The Egyptian fruit bat exhibits a large repertoire of naturally emerging social and spatial behaviors that likely involve large populations of neurons throughout different brain regions. To date, wireless electrophysiology in freely flying Egyptian fruit bats has originated (Yartsev & Ulanovsky, 2013), and has since been limited to, tetrode-based techniques that can be difficult to scale to large numbers of neurons and across multiple brain regions simultaneously. Recent advancements in large-scale electrophysiology techniques such as Neuropixels have enabled researchers to record hundreds to thousands of neurons from many brain areas simultaneously in freely behaving animals. However, whether such techniques can be implemented in freely flying

animals remains unknown. Here we describe the establishment of wireless electrophysiological recordings using Neuropixel 1.0 (NP1) probes in freely flying bats. Using custom implant geometries, we find that bats can carry multiple Neuropixel probes with little to no impact on their flight abilities. Our goal is to evaluate the feasibility of using Neuropixels for recording large populations of neurons across multiple brain regions simultaneously in flying bats. Our current efforts begin with recordings that include the hippocampus and entorhinal cortex, as these areas have been previously studied in this species using tetrodes and hence provide a basis for comparison. We will examine the feasibility of wireless NP1 recordings during (i) 3D spatial foraging (ii) 2D behavior and (iii) social interactions. We will present preliminary data on (a) validating the quality of NP1 recordings during flight and (b) recording populations of neurons simultaneously from multiple brain regions in bats.

References

Michael M. Yartsev & Nachum Ulanovsky. Representation of three-dimensional space in the hippocampus of flying bats (2013). *Science*., 340, pages 367-372.

Disclosures: K.K. Qi: None. **T. Yoshida:** None. **M.M. Yartsev:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

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Topic: H.08. Learning and Memory

Support: New York Stem Cell Foundation (NYSCF-R-NI40)
Air Force Office of Scientific Research (FA9550-17-1-0412)
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The Office of Naval Research (N00014-21-1-2063)
Searle Scholars Program (SSP-2016-1412)
Human Frontiers Fellowship (LT000302/2020))
EMBO (1022-2019)

Title: Hippocampal Representation During Collective Spatial Behavior in Bats

Authors: *A. FORLI[1], M. M. YARTSEV[2];
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Abstract: Social animals live and move through spaces shaped by the presence, motion, and sensory cues of multiple other individuals. Neural activity in the hippocampus is known to reflect spatial behavior yet has not been studied in such dynamic group settings, which are

ubiquitous in natural environments. Here, we studied hippocampal activity in groups of bats engaged in collective spatial behavior. We find that under spontaneous conditions a robust spatial structure emerges at the group level where behavior is anchored to specific locations, movement patterns and individual social preferences. Using wireless electrophysiological recordings from both stationary and flying bats, we find that many hippocampal neurons are tuned to key features of group dynamics. These include the presence or absence of a conspecific, but not typically of an object, at landing sites, shared spatial locations, individual identities, and sensory signals that are broadcasted in the group setting. Finally, using wireless calcium imaging, we find that social responses are anatomically distributed and robustly represented at the population level. Combined, our findings reveal that hippocampal activity contains a rich representation of naturally emerging spatial behaviors in animal groups which could in turn support the complex feat of collective behavior.

Disclosures: A. Forli: None. **M.M. Yartsev:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

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Program #/Poster #: PSTR371.13/VV43

Topic: H.08. Learning and Memory

Support: NIH Grant F32MH126643
NIH Grant DP2NS111657
NIH Grant RF1NS127123
The Whitehall Foundation
The Searle Scholars Program
The Sloan Foundation
The University of Chicago

Title: Place field dynamics as a window on synaptic plasticity in the hippocampus

Authors: *A. D. MADAR[1], C. DONG[2], M. E. J. SHEFFIELD[1];
[1]Neurobio., Univ. of Chicago, Chicago, IL; [2]Neurobio., Stanford Univ., Stanford, CA

Abstract: Changes in synaptic efficacy are widely thought to be a substrate for memory storage in the brain, but the rules and impact of synaptic plasticity are difficult to measure in vivo. We considered the dynamics of hippocampal place fields (PFs) as an indirect indicator of ongoing plasticity during memory formation and familiarization. By comparing 2-photon calcium recordings of CA1 and CA3 neurons in running mice with computational models of spiking place cells with plastic inputs, we found that Behavioral Timescale Synaptic Plasticity (BTSP), not classic Hebbian STDP, is the main mechanism underlying trial-by-trial PF shifting dynamics. Nonlinearities in these dynamics show that the probability of BTSP-triggering events exponentially decays after PF emergence and decreases with familiarity but continually drives

representational drift. Overall, our study uncovers the importance of BTSP in updating hippocampal representations and points to phenomenological differences between CA1 and CA3 in this form of plasticity.

Disclosures: **A.D. Madar:** None. **C. Dong:** None. **M.E.J. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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Support: NIH Grant F32NS124752
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The Whitehall Foundation
The Searle Scholars Program
The Sloan Foundation
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Title: Inhibition of dentate gyrus mossy cells disrupts CA1 place cell stability

Authors: *D. GOODSMITH, M. E. J. SHEFFIELD;
Univ. of Chicago, Chicago, IL

Abstract: The accurate formation and storage of memory requires a tradeoff between stability and flexibility; novel experiences must be flexibly encoded while allowing retrieval of stable representations of familiar events. Encoding and retrieval are often associated with the dentate gyrus (DG) and CA3 of the hippocampus, respectively. However, a balance between these processes must constantly be maintained within the broader DG/CA3 circuit. Mossy cells (MCs) in the DG hilus represent a crucial and understudied node in the DG/CA3 circuit. They receive convergent inputs from DG granule cells and CA3 pyramidal cells and can bidirectionally regulate granule cell activity. MCs may, therefore, coordinate the balance between memory encoding and retrieval in the DG/CA3 circuit based on novelty and task demands - promoting encoding in novel environments and retrieval in familiar environments. Despite their significance in the DG circuit, few studies have examined MC activity in awake, behaving animals. While MCs have been shown to have place fields in familiar environments, the spatial activity of MCs in novel environments and their influence on place cells in CA1, the primary output region of the hippocampus, remains unclear.
Using Drd2-cre mice to selectively image and manipulate MC activity, we performed two-photon calcium imaging in the dorsal hippocampus of head-fixed mice while they explored novel and familiar virtual linear tracks. We first recorded activity from 460 MCs in 8 mice. In novel environments, mossy cell activity and the stability of mossy cell place fields within a session were slightly decreased relative to familiar environments, suggesting that mossy cell activity is

regulated by environmental novelty. We next aimed to determine how MCs regulate CA1 circuit dynamics during novel and familiar environments. We recorded CA1 pyramidal cell activity following DREADD-mediated unilateral inhibition of MCs (3,796 cells from 7 mice) and in saline-injected control sessions in the same mice (2,759 cells from 5 mice). In both novel and familiar environments, mossy cell inhibition increased pyramidal cell activity in CA1 and caused the emergence of more place fields relative to saline-injected sessions. Mossy cell inhibition also reduced the spatial stability of CA1 place cell activity, both within a single session and across repeated exposures to the same environment. Intriguingly, this decrease in the stability of the CA1 population was more apparent in novel than familiar environments, suggesting that disruption of the DG/CA3 circuit via mossy cell inhibition prevents the development and stabilization of a new spatial map of novel experiences in CA1.

Disclosures: D. Goodsmith: None. **M.E.J. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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Topic: H.08. Learning and Memory

Support: NIH Grant 1DP2NS111657-01
T32 training grant T32DA043469
BRAIN initiative grant RF1NS127123

Title: Distinct sets of dopaminergic inputs in hippocampal CA1 transmit contrasting signals during behavior in a changing world

Authors: *C. HEER, S. KRISHNAN, M. SHEFFIELD;
Neurobio., Univ. of Chicago, Chicago, IL

Abstract: Dopaminergic activity in the hippocampus impacts synaptic plasticity, alters hippocampal neuron activity, and effects hippocampal dependent learning and memory processes. These influences have previously been attributed to dopamine release from sparse projections from the ventral tegmental area (VTA) to the hippocampus. However, it has recently been shown that locus coeruleus (LC) inputs to the dorsal hippocampus also release dopamine and impact hippocampal dependent learning and memory. To dissect the impacts of VTA and LC dopaminergic circuits on hippocampal activity and memory, it is necessary to first compare how these inputs are differently active during the exact same behaviors and learning contexts. Therefore, using 2-photon microscopy, we functionally imaged the activity of VTA and LC axons in the CA1 of dorsal hippocampus in head fixed mice as they navigated linear tracks in virtual reality (VR) environments. In familiar environments, during the approach to learned rewarded locations, VTA axons in CA1 exhibit ramping activity that peaks near reward locations, while LC axons in CA1 show no such ramping-to-reward activity. Instead, LC axon

activity is correlated to the animals' running speed. However, when mice are switched to a novel VR environment, LC axon activity abruptly increases and remains elevated for more than 1 min. On the other hand, the ramping-to-reward activity in VTA axons disappears upon the switch to a novel environment. Together these findings reveal that the two sources of dopamine in dorsal CA1 of the hippocampus encode distinct information and therefore likely play different roles in modulating hippocampal activity during behavior and learning.

Disclosures: C. Heer: None. **S. Krishnan:** None. **M. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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Support: The Whitehall Foundation
The Searle Scholars Program
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1DP2NS111657-01
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Title: Thalamic Nucleus Reuniens Inputs to Hippocampal CA1 Suppress Contextual Fear Memory Retrieval

Authors: *H. C. RATIGAN[1], **Y. WANG**[2], V. BARRETO[3], M. E. J. SHEFFIELD[4];
[1]Univ. of Chicago Committee On Neurobio., Chicago, IL; [2]Univ. of Chicago Committee on Neurobio., Chicago, IL; [4]Dept. of Neurobio., [3]Univ. of Chicago, Chicago, IL

Abstract: Retrieving memories of fearful experiences is essential for survival but can become maladaptive when these memories trigger lasting inappropriate fear responses. Fear memories can be acquired through contextual fear conditioning (CFC) and their retrieval and modification depend on both the medial prefrontal cortex (mPFC), and dorsal hippocampal CA1 (dCA1). However, there is no direct mPFC to CA1 path, leaving unclear how these regions coordinate to retrieve or alter fear memories. One route is through the Nucleus Reuniens (NR), a higher-order thalamic region well-connected to emotional regulation areas. MPFC drives NR and sends an excitatory monosynaptic input to the Stratum Lacunosum Moleculare (SLM) of CA1, where it synapses with CA1 dendrites. Both the mPFC-NR pathway and NR itself are necessary for contextual fear memory extinction, but the NR-CA1 pathway's role is unknown. To test the role of NR during fear memory formation and subsequent modification, we developed a novel virtual reality contextual fear memory paradigm (VR-CFC) using mild tail-shocks to induce context-dependent fear (freezing). Then, by using targeted chemogenetic NR-CA1 inactivation and in-

vivo 2-photon calcium imaging, we examined the activity of NR-CA1 axons, dCA1 pyramidal somas, and their corresponding dendrites in SLM during VR-CFC. We found that inactivation of the NR-CA1 pathway prolongs fearful freezing epochs, induces fear generalization, and delays fear extinction in mice. We also found that NR-CA1 axons become selectively tuned to freezing only after CFC, and this activity is well-predicted by an encoding model. This suggests the NR-CA1 pathway actively suppresses fear responses, possibly by disrupting contextual fear memory retrieval during freezing. To further test this idea, we are collecting data from large populations of CA1 pyramidal cells and their dendrites in SLM during fear memory retrieval both with and without NR-CA1 inactivation. We aim to determine how NR interacts with CA1 to exert its memory suppression effects at both the synaptic and population level.

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Poster

PSTR371. Hippocampal Formation Circuitry I

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Topic: H.08. Learning and Memory

Support: DP2(5-20335)
R21(5-21302)
RFI(5-20064)
RF1NS127123

Title: Synchronous ensembles of hippocampal CA1-CA3 neurons support memory encoding and retrieval

Authors: *C. DONG[1,2], S. KRISHNAN[3], M. E. J. SHEFFIELD[2];
[1]Stanford Univ., Santa Clara, CA; [2]Neurobio., [3]Univ. of Chicago, Chicago, IL

Abstract: The hippocampus is critical for spatial and episodic memory and consists of different subregions (dentate gyrus, and the cornus ammonis CA regions: CA1, CA2/CA3) that have been theorized to participate in different memory processes owing to their distinct inputs and internal circuitry. CA1 receives input from CA3 and while theoretical models have implicated these two regions to have different processing capabilities and functions, it is unclear how interactions between these two regions may contribute to episodic memory formation and recall. Here, we implemented a novel behavioral and imaging paradigm that enables contextual fear conditioning (CFC) in virtual reality (VR-VFC) with simultaneous two-photon calcium imaging of hippocampal CA1 and CA3. Head-fixed mice navigating virtual contexts underwent VR-CFC and fear memory recall was assessed across multiple days until fear memory extinction. We observed fear behaviors in head-fixed mice in VR similar to freely moving animals. Neural recordings revealed ensembles of neurons with synchronized activity within and across both

regions. CA1 neurons had more synchronous events compared to CA3, however a higher proportion of CA3 neurons were recruited into ensembles during each synchronous event. Connecting ensemble activity to CFC, we found the frequency of synchronous events occurring simultaneously across both CA1 and CA3 during fear memory acquisition predicted the degree of fear memory retrieval in each mouse (as assessed by the degree of freezing in the fear conditioned environment). Thus, we propose that CA1-CA3 ensemble synchrony is critical for the encoding and retrieval of memories, possibly through facilitating synaptic plasticity between ensembles in the hippocampus. Our findings provide new empirical data to build upon existing theoretical models on the role of hippocampal subregion interactions in episodic memory.

Disclosures: C. Dong: None. **S. Krishnan:** None. **M.E.J. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR371.18/VV48

Topic: H.08. Learning and Memory

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Title: Contributions from the left and right CA3 on the formation and refinement of CA1 place fields during learning

Authors: *A. JIANG, M. E. J. SHEFFIELD;
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Abstract: In a novel environment new place fields in hippocampal CA1 form during navigation and become more precise and stable with experience. This process is thought to reflect spatial learning. Existing literature suggests CA3, which sends bilateral glutamatergic inputs to CA1, is the main input source driving the dynamics of CA1 place fields. However, the independent contributions from the left and right CA3 inputs to the formation, precision, and stabilization of place fields in CA1 is unknown. In this study, we used 2-photon calcium imaging to record from large populations of CA1 pyramidal cells while optogenetically inactivating either the left contralateral CA3 inputs, or right ipsilateral CA3 inputs, in right CA1 during navigation in a novel environment. We found that inhibiting either the left or right CA3 inputs to CA1 during the initial moments in the novel environment significantly delayed the emergence of CA1 place fields, suggesting both hemispheres of CA3 are involved in the rapid formation of CA1 place fields. Next, we examined the roles of left and right CA3 inputs after place fields had formed in CA1. Inactivating left CA3 inputs significantly reduced the gradual improvements of CA1 place field precision and stability that normally occur with experience, but inactivating right CA3 inputs had no such effect. Further, when tracking the same CA1 cells over days, we found that

the optogenetic inhibition of left CA3 inputs on the first day had long-term effects in reducing the precision and stability of CA1 place fields on day 2, yet this was not observed with right CA3 inhibition. This result suggests the left CA3 input to right CA1 is specialized in refining and stabilizing CA1 place fields during familiarization to a novel environment, whereas the right CA3 is not. In summary, our research shows that both hemispheres of CA3 are involved in the rapid formation of CA1 place fields upon exposure in a novel environment, but left CA3 inputs are required for place field refinement and stabilization during experience-based learning.

Disclosures: A. Jiang: None. **M.E.J. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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University of Chicago Institute for Neuroscience

Title: Internal states shape hippocampal spatial representations through neuromodulation

Authors: *S. KRISHNAN[1], C. M. HEER[1], A. CAO[2], A. JAMES, Jr[1], M. E. J. SHEFFIELD[1]; [1]Univ. of Chicago, Chicago, IL; [2]Northwestern Univ., Chicago, IL

Abstract: Internal states play a critical role in the formation and retrieval of episodic memories. Rewards and punishment are major influences on internal states. For instance, events that lead to rewards may result in an internal state of reward expectation, or events where predators are encountered may lead to a fearful internal state. Memories associated with these internal states are better remembered as they are essential for survival. The hippocampus is critical for the formation and retrieval of such episodic memories, with representations of space in the CA1 subregion thought to be a key component of these memories. However, at the cellular level, how internal states influence spatial memory encoding in the hippocampus is yet to be fully understood. To investigate this, we performed two experiments while imaging from hippocampal neurons in mice traversing VR environments. In experiment 1, we designed a task to extinguish a previously established internal state of reward expectation. We found that when reward expectation is extinguished, hippocampal spatial representations of the environment become

unreliable across trials and unstable across days at the single cell level, indicating that reward expectation is necessary for reliable encoding of memories by the hippocampus. We also found that dopamine axons from the Ventral Tegmental Area (VTA) in CA1 exhibited a ramping-to-reward signal that is dependent on reward expectation and inhibiting VTA dopamine neurons largely replicated the effects of reward expectation extinction on hippocampal neurons. This provides a mechanism by which dopamine modulates hippocampal spatial representations based on internal state. In experiment 2, we trained mice in an alternating reward task, where mice received a reward on one trial but not on the next. Mice that learnt the task, were able to behaviorally distinguish between the rewarded and unrewarded trials by exhibiting reward expectation specifically on rewarded trials and not in the unrewarded trials. We found that these mice had distinct hippocampal representations of the environment on the two trials compared to mice that did not learn the task. This shows that distinct internal states can lead to distinct representations in the hippocampus of the same environment, and mice are able to rapidly switch between them. Efforts are ongoing to investigate the influence of dopamine neurons in the emergence and maintenance of these distinct representations. Thus, we conclude that internal states of reward expectation influence the encoding of hippocampal dependent memories by modulating spatial representations through neuromodulatory circuits that project to the hippocampus.

Disclosures: **S. Krishnan:** None. **C.M. Heer:** None. **A. Cao:** None. **A. James:** None. **M.E.J. Sheffield:** None.

Poster

PSTR371. Hippocampal Formation Circuitry I

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Topic: H.08. Learning and Memory

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Title: Formation of hippocampal cognitive maps revealed by longitudinal cellular imaging during learning

Authors: *W. SUN, J. WINNUBST, M. NATRAJAN, C. LAI, K. KAJIKAWA, M. MICHAELOS, R. GATTONI, J. E. FITZGERALD, N. SPRUSTON;
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Abstract: The hippocampal formation is essential for an animal's ability to navigate and forage effectively in complex environments. It contributes by forming structured representations of the environment, often called cognitive maps. While many experimental and theoretical aspects of learned hippocampal cognitive maps are well established, the exact learning trajectories for their formation and usage remain unknown. We performed large-scale 2-photon calcium imaging of thousands of neurons in mouse CA1 and tracked neural activity of the same neurons over several

weeks, while the animals learned a linear two-alternative choice task in virtual reality (VR) environment. We used various manifold discovery techniques to visualize the high-dimensional neural data over the entire learning period and found that each animal went through a stereotyped transition of learning stages, demarcated by distinct low-dimensional embeddings and decorrelations of neural activity at key positions along the VR track, which paralleled improvements in task performance. Our results indicate that the evolution of hippocampal representations during learning reflects the extraction of task-related features that correlate temporally with the animal's evolving performance. Furthermore, the learned structures appear to be reused in novel tasks, suggestive of transfer learning. By designing and simulating a variety of cognitive models and neural network models, we could reproduce key features of both animal behavior and neural activity. The ability to monitor the formation of cognitive maps over weeks-long periods of learning provides a platform for developing and testing hypotheses regarding the underlying plasticity mechanisms, cell types, circuits, and computational rules responsible for adaptive learning.

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Poster

PSTR371. Hippocampal Formation Circuitry I

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Title: Decoding and manipulating memory retrieval in the subiculum with multiphoton holography

Authors: *D. A. O'NEIL, R. YUSTE;
Neurotechnology Center, Dept. of Biol. Sci., Columbia Univ., New York, NY

Abstract: The primary output node of the hippocampus—the subiculum—has proven critical to the integrity of memory retrieval and is robustly associated with the memory impairments observed in Alzheimer's disease. However, despite a rich history of memory research in the hippocampus, the subiculum and its role in memory remain critically understudied. To this end, we leverage large-scale multi-photon calcium imaging alongside holographic photostimulation to decode and manipulate subicular activity in behaving mice. We have constructed an experimental setup in which we are able to simultaneously measure (calcium imaging) and

manipulate (holographic optogenetics) neural activity in mice during trace fear conditioning. In this assay, mice learn to associate a 15 second auditory stimulus and an innately aversive stimulus (air-puff) despite their separation by a 10 second stimulus-free trace period. Following successful learning, the presentation of this auditory stimulus evokes a conditioned fear response (i.e., fear memory recall). In our implementation, the conditioned fear response takes the form of burrowing: head-fixed mice retract a lightweight "burrow" that slides along a frictionless rail. This behavior is innate, requires no training, and is clearly demarcated from an induced-startle response and escape behavior. Our behavioral timeline consisted of a 10 trial pre-exposure stage, 20 trial encoding stage, and 10 trial retrieval stage on three consecutive days. Each trial was initiated only after the mouse has determined it was safe (15 seconds without retreat) and were separated by a 90 second inter-trial interval. Comparison of the behavioral response to the conditioned stimulus (CS+) vs neutral stimulus (CS-) revealed mice reliably associated the CS+ with the UCS ($n=7$, $p<0.01$). Our calcium imaging data (>500 neurons / mouse) demonstrates that subicular activity contains a diversity of dynamical subtypes and mixed-selective tuning profiles. We find a variety of task-relevant features are reliably decodable from this data using linear support vector classification—even when disentangling spatial information. Our preliminary conclusion is that the diversity of subicular coding relays memories to diverse downstream targets in a manner that is robust and easily-interpretable. By developing a better understanding of subicular contributions to memory recall, the studies proposed here will solidify our understanding of memory retrieval and illuminate new avenues for understanding its pathological dysfunction.

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Poster

PSTR371. Hippocampal Formation Circuitry I

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John R. Evans Leaders Fund 38369).

Title: Atypical excitatory neurons of the hippocampus represent novelty and toggle novelty seeking

Authors: *A. KINMAN[1], S. ERWIN[2], D. N. MERRYWEATHER[1], R. CAMPBELL[1], L. KRAUS[1], K. SULLIVAN[3], M. ELDER[1], S. WOOD[1], B. BRISTOW[1], E. KIM[1], W. DANIELS[1], M. ANWER[3], C. GUO[5], M. S. CEMBROWSKI[4];
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Abstract: Hippocampal neural representations are typically studied from the perspective of pyramidal cells, wherein a single class of cells can flexibly represent diverse features of space and memory. Recent work suggests that pre-assigned subtypes of excitatory hippocampal neurons exist, defying the view that relative cellular homogeneity drives the diverse functions of the hippocampus. Here, we reveal an excitatory non-pyramidal subtype of hippocampal neuron that defies this conventional cell-type structure and functionality flexibility. This neuron subtype — the "ovoid" neuron — is located spatially adjacent to subiculum pyramidal cells, yet markedly varies in gene expression, morphology, electrophysiology, connectivity, and function. Ovoid cells are transcriptomically and morphologically unique from pyramidal cells with uncharacteristically singular long-range projections. Functionally, ovoid cells are remarkably excitable compared to their pyramidal counterparts when assayed via electrophysiology and show slow, sustained activity that acts on the order of tens of seconds at a time when imaged with 1-photon calcium imaging. Despite the subiculum being heavily implicated in spatial navigation, ovoid neurons show prominent tuning to object novelty but not spatial novelty, such that novel object encounters selectively drive ovoid neuron responses, whereas familiar objects fail to elicit activity even months after single-trial learning. Remarkably, for objects encountered in either the recent (24 hours) or remote past (50 days), exogeneous activation of ovoid neurons via optogenetic excitation is sufficient to evoke familiarity seeking over the expected novelty seeking response. These results highlight a novel cell type that defies conventional hippocampal timescales, functions, and illustrates subtype- and experience-specific feature selectivity in the hippocampus. Thus, ovoid neurons form a new subtype-specific layer of hippocampal computation operating across multiple behavioral timescales, selectively embodying the cellular expression, and controlling the behavioral expression of a non-spatial memory.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Title: Functional organization and local processing of hippocampal output signals in medial entorhinal cortex layer VI

Authors: M. RANNAP, *A. DRAGUHN, A. V. EGOROV;
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Abstract: The deep layers (V/VI) of the entorhinal cortex constitute a major relay station for routing neuronal activity from the hippocampus to downstream networks. We recently found marked dorsoventral differences in the structural and functional organization of hippocampal projections to layer V (LV) of the medial entorhinal cortex (MEC) (Ohara, Rannap et al., Cell Rep. 2023). In comparison, little is known about potential dorsoventral gradients of the hippocampal output to MEC layer VI (LVI) and how LVI neurons are integrated into the MEC micro-network. Here, we performed whole-cell patch-clamp recordings from MEC LVI neurons in horizontal brain slices of adult male mice. LVI neurons comprised mainly horizontal or multipolar cells. The axons of LVI neurons travelled towards the subiculum and to superficial layers of MEC, indicating a role in entorhinal-hippocampal feedback loops. Reciprocal connections between excitatory LVI neurons in paired recordings were relatively sparse (7/119; 6%) and no interconnections between LVI and LVb (0/112) or LVI and LVa (0/57) glutamatergic neurons were found. However, weak responses of LVI neurons were found after optogenetic activation of multiple LVa neurons, which were selectively infected with a ChR2-expressing AAV using the Rbp4-Cre mouse line. These responses were present at both dorsal and ventral MEC levels. To study hippocampal-MEC LVI projections along the dorsoventral axis, we injected the ChR2-expressing AAV into the dorsal or ventral hippocampus (CA1/subiculum) and activated the axons of infected neurons with blue light. Dorsal hippocampal projections were confined to the dorsal MEC and their activation elicited weak monosynaptic responses in only half of recorded LVI neurons (10/22). In contrast, ventral hippocampal projections innervated both dorsal and ventral MEC and elicited strong monosynaptic responses in nearly all recorded LVI neurons (30/31). Our findings reveal a specific functional architecture and network integration of MEC LVI and suggest that MEC LVI-mediated signal propagation is preferentially controlled by the ventral hippocampus.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Title: Comparing Visual Associative Learning in parahippocampal networks

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Abstract: Combinding sensory information with valence is essential to learn and develop behaviors. The established view suggests that contextual information is processed by the lateral entorhinal cortex (LEC) and spatial information by medial entorhinal cortex (MEC). Recent work in the rat shows that POR, believed to carry most of the visiospatial information to the MEC, predominantly projects to the LEC with only minor inputs to the MEC. This questions the functional differences between the MEC and LEC in processing of contextual information. We therefore aim to investigate how non-spatial visual association learning is represented in MEC and LEC in comparison to the upstream area POR.We used 2-photon calcium imaging to track neural activity while mice learned a visual association task. Head-fixed mice are trained over multiple sessions while large neuronal populations are recorded during and after training using two-photon imaging. Utilizing custom right-angle prism assemblies and local injections of GECIs allow tracking the same neuronal population over time. Cue-selective tuning was present in a fraction of POR cells even in naive animals. In contrast, MEC exhibited only a small fraction of cells with cue-related but not cue-specific tuning at this stage. The results from ongoing analyses unveiled learning-induced changes in the POR and MEC populations. These changes were evident as selective tuning to the rewarded visual cue within a substantial portion of the imaged cell population. Notably, we observed a difference in timing of the appearance of selective tuning. The POR neurons increased their responses to salient cues early in training, while the tuning in MEC neurons appeared later in concert with behavioral improvement. To investigate how these learning-induced changes are integrated into the network activity, we also recorded in the offline state, after daily training. We observe cue-selective reactivations of neurons in POR, as previously reported. Future analysis will reveal if and how reactivations shape the learning-induced activity changes in EC. Data from LEC has been collected and will be compared with that of MEC and POR.Our results show that performance is closely reflected i the development of neuron response properties suggesting that these areas are involved in visual associative learning.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Research Council of Norway

Title: Visual Associative Learning in The Medial Entorhinal Cortex

Authors: *I. NYMOEN[1], *I. NYMOEN[1], F. ROGGE[1], M. E. LEPPERØD[2], T. HAFTING-FYHN[2], K. K. LENSJØ[3], M. FYHN[4];
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Abstract: Mammals show a significant capacity for contextual knowledge transfer, effectively applying acquired information to novel situations. Such generalization involves extracting common features from diverse experiences and utilizing them to make predictions or guide behavior in unfamiliar contexts. It remains unknown which brain areas are responsible for such abstractions. Neural representations in the medial entorhinal cortex (MEC) constitute a generalized map of the local space, but recent findings may suggest that the MEC possesses non-spatial dimensions suggesting a generalized network for learning state spaces irrespective of modalities.
In this study, we investigate the functional properties of the dorsal layer II/III MEC neurons during learning of a non-spatial, go/no-go visual association task. Head-fixed mice are trained over multiple sessions while recording neural activity during and after training using two-photon imaging. Utilizing our newly developed soma-targeted calcium sensor (Soma-GCaMP8s) we have recorded the activity of identified neurons and populations from the animal's initial naive state to an expert level performance in the behavioral task. To examine the generalization of structural components within the task, we introduce rule changes while monitoring performance and neural responses during learning the new rules.
We find profound learning-induced changes in the MEC population, with an increasing fraction of the neuron population showing tuning to the rewarded visual cue. Interestingly, many of the responsive neurons exhibit a dichotomy of positive and negative response profiles. Strikingly, following the same neuron population over the course of learning, shows that the development of the response pattern is reflected in discrete anatomical cell clusters in the field of view. Upon rule switching, a fraction of the population follows the cue-outcome while a small number of units follows the cue identity.
In summary, our data show that the neurons in MEC develop strong and highly selective responses to salient visual cues in a non-spatial task.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Alzheimer's Association Research Grant AARG-17-532932

Title: Disrupted associative memory encoding in the lateral entorhinal cortex of amyloid precursor protein knock-in mice

Authors: *T. NAKAGAWA, J. L. XIE, M. SAVADKOHI, J. Y. LEE, H. JUN, K. M. IGARASHI;
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Abstract: Alzheimer's disease (AD) is a leading cause of dementia. Previous fMRI studies have shown that the lateral entorhinal cortex (LEC) is the primary site of dysfunction in patients with early-stage AD. However, the circuit mechanisms involved are unknown. We recently found that the LEC neurons in healthy animals are critically involved in associative memory encoding (Lee et al., Nature 2021). To test if this LEC activity is affected in AD, we recorded LEC neurons in amyloid precursor protein knock-in (APP-KI) mice engaging in an odor cue-reward task. APP-KI mice showed associative memory impairment. LEC neurons in APP-KI mice showed the disruption of associative memory encoding. Our results suggest that the disruption of encoding in LEC neurons may lead to associative memory deficit in APP-KI mice.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Title: Associative memory encoding in lateral entorhinal cortex deep layers

Authors: *J. LEE, H. JUN, N. BLEZA, A. ICHII, K. M. IGARASHI;
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Abstract: Where are associative memories formed in the brain? We previously found that lateral entorhinal cortex (LEC) layer 2a (L2a) neurons projecting to the hippocampus develop activity which dynamically represents learning of a cue-reward association (Lee, Jun et al, Nature 2021).

However, the role of neurons comprising the deep layer of the LEC remains unclear. The layer 5/6 (L5/6) neurons of the LEC receive major input from the hippocampus and relay to various neocortical regions. To identify the memory encoding properties of LEC L5/6, we performed opto-tagged spike recordings using LEC L5/6-specific genetic marker Rbp4-cre mice. As mice learned associations, the LEC L5/6 neuron population dynamically represented rewarded cues together in one category, and non-rewarded cues together as a separate category. This contrasts with L2a neurons, which lacked categorization of non-rewarded cues. These results suggest a critical role of deep layer LEC in representing behaviorally relevant category information in support of associative learning.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Program #/Poster #: PSTR372.06/VV58

Topic: H.08. Learning and Memory

Title: Lateral entorhinal cortex projections to CA1 encode stronger odor and time-specific information compared to medial entorhinal cortex during non-spatial working memory task performance

Authors: *C. DORIAN[1], J. TAXIDIS[2], P. GOLSHANI[3];
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Abstract: Hippocampal CA1 neurons generate sequential firing during the performance of an odor-based working memory task, encoding both odor and the passage of time. How does CA1 integrate its various inputs to give rise to sensory and temporal representations? Entorhinal cortex (EC) neurons project to CA1 distal dendrites through the temporoammonic path and have been shown to represent the passage of time over long durations. Yet it is still poorly understood how lateral and medial EC (LEC and MEC) inputs to CA1 encode odor or temporal information. By comparing activity patterns of LEC and MEC projections to CA1, we investigated the relative strength of odor and temporal specific information encoding during working memory performance. We therefore conducted 2-photon calcium imaging of LEC and MEC axons expressing GCaMP7s within the stratum lacunosum moleculare layer of CA1, while mice performed an olfactory-based delayed non-match to sample working memory task with a 5 second delay. While both groups of axons significantly encoded odor information throughout the odor presentation and delay period, LEC axons were more odor-selective compared to MEC axons. We trained a linear decoder to predict odor-identity during the odor and delay periods; we found that LEC axons carry stronger odor-specific information during odor presentation and the

delay period than MEC axons. While both LEC and MEC inputs show sequential activity during the delay period, the slopes of these sequences were dramatically different between groups. In LEC, 23% +/- 4% of axons have significant firing fields during the first odor presentation or delay period. Of those, 47% +/- 5% fire maximally during odor presentation, and the other 53% +/- 5% during the delay period. In MEC, a surprising 48% +/- 3% of axons have significant firing fields, but the vast majority at 75% +/- 3% have their maximal firing field during the first odor presentation. Despite this over-representation at odor presentation, these MEC axons were poorly selective for odor. This suggests that MEC inputs to the hippocampus may provide reliable excitation to CA1 at stimulus presentation, coincident with the arrival of odor-specific information from LEC. We are currently performing experiments to silence LEC or MEC while imaging calcium activity of CA1 pyramidal neurons to determine how these direct pathways drive multimodal sequential activity seen in CA1.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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PENN Rad Onc Dept (PI: Eisch. & Fan)

Title: Investigating the role of the lateral entorhinal cortex-hippocampal circuit in the memory stages of behavioral pattern separation

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Abstract: The ability to discriminate highly similar episodes is termed behavioral pattern separation (BPS). This episodic memory process occurs in stages (encoding, consolidation, retrieval), is altered in humans with and rodent models for aging and many brain disorders. In BPS, it is more challenging to discriminate episodes that are very similar (high load) vs. different (low load). Defining the neural circuitry that supports memory stage- and load-specific BPS is a key step to a future where neuropathology-induced cognitive dysfunction may be treated via circuit-based manipulations. Here we interrogate how BPS in mice is influenced by the activity of the lateral entorhinal cortex (LEC) - an integrator of spatial and nonspatial information - and its projections to the hippocampus. We focus on this circuit because correlative studies in humans and lesion studies in rodents implicate LEC-dentate gyrus (DG) circuit integrity in BPS. Also, chronic stimulation of LECIIa fan cells (whose target regions include the DG) improves BPS. This evidence shapes our central hypothesis: LECIIa fan cells play a memory stage- and load-specific role in BPS in mice. To test this, we utilize the spontaneous location recognition test (SLR), which allows relative isolation of distinct memory stages of BPS, and chemogenetic manipulation of LECIIa fan cells that project to the hippocampus and DG. Viral-mediated expression of an inhibitory chemogenetic DREADD (hMDi) or a control protein (mCherry) was infused into the LEC of Sim1-cre male mice (7-wks-old). Four weeks post-infusion, mice underwent SLR with three different loads: low (dissimilar [d-]), high (extra-similar [xs-]), and medium (similar [s-]). For each load, mice underwent encoding (or sampling), consolidation (45 min), and retrieval (or testing). To test the acute necessity of LEC fan cells for all stages, a DREADD actuator (C21) was given 30min prior to sampling phase of each SLR test. Control mice explored a novel location more than a familiar location in d- and s-SLR but not in xs-SLR, indicating xs-SLR is not appropriate for this BPS test. hM4Di and mCherry mice spend the same time exploring the novel location in d-SLR. However, in s-SLR hM4Di mice spent less time exploring the novel location relative to mCherry mice. Thus, LEC fan cell activity during sampling and testing is required for BPS. We are now harnessing the temporal specificity of optogenetics to assess the role of LEC-DG circuit activity in more discrete memory stages, such as consolidation. Our study provides essential behavioral and mechanistic insight to understand BPS and to fuel therapeutics to combat disease-induced cognitive dysfunction.

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Poster

PSTR372. Hippocampal–Entorhinal Cortex Circuit

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Program #/Poster #: PSTR372.08/VV60

Topic: H.08. Learning and Memory

Support: DFG SFB 1089 – Project A05

Title: HCN channels in the medial entorhinal cortex are crucial for the consolidation of single-trial spatial learning.

Authors: *R. M. NEVES[1,2], H. KANEKO[1,3], K. MEIER[4], A. MERSEBURG[1,2], F. MORELLINI[4], S. REMY[1,3], D. ISBRANDT[1,2], S. MARGUET[1,2];
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Abstract: Hyperpolarization-activated cyclic nucleotide-gated (HCN) channels conduct the h-current (I_h) which is an important regulator of neuronal excitability as it contributes to the resting membrane potential, dendritic integration, and subthreshold resonance in the theta-frequency range. To investigate the functional role of I_h in the medial entorhinal cortex (MEC), we generated a transgenic mouse line expressing a dominant-negative HCN subunit (HCN-DN) specifically in MEC LII stellate cells (MEC-SCs) under the control of the Neuropsin (Nop) promoter. The HCN-DN subunit, upon assembly with endogenous HCN1-4 alpha subunits, renders the channel tetramer non-functional. We found that mice expressing HCN-DN in MEC-SCs exhibited a specific impairment in short/single-trial but not in multi-trial long-term spatial learning. In addition, Nop-HCN-DN mice showed significantly fewer c-fos positive labeled neurons in the hippocampal CA1, CA3 and Dentate Gyrus (DG) subregions following single-trial fear conditioning, and showed a suppression of delta (<4 Hz) and high gamma (>90 Hz) oscillations in the DG of awake head-fixed mice. Although HCN-DN expression abolished the theta resonance and the “sag” potential in acute MEC slice recordings, I_h ablation in MEC-SCs showed no effect on theta oscillations in the MEC or hippocampus. In order to test whether the effect of I_h loss can be replicated or rescued by altering MEC-SC excitability, we co-expressed our transgene with an excitatory or inhibitory (hM3Dq or hM4Di, respectively) DREADD receptor (designer receptor exclusively activated by designer drug) and we activated with compound 21 (C21). We found that the administration of C21 prior to memory acquisition rescues behavioral performance and c-fos expression levels, and normalizes DG delta and high gamma oscillations. Moreover, C21 administration immediately after behavioral acquisition equally restored spatial memory, indicating that loss of I_h in MEC-SCs disturbed memory consolidation. Finally, C21-induced activation of MEC-SCs expressing hM3Dq alone impaired behavioral performance, increased c-fos baseline expression levels, and augmented high gamma activity; whereas, conversely, C21-induced inhibition reproduced the behavioral and physiological effects of HCN-DN induced I_h ablation. Together, these results indicate that I_h in MEC-SCs is crucial for maintaining an optimal excitability balance necessary for rapid acquisition of spatial information following brief exposure to the environment.

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Poster

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Topic: H.08. Learning and Memory

Title: Phase-locking of entorhinal neurons to CA1 and CA3 theta oscillations during recent and very remote memory retrieval - correlates of memory precision?

Authors: *H. MULLA-OSMAN[1], S.-P. KU[1], E. ATUCHA[1], S. CALABRESE[1], A. REBOREDA[1], K. ALLEN[2], M. YOSHIDA[1], M. SAUVAGE[1];
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Abstract: Recent studies show that CA3 is engaged for retrieving recent and early remote memories in mice (i.e. up to 1-month-old memories) while CA1 is involved independently of the age of the memory (i.e. up to 1-year-old memories, comparable to 40-year-old memories in humans based on life expectancy; Bartsch et al., 2011; Lux et al., 2016). These studies also indicate that parahippocampal areas, including the medial entorhinal cortex (MEC), provide support to CA1 for the retrieval of the most remote memories (Lux et al., 2016; Atucha et al., 2021). The selective disengagement of CA3 over time suggests a shift from a greater contribution of the trisynaptic (EC-DG-CA3-CA1) and the temporoammonic (EC-CA1) pathways for the retrieval of recent memories to a preferential engagement of the temporoammonic pathway for recalling very remote memories that have naturally lost their precision (gist-like memories). Hippocampal theta oscillations play an important role in spatial navigation and are associated with memory processes, but recent findings also indicate that the synchronization of population spiking activity with theta oscillations (spike-to-theta phase-locking) across brain regions is crucial for successful recognition memory (Ku et al., 2023). Here, we record single units and LFP signals *in-vivo* simultaneously in CA1, CA3 and MEC during contextual fear memory retrieval 1 day and 1 year after memory formation in distinct groups of mice and compare the cross-regional interaction between the spiking of MEC cells to CA1 or CA3 theta oscillations. Preliminary data indicate stronger changes in the coordination of MEC activity with CA3 than with CA1 when comparing precise (1-day-old) and gist-like (1-year-old) memories. This supports the hypothesis of a shift over time between MTL pathways for the retrieval of memories. These results bring further insights into the contribution of the trisynaptic and temporoammonic pathways in memory retrieval as memories age and suggest a differential contribution of these pathways to memory precision. Moreover, our data indicate that the coordination of MEC spikes with CA1 or CA3 theta oscillations might be one of the underlying mechanisms supporting recognition memory.

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Poster

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Title: Structural plasticity in the monkey entorhinal and perirhinal cortices following selective hippocampal lesion

Authors: *J. VILLARD[1], L. J. CHAREYRON[1], O. PIGUET[1], P. LAMBERCY[1], G. LONCHAMPT[1], P. BANTA-LAVENEX[2], D. G. AMARAL[3], P. LAVENEX[1];
[1]Univ. of Lausanne, Lausanne, Switzerland; [2]UniDistance Suisse, Brig, Switzerland; [3]Univ. of California Davis, Sacramento, CA

Abstract: Immature neurons expressing the Bcl2 protein are present in various regions of the mammalian brain, including the amygdala and the entorhinal and perirhinal cortices. Their functional role is unknown but we have previously shown that neonatal and adult hippocampal lesions increase their differentiation in the monkey amygdala. Here, we assessed whether hippocampal lesions similarly affect immature neurons in the entorhinal and perirhinal cortices. Since Bcl2-positive cells were found mainly in areas Eo, Er and Elr of the entorhinal cortex and in layer II of the perirhinal cortex, we also performed stereological analyses on Nissl-stained sections to determine the number and soma size of immature and mature neurons in layer III of area Er and layer II of area 36 of the perirhinal cortex. We found different structural changes in these regions following hippocampal lesions, which were influenced by the time of the lesion. In neonate-lesioned monkeys, the number of immature neurons in the entorhinal and perirhinal cortices was generally higher than in controls. The number of mature neurons was also higher in layer III of area Er of neonate-lesioned monkeys but no differences were found in layer II of area 36. In adult-lesioned monkeys, the number of immature neurons in the entorhinal cortex was lower than in controls but did not differ from controls in the perirhinal cortex. The number of mature neurons in layer III of area Er did not differ from controls, but the number of small, mature neurons in layer II of area 36 was lower than in controls. In sum, hippocampal lesions impacted populations of mature and immature neurons in discrete regions and layers of the entorhinal and perirhinal cortices, which are interconnected with the amygdala and provide major cortical inputs to the hippocampus. These structural changes may contribute to some functional recovery following hippocampal injury in an age-dependent manner.

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Poster

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Title: Investigating the role of medial septum glutamatergic neurons in spatial and speed coding in the medial entorhinal cortex

Authors: *J. ROBINSON[1], M. E. HASSELMO[1], M. P. BRANDON[2];
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Abstract: The medial entorhinal cortex (MEC) is integral in spatial navigation and memory processing. The MEC contains several cell types which together represent different spatial aspects of the current environment, these neurons include 1) grid cells, 2) boundary vector cells, and 3) head direction cells. Grid cells may play a pivotal role in spatial navigation by providing idiothetic cues for path integration. Path integration is a form of navigation that relies on the continuous integration of self-motion information about distance travelled and running speed. The medial septum plays a pivotal role in spatial and speed coding in the MEC and has also been linked to locomotion behaviours. The MS consists of three separate populations: cholinergic, GABAergic and glutamatergic neurons. MS glutamatergic terminal activity has been linked to locomotion initiation in the MEC. We therefore aim to evaluate the contribution of septal glutamatergic neurons to speed signalling in the MEC. Specifically, we target glutamatergic neurons in the MS using optogenetic activation of Archaerhodopsin to selectively silence these neurons while recording neurons in the MEC. To target the MS, an optic fiber was placed above the MS for light delivery, and a four-tetrode microdrive was implanted into the MEC for grid cell recordings. We used a stimulation protocol of 30-second inactivation followed by a 30-second recovery period throughout the recording session. We examine the role of glutamatergic input in velocity coding in the entorhinal cortex across spatially modulated cells in the MEC. Our results show that silencing septal glutamatergic neurons did not affect MEC theta power nor frequency. Interestingly, silencing this population results in a slight distortion of grid fields and also alters the neural coding of running speed. We further examine if disrupting MS glutamatergic signalling to the MEC results in deficits in performance of path integration. Together, these data highlight the importance of input from the MS in spatial and speed coding in the entorhinal cortex.

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Poster

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Title: Associative and predictive hippocampal codes support memory-guided behaviors

Authors: *C. LIU[1], R. TODOROVA[2], W. TANG[3], A. OLIVA GONZÁLEZ[4], A. FERNANDEZ-RUIZ[4];
[1]Cornell Univ. Neurobio. and Behavior, Ithaca, NY; [2]Dept. of Neurobio. and Behavior, [4]Neurobio. and Behavior, [3]Cornell Univ., Ithaca, NY

Abstract: Generating an internal model of the world requires learning both the states of the world and the transitional relationships between these states. Such an internal model can be used to guide flexible behavioral decisions and support novel inferences. Two different lines of evidence have demonstrated the critical role of the hippocampus in forming the internal model, encoding positional states by coactive place-cell assemblies and generating neuronal sequences concatenating a series of locations. However, whether these two hippocampal coding schemes are supported by the same or distinct underlying circuit mechanisms remains unknown. To address this question, we deployed an optogenetic manipulation in rats that could dissociate the two different codes in the hippocampus. By entraining medial entorhinal cortex interneurons, we disrupted hippocampal theta sequences while rats navigated specific spatial trajectories, but preserved place-cell tuning curves and global network dynamics. Interestingly, this perturbation resulted in impaired subsequent sequential replay of those specific trajectories but spared the reactivation of coactive neuronal assemblies representing discrete locations along those trajectories. Furthermore, the disruption of hippocampal sequences led to impaired learning of new optimal trajectories during memory-guided navigation, but it did not affect reward-context associative learning. Finally, computational simulations indicated that distinct network Hebbian plasticity mechanisms mediate hippocampal assembly reactivation and sequence replay. Together, our results provide the first mechanistic and functional dissociation of associative and predictive codes in the hippocampus. This new framework of understanding how episodic associations develop into predictive models of the world helps reconcile disparate views on hippocampal functions.

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Poster

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Title: Distinct population firing modes of CA2 pyramidal cells correlate with anatomical diversity and have implications for memory consolidation

Authors: *R. HARVEY, L. KARABA, H. ROBINSON, A. FERNANDEZ-RUIZ, A. OLIVA; Cornell Univ., Ithaca, NY

Abstract: The hippocampal formation plays a crucial role in memory processing, with CA1, CA3, and dentate gyrus traditionally receiving significant attention. However, recent evidence has highlighted the importance of investigating the unique properties of other subregions, such as CA2. In this study, we aimed to explore the role of CA2 pyramidal cells in memory consolidation and their potential influence on hippocampal-entorhinal cortex communication. Contrary to the traditional notion of homogeneity, we discovered that CA2 pyramidal cells exhibit notable anatomical diversity. Through electrophysiological recordings, we observed a novel population event in the hippocampus, during NREM sleep, wherein ensembles of CA2 pyramidal neurons display a ‘barrage’ of action potentials. These barrages were found to be particularly prevalent in the deep sublayer of CA2. On the contrary, deep and superficial CA2 cells displayed the opposite modulation during sharp-wave ripples, with CA2 cells particularly active. Importantly, we propose that these barrages serve as a mechanism to facilitate the down-scaling of coordinated firing associated with memory consolidation processes. To elucidate the functional implications of CA2 barrages, we investigated the connectivity patterns between CA2 and the medial entorhinal cortex (MEC). Based on our preliminary analyses, the anatomical diversity observed within CA2 pyramidal cells may significantly influence the strength and specificity of their outputs to the MEC. These specialized outputs may potentially play a crucial role in shaping the flow of information between the hippocampus and the cortex, thus contributing to memory-guided behaviors. Our findings shed light on the distinctive properties of CA2 pyramidal cells and their functional significance in memory consolidation and hippocampo-cortical communication. Understanding the mechanisms underlying the firing of CA2 pyramidal cells and their interactions with the cortex may provide valuable insights into the computational flexibility and memory capacities of the hippocampal formation as a whole. Further investigations are warranted to fully unravel the complex dynamics of CA2 and its role in memory processing.

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Poster

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Title: A hippocampal compensatory mechanism to balance sharp-wave ripple reactivation

Authors: *L. A. KARABA, R. E. HARVEY, H. L. ROBINSON, A. FERNANDEZ-RUIZ, A. OLIVA GONZÁLEZ;
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Abstract: The hippocampus plays a key role in the formation and consolidation of memories. The currently accepted mechanism of memory consolidation is the reactivation of experience-related cell ensembles during sharp-wave ripples (SWRs) in subsequent periods of sleep. However, it is not yet known how the reactivation of these cells, which results in an increase in their coordinated firing rate, returns to baseline. We hypothesized that an alternative hippocampal mechanism is necessary to balance SWR reactivations at the network level. We additionally hypothesized that, due to its heterogeneous response to SWRs, the CA2 subregion could contribute to this mechanism. To test this, we bilaterally implanted silicon probes in dorsal hippocampal regions CA1 and CA2 and recorded neuronal activity during a variety of hippocampus-dependent tasks. We found a novel electrophysiological event in non-REM sleep (termed a “CA2 barrage”) comprised of persistent CA2 pyramidal cell firing and reduced CA1 pyramidal cell activity. These events are distinctly anticorrelated in time with SWRs and last over 300 milliseconds on average. We find that within CA2 barrages, task-related cells and assemblies are inhibited. Furthermore, the reactivation of a given cell during SWRs in the sleep period following a task was anticorrelated with their activity during CA2 barrages. To better test the direct relationship between SWRs and CA2 barrages, we additionally performed optogenetic experiments. We found that artificial generation of SWRs increases CA2 barrage rate and length. Our results suggest that CA2 barrages are important for regulating ensemble activity that is being reactivated following a given experience.

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Poster

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Title: Hippocampal sequences support the formation of a predictive map

Authors: *W. TANG, C. LIU, R. TODOROVA, A. OLIVA, A. FERNANDEZ-RUIZ; Neurobio. & Behavior, Cornell Univ., Ithaca, NY

Abstract: Creating a predictive map (*Stachenfeld et al., 2017*) and generating neuronal sequences (*Buzsaki and Tingley, 2018*; *Tang and Jadhav, 2022*) are believed to be two main functions of the hippocampus. While both functions can support flexible navigation, whether they are linked by similar circuit mechanisms is not known. Past studies to probe their causal dependencies have proven to be difficult, because these manipulations profoundly interfere with various aspects of hippocampal physiology at the same time. Here, we leveraged a novel optogenetic approach (*Fernandez-Ruiz et al., 2021*) to selectively disrupt hippocampal sequences in rats (n = 5). We found that while coactivity of hippocampal cells at the theta timescale was not affected by this manipulation, the "look-ahead" of theta sequences that was predictive of the animal's future locations was severely impaired. Along with the disrupted sequences, the predictive properties of place fields, including backward shift on a linear track and elongation near boundaries in an open arena, were also selectively disrupted, despite the preserved representation of spatial locations. Taken together, these results provide a direct causal link between hippocampal sequences and the formation of a predictive map, suggesting a unifying mechanism underlying these two seemingly disparate neural codes.

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Poster

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Title: Pupillometry as a readout of brain state dynamics across sleep-wake cycles in freely moving mice

Authors: *H. CHANG[1], W. TANG[2], A. WULF[2], M. WOLF[2], A. FERNANDEZ-RUIZ[2], A. OLIVA[2];
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Abstract: Pupil fluctuations are known to reflect state changes in the brain. However, the majority of the evidence comes from neural recordings in head-fixed animals, leaving it unclear whether and how pupillary dynamics interact with memory and cognitive processing during natural behaviors, such as memory-guided navigation. In addition, while sleep stages (such as Rapid Eye Movement, or REM, state, and Non-REM state) are associated with distinct pupil dynamics, the finer time scales of neural processes that support learning and memory and their correlation with pupil features are less characterized. Here, we developed a method to simultaneously monitor pupil dynamics and large neuronal ensembles (up to ~300 cells per brain region) across sleep and waking states in freely moving mice, performing memory-guided navigation. We found a tight temporal correlation between pupil diameter (and speed) with hippocampal neural patterns at various timescales during behavior and sleep. Our findings highlight the potential of pupil measurements for reading-out neural circuit operations in rodents. Importantly, this study could open the possibility to study and interfere with the interactions between brain state and cognitive processing in freely behaving animals guided by non-invasive pupillometry.

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Poster

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Title: Closed-loop stimulation of sharp-wave ripples enhances spatial memory via endogenous cortical consolidation mechanisms

Authors: *H. L. ROBINSON[1], R. TODOROVA[1], A. GRUZDEVA[1], G. A. NAGY[2], A. OLIVA GONZÁLEZ[1], A. FERNANDEZ-RUIZ[1];
[1]Cornell Univ., Ithaca, NY; [2]Sch. of Medicine, Univ. of Szeged, Szeged, Hungary

Abstract: Animals experience many events throughout their lives. Many of these events are consolidated into long-term memories in a two stages process: an initial encoding phase during awake behavior and a subsequent consolidation phase during sleep. The immediate consolidation of recent memories and their transfer to the neocortex for long-term consolidation is facilitated by hippocampal sharp-wave ripples (SWRs), synchronous events generated in the hippocampus and propagated to the cortex. However, the precise mechanisms by which SWRs coordinate the reactivation of neuronal ensembles across different brain structures are not yet fully understood. We investigated how SWRs mediate the consolidation of memories within hippocampal-cortical structures using the Spatial Object Recognition (SOR) memory task. We designed an experiment to causally test the role of SWR-associated memory reactivation in hippocampus and cortex. Specifically, animals underwent different levels of training while simultaneously stimulating, or silencing SWR-associated neuronal activity in both the hippocampus and the medial prefrontal cortex (mPFC) using closed-loop optogenetics. Our findings revealed that inhibiting the activity in the mPFC during SWRs impaired memory performance in the SOR task. Conversely, we observed that enhancing hippocampal SWRs through optogenetic methods during post-learning sleep improved memory performance to levels of increased training. Importantly, this beneficial effect was suppressed when we simultaneously silenced the mPFC during hippocampal SWRs. Additionally, delivering optogenetic stimulation outside of SWRs had no impact on behavior, underscoring the significance of precise temporal coordination between these brain structures for effective memory consolidation. Furthermore, increased training or enhancement of SWRs led to increased memory reactivation in the cortex, measured by neuronal cell assembly reactivation and SWR-associated cortical firing rates. Taken together, our results provide support for the hypothesis that the reactivation of mPFC ensembles during SWRs is crucial for memory consolidation. However, further research is still needed to gain a comprehensive understanding of the mechanisms underlying the coordination between the hippocampus and the mPFC.

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Poster

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Title: A cortical consolidation process mediated by sharp-wave ripples

Authors: *R. TODOROVA[1], H. ROBINSON[1], A. OLIVA[1], A. FERNANDEZ-RUIZ[2];
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Abstract: Hippocampal sharp wave-ripples have been shown to be involved in learning, consolidation, and retrieval. In each of these processes, hippocampal activity is part of a coordinated mechanism spanning multiple brain areas, including the prefrontal cortex. In waking behavior and sleep, cortical ensembles can be recruited and reactivated following hippocampal sharp wave-ripples, and in NREM sleep, this reactivation is followed by a cortical delta wave. Here, we recorded local field potentials and spiking activity of hippocampal area CA1 and the medial prefrontal cortex during learning, consolidation, and retrieval, to investigate the cross-regional coordination in these different stages. Cortical recruitment in temporal proximity to hippocampal sharp wave-ripples was striking, suggesting that ripples may orchestrate and serve as an anchoring point to cortical cell assemblies. To test this causally, we took advantage of a novel technique to optogenetically boost ripples detected on-line (Fernandez-Ruiz*, Oliva* et al., 2019), which we applied in mice following training on a memory task. The stimulation resulted in an enhanced ripple activity in line with the enhancements observed following natural learning. In the prefrontal cortex, these boosted events resulted in enhanced cortical assembly reactivation and hippocampo-cortical coordination consistent with an artificially induced memory consolidation. Overall, our results shed light on the how the hippocampo-cortical dialogue underlies the different stages of memory through hippocampal sharp wave-ripples.

Disclosures: R. Todorova: None. **H. Robinson:** None. **A. Oliva:** None. **A. Fernandez-Ruiz:** None.

Poster

PSTR373. Intrinsic Hippocampal Circuits

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR373.08/WW7

Topic: H.08. Learning and Memory

Support: NIH grant 4R00MH122582
New Frontiers Grant
NIH grant 4R00MH120343

Title: Social dynamics and memory consolidation: a study in semi-natural outdoor settings

Authors: *P. PAUDEL, C. VOGT, C. LIU, M. J. SHEEHAN, A. FERNANDEZ-RUIZ, A. OLIVA;
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Abstract: Social behavior is a fundamental feature of the lives of many animal species. Understanding the neural mechanisms of social behaviors is key to understanding animal behavior within groups and neuropsychiatric diseases involving social cognitive deficits. However, most studies investigating the neural mechanisms of social behavior are conducted in socially and spatially restricted laboratory conditions which limit the range and complexity of natural behaviors model organisms can express. Here, we overcome this limitation by investigating how the properties of hippocampal brain patterns important for learning and memory are modulated by the changes in the spatial and social organization of a population of male mice living in an outdoor field enclosure with monopolizable resource zones. We studied males in two field contexts: an “abundance” context with 8 males and 12 resource zones, and a “scarcity” context with 12 males and 8 resource zones. These contexts resulted in dichotomous male behavioral patterns in terms of the group's spatial and social structure and each individual's distinct behavior. In abundance, multi-territory males patrolled more, whereas single-territory males displayed reduced mobility. In the scarcity context, a subset of males established single-zone territories, while the remainder largely failed to establish territories and experienced high mortality rates. We conducted extracellular electrophysiological recordings during sleep before and after exposure to the field enclosure. We focused our analysis on the properties of hippocampal sharp-wave ripples (SWRs) due to their established role in encoding recent experiences. Our initial results indicate elevated SWR duration, rate, and proportion of long ripples post-exposure. Furthermore, different properties of SWRs changed in relation to different socio-spatial behavioral patterns expressed during the field experience. In addition, to evaluate the effects on memory function, we examined object displacement and object novelty behaviors pre- and post-exposure to field enclosure. We find no significant changes in object novelty but detect a marked enhancement in object displacement discrimination. These results contribute to our understanding of how environmental factors and social dynamics modulate hippocampal memory processes. Importantly, it allows investigation of the neural mechanisms of rodent social behaviors under ecologically valid conditions.

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Poster

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Program #/Poster #: PSTR373.09/WW8

Topic: H.08. Learning and Memory

Support: NIH NIMH R01MH128187

Title: Human neurons encode economic risk prediction and error

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Abstract: Surviving and succeeding in a complex world requires inferring a way to act based on the prospect of uncertain or delayed rewards. In economics, the amount of uncertainty associated with a potential reward defines its riskiness. The dopamine system is well-known to compute predicted reward and deviations from such predictions. However, how the dopamine system and prefrontal cortex interact to generate reward predictions under uncertainty is an active area of study. Neuroimaging studies in humans have indicated the anterior insula and dorsal striatum specifically represent the amount of risk, or quadratic encoding of reward probability, related to a decision. Here we study single neuron recordings from the prefrontal cortices and hippocampi of human neurosurgical patients playing the balloon analog risk task in order to understand how reward probabilities and outcomes are represented and computed. We recorded 213 well-isolated single units from 24 participants. We grouped these recordings into four general anatomical areas: The Orbitofrontal Cortex (OFC; 46 units), The Medial Frontal Cortex (MFC; 38 units), the Anterior Cingulate Cortex (ACC; 46 units), and the Mesial Temporal Lobe (MTL; 84 units). In fitting generalized linear models to the firing rates from each of these areas, we found significant proportions of units that monotonically and quadratically encoded reward probability. Across units, we found significant encoding of reward probability in most brain areas. Outcome related firing in MFC was the only brain are in which significant proportions of encoding neurons were not observed, suggesting MFC representations are only predictive. The majority of neurons that significantly predicted the reward probability categories in response to the cue exhibited a reversal of their reward probability encoding (regression betas) in response to the outcome (100% of units in ACC, 16% in MFC, 75% in MTL, and 75% in OFC). These dynamics mirror the classic prediction and resolution motifs of Ventral Tegmental Area and Substantia Nigra neurons, despite their nonlinear encoding of reward probability.

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Poster

PSTR373. Intrinsic Hippocampal Circuits

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Topic: H.08. Learning and Memory

Support: NSF GRFP 1650113
NIH F31MH124366
UCSF Discovery Fellowship
Simons Foundation 542981
Howard Hughes Medical Institute

Title: Dynamic engagement of non-local spatial representations in the hippocampus during value-guided foraging decisions

Authors: *A. E. COMRIE[1], E. J. MONROE[2], E. L. DENOVELLIS[3], A. JOSHI[3], J. A. GUIDERA[2], A. E. KAHN[4], T. A. KRAUSZ[1], J. D. BERKE[2], N. D. DAW[4], L. M. FRANK[3]; [1]Physiol., Univ. of California San Francisco Neurosci. Grad. Program, San Francisco, CA; [2]Univ. of California San Francisco, San Francisco, CA; [3]Univ. of California San Francisco, HHMI, San Francisco, CA; [4]Princeton Univ., Princeton, NJ

Abstract: Adaptive decisions are guided by prior experience. To decide where to seek out food, for instance, an animal may retrieve memories of past locations and mentally simulate where it could go next. The hippocampus (HC) is known to be important for such experience-guided navigation. Consistent with this, neural activity in the HC can not only represent an animal's current position, but can also express "non-local" representations of other locations. For example, when a rat approaches a maze bifurcation, non-local representations can serially sweep down the two paths ahead. These representations are hypothesized to simulate potential choices during deliberative decision-making, when animals are uncertain of the best path to take. However, it remains unknown whether non-local representations are modulated by uncertainty over learning and across different navigational decisions. To address this, we developed the Spatial Bandit Task with corridors connecting several foraging patches with different reward probabilities. By changing the reward probabilities, we systematically introduced uncertainty about the values of spatial choices. Rats (n=5) explored patches to learn which locations to exploit for reward, and adapted their foraging decisions based on their changing reward experiences. We simultaneously recorded hundreds of HC and prefrontal neurons throughout foraging, beginning on the first day of exposure to the maze environment. Using a state-space algorithm, we decoded spatial representations during locomotion at the millisecond-timescale from HC spiking. We found that uncertainty engaged HC non-local spatial representations in two ways. First, the extent of non-local representations was greater in early days of task experience and learning. Second, even after the task became familiar, non-local representations were specifically recruited upon entering and exploring patches of uncertain value. Strikingly, these non-local representations were not limited to prospective paths ahead of the animal; representations also extended behind the animal, along counterfactual paths, and to distant locations in alternative patches. Our results reveal a diversity of non-local representations that are preferentially engaged during periods of learning and uncertainty. These HC activity patterns

may sample an internal spatial model, linking alternative locations with ongoing experiences, to support flexible decision-making behavior.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant 5F32MH123003
Howard Hughes Medical Institute
Simons Foundation

Title: Closed-loop modulation of hippocampal representations through neurofeedback

Authors: *M. E. COULTER[1], A. K. GILLESPIE[2], J. CHU[3], E. DENOVELLIS[1], T. NGUYEN[1], D. F. LIU[1], K. WADHWANI[1], B. SHARMA[1], K. WANG[4], X. DENG[5], C. KEMERE[3], U. T. EDEN[5], L. M. FRANK[1];
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Abstract: Memories are useful because they allow past experiences to be retrieved and used to guide decisions about future actions. In humans, retrieval can be cued verbally, but in animals it remains unclear if specific past experiences are retrieved at specific times. The retrieval of complex spatial memories depends on the hippocampus and is thought to engage reactivation of neural activity patterns like those seen during the experience. Current theories propose the hippocampus can be instructed to reactivate specific activity patterns relevant to, but distinct from, ongoing experience (e.g., remote reward locations). However, whether instructed reactivation can occur remains unknown. We therefore asked whether animals could be trained to retrieve representations of specific remote spatial locations. To answer this question, we designed a closed-loop neurofeedback system and used it for operant conditioning of hippocampal content in rats. This feedback system utilized fast, online decoding of hippocampal CA1 spiking to decode spatial location represented in the hippocampus, then triggered a cue and food reward when the decoded location matched a specific target location. If remote hippocampal content can be retrieved when needed, animals should be able to learn to generate the associated spiking patterns in response to external cues. Indeed, using this neurofeedback system, we found that rats can increase generation of specific remote hippocampal spatial representations in response to conditioning. Over 1-3 weeks of training, 5 of 6 rats increased representation of a remote target location. This change was specific; we did not observe

consistent increased representation of non-rewarded locations. In addition, the amount of time when the hippocampus expressed a representation of the target was higher in all rats during neurofeedback sessions compared to control training sessions with equal reward but no neurofeedback. In summary, we show that rats can increase generation of specific remote spatial representations in hippocampus through neurofeedback. This finding provides evidence that specific hippocampal representations can be retrieved and expressed when needed, indicating that the hippocampus can generate targeted retrieval of activity patterns in a way that could support online memory processes such as memory-guided decision-making and offline processes such as maintenance of stored memories. We hypothesize that targeted retrieval is essential for both processes, and we are investigating which brain states were active during remote target representations in this task (online: periods of high theta band power; offline: sharp wave ripples).

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Poster

PSTR373. Intrinsic Hippocampal Circuits

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Topic: H.08. Learning and Memory

Support: NIDA
NINDS
NIMH

Title: Hippocampal non-local representations retrieve and update dopaminergic place values

Authors: *T. A. KRAUSZ[1], D. EGERT[2], A. E. COMRIE[3], E. L. DENOVELLIS[4], N. D. DAW[5], L. M. FRANK[6], J. D. BERKE[2];
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Abstract: Dopamine in the nucleus accumbens (NAc DA) helps motivate behavior based on estimates of future reward (”values”). We recently found that as rats navigate a novel complex, changing environment, NAc DA scales in proportion to the value of the current location. These place values are inferred, in part, using internal models of the spatial relationships between available paths and potential rewards. We sought to understand how the brain implements such

inferential valuation. The activity of place cells in dorsal hippocampus CA1 (dCA1) provides a candidate spatial representation that could be used for this inference. In particular, the dCA1-represented location has been observed to sweep ahead of actual location into potential future locations at each cycle of the 8Hz theta rhythm, as if rapidly simulating options to retrieve values. In addition, during sharp-wave ripple (SWR) events following reward consumption, the dCA1-represented location can also represent non-local places throughout the current environment, as if updating the values of distant locations. We simultaneously monitored NAc DA using dLight fiber photometry, and dCA1 place cells using a custom 256-channel silicon electrophysiology probe, while a rat (n= 6 sessions) foraged for reward in our maze task. We first observed that NAc DA release was entrained to dCA1 theta during running. We then used a novel two-dimensional spatial state-space algorithm to achieve robust millisecond-timescale decoding of hippocampal place representations. These included theta-associated sweeps ahead of the animal into available paths, and representations of distant locations following reward. When dCA1 represented a higher-value available future path, NAc DA increased more than when dCA1 represented a lower-value path. Finally, if a location was represented in dCA1 following reward, NAc DA was higher the next time the rat traversed that location, compared to traversals when that location had not previously been represented. These results provide striking new evidence for specific neural mechanisms that implement inference through simulation, and may therefore underlie intelligent learning and decision making.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH R01NS39600
NIH U01MH114829

Title: Hippocampome.org v2.0: a knowledge base enabling data-driven spiking neural network simulations of rodent hippocampal circuits

Authors: *D. WHEELER, J. D. KOPSICK, N. SUTTON, C. TECUATL, A. O. KOMENDANTOV, K. NADELLA, G. A. ASCOLI;
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Abstract: Hippocampome.org is a mature open-access knowledge base of the rodent hippocampal formation (dentate gyrus, CA3, CA2, CA1, subiculum, entorhinal cortex) focusing on neuron types and their properties. Hippocampome.org v1.0 established a foundational classification system identifying 122 hippocampal neuron types based on their axonal and

dendritic morphologies, main neurotransmitter, membrane biophysics, and molecular expression. Releases v1.1 through v1.12 furthered the aggregation of literature-mined data, including among others neuron counts, spiking patterns, synaptic physiology, in vivo firing phases, and connection probabilities. Those additional properties increased the online information content of this public resource over 100-fold, enabling numerous independent discoveries by the scientific community. Hippocampome.org v2.0 incorporates over 50 new neuron types and extends the functionality to build real-scale, biologically detailed, data-driven computational simulations. In all cases, the freely downloadable model parameters are directly linked to the specific peer-reviewed empirical evidence from which they were derived. Possible research applications include quantitative, multiscale analyses of circuit connectivity and spiking neural network simulations of activity dynamics. These advances can help generate precise, experimentally testable hypotheses and shed light on the neural mechanisms underlying associative memory, spatial navigation, and other cognitive functions.

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Poster

PSTR373. Intrinsic Hippocampal Circuits

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Topic: H.08. Learning and Memory

Support: U01MH114829
R01NS39600
RF1MH128693
R00NS116129

Title: A continuous attractor model with realistic neural and synaptic properties quantitatively reproduces grid cell physiology

Authors: *N. SUTTON[1], B. GUTIÉRREZ-GUZMÁN[1], H. DANNENBERG[2], G. A. ASCOLI[2]; [1]Bioengineering, [2]Bioengineering and Neurosci. Program, George Mason Univ., Fairfax, VA

Abstract: A deeper understanding of the neural mechanisms involved in spatial navigation can be achieved through advancing current approaches to simulating cognition with lower-level physiological detail. A wealth of data about neural properties can be found in Hippocampome.org. This study applies neural characteristics from Hippocampome.org, along with high-performance computing via graphical processing units (GPUs), to investigate neural codes in spatial navigation with spiking neural network models. GPUs provide the computing power needed for large-scale simulation with biologically realistic properties. Physiological characteristics studied here include the excitability, connectivity, and synaptic signaling of neuron types defined primarily by their axonal and dendritic morphologies. The research

investigates the spiking dynamics when specific neuron types communicate together and the directions in communication flow between groups of neuron types. Modeling the rodent hippocampal formation keeps the simulations to a computationally reasonable scale while also anchoring the parameters and results to experimental measurements. At the same time, findings in a rodent animal model may translate into insights into human cognition. We report that our spiking neural network implementation of the continuous attractor model based on Hippocampome.org data generates grid cell firing that matches well the spacing, size, and firing rates of grid fields observed in animal recordings. Our simulations can also recreate different scales of those properties, e.g., small to large, as found along the dorsoventral axis of the medial entorhinal cortex. Computational exploration of neuron and synapse model parameters reveals that a broad range of neural properties produce grid fields in the simulation. Further investigation in animal studies of the model parameters will be valuable to test hypotheses generated by the simulation. Our biologically realistic spiking neural network model of grid cell physiology will contribute to our understanding of neural dynamics in spatial cognition and spatial navigation. This is important because spatial navigation deficits have been reported to occur early in Alzheimer's disease, and a better understanding of neural dynamics in spatial cognition might contribute to finding novel diagnostic and treatment approaches. Furthermore, general principles of learning and memory may be gleaned from simulation findings. We plan to release the software as open source to help accelerate research and foster community application of this work to a greater number of studies.

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Poster

PSTR373. Intrinsic Hippocampal Circuits

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Program #/Poster #: PSTR373.15/WW14

Topic: H.08. Learning and Memory

Title: Representational drift leads to sparsely engaged representations that enable robust maintenance of memories

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Abstract: Memories are commonly believed to be stored in synapses and retrieved through the activation of neural ensembles. However, both synapses and neural representations exhibit extensive dynamics even when animals are not explicitly learning or forgetting, resulting in a phenomenon known as representational drift. Previous studies have demonstrated that synaptic weight updates, incorporating random and performance-dependent components, enable continued exploration of the weight solution space following initial learning. Nevertheless, the

nature of the representations generated through this weight exploration remains elusive. Representational drift has been proposed as a consequence of utilizing the same network to learn additional tasks. However, it remains uncertain whether representational drift can actively facilitate maintenance of previously stored memories despite continuous acquisition of new information. Here, we modeled drift as uniform sampling of equivalent performance weight solutions and showed that drift favors sparse representation solutions that are robust to weight changes.
For a clipped threshold linear activation function, there are numerous input currents that correspond to inactive or saturated neurons. Consequently, when the weight norm limit is sufficiently large, there are many weight configurations that result in sparsely engaged representations, where most neurons are either inactive or saturated. Hence, uniform sampling of the weight solution space by drift tends to favor these sparsely engaged representations. Furthermore, weight perturbations have less impact on the activity of inactive and saturated neurons, making these sparsely engaged representations more resilient to weight changes caused by noise or continual learning. Additionally, when initial representations of different stimuli are correlated, drift leads to orthogonalized representations, thereby improving discriminability and robustness to changes in downstream classifier weights. Our model predicts that if learning disturbs the fraction of engaged neurons, drift will bring it back to a fixed level of sparsity. Moreover, initial drift produces more substantial changes to the representation, while subsequent weight modifications have a diminishing impact on the representation. Overall, our results demonstrate that sparsity can naturally arise as a result of drift, and drift can be leveraged in both biological and artificial agents to facilitate robust maintenance of existing memories despite weight changes due to acquisition of new ones.

Disclosures: **M. Natrajan:** None. **J. Fitzgerald:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.01/WW15

Topic: H.08. Learning and Memory

Support: NIH R01 NS109965
NIH DP2 NS111134

Title: Variation in learning behavior across inbred and outbred mice performing a live-in sensory-guided goal-directed task.

Authors: *A. E. CAREY[1,2], D. G. LEE[3,2], K. M. DELGADO[1], N. TAN[1], D. E. POWERS[1], G. HOUSE[1], J. L. CHEN[1,3,2];
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Abstract: Capacities and behavioral strategies for learning complex tasks can vary greatly across a population of individuals. This variation could reflect intrinsic or environmental factors. Comparing learning behavior between individual outbred mice or between groups of different inbred mice provides insight into aspects of learning that is influenced by genetics. To gain an understanding of the neurobiology of individual differences and examine genetic contributions to behavior, we developed an automated home-cage training system to study goal-directed, sensory-guided task learning in a high throughput manner. A motorized rotor is used to sequentially deflect whiskers in either an anterior or posterior direction during a 'sample' and a 'test' period. Mice are trained through multiple stages over 6 weeks to report by 2-alternative forced choice designed to assay different aspects of learning. Advancement to further stages of training is contingent on reaching performance criteria defined in the training software. Animals are tested on one non-match stimulus condition and two match conditions which mice are required to unitize the sample and test stimuli and pair it with a reward (procedural learning). Following successful procedural learning, the remaining omitted non-match condition is introduced to test whether the animal can generalize the task rule (rule learning). Following successful rule learning, delays between the sample and test stimuli are gradually extended temporally and spatially to test the animal's ability to retain memory of the sample stimulus (working memory learning). We assayed learning in Diversity Outbred (DO) mice (*n*=244) along with groups consisting of the 8 founder inbred lines (129S1, NZO, CAST, A/J, WSB, C57, NOD, PWK, *n*=6 per strain). Learning performance was highly variable for DO mice. 24.6% of DO mice complete all stages of training, 13.1% mice accomplished procedural learning but failed to progress beyond rule learning, 62.3% failed to procedurally learn. Group differences were observed between inbred lines that spanned the variation observed in DO mice. We found that NZO and CAST lines learned the task to expert levels more frequently compared to the total inbred founder lines (100% NZO, 66.7% CAST, 38.7% total), while PWK and NOD lines failed to advance through procedural learning more frequently compared to the total (100% PWK, 100% NOD, 48.9% total). Behavioral analysis from lick sensors and videography suggest that individuals employ a range of motor strategies to perform the task. This work provides evidence for intrinsic factors that govern aspects of learning that can be further investigated using genetic strategies.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.02/WW16

Topic: H.08. Learning and Memory

Support: NIH Grant DA053743

Title: Reelin protein is required for normal cellular and behavioral function in the striatum

Authors: *K. BRIDA, E. T. JORGENSEN, M. ZIPPERLY, R. A. PHILLIPS, III, J. TUSCHER, M. DAVIS, J. J. DAY;
Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Reelin is a large, secreted glycoprotein with a well-characterized role in brain development and links to numerous neuropsychiatric disorders. While Reelin is abundant in the adult striatum, Reelin's functional role in this brain region remains poorly characterized. Using a recently generated cellular atlas of the rat nucleus accumbens (NAc) following cocaine experience, we identified *Reln* mRNA as a marker of cocaine-responsive *Drd1+* medium spiny neurons (MSNs). Here, we sought to define Reelin's role in striatal functions, and to determine its contribution to cocaine behavioral response. We designed a CRISPR sgRNA targeting the *Reln* promoter to enable bidirectional manipulation of *Reln* mRNA and protein levels with CRISPR activation (CRISPRa) or CRISPR interference (CRISPRi). We first used these CRISPR systems in primary striatal neuron cultures to assess the impact of *Reln* overexpression and knockdown on dopamine response. Notably, CRISPRa overexpression of *Reln* in rat primary striatal neuron cultures enhanced stimulus-dependent transcription of immediate early genes following dopamine stimulation. In contrast, CRISPRi-mediated *Reln* decreased overall cell firing rate and suppressed dopamine-induced increases in firing. Using whole-cell patch clamp in *Reln* knockdown rat NAc tissue, we found no alterations in passive membrane properties or action potential properties. However, following current injection, cells lacking *Reln* exhibited decreased intrinsic excitability and an inability to maintain sustained firing. Similarly, targeted knockdown of *Reln* in the NAc decreased conditioned place preference for cocaine in Sprague-Dawley rats. Taken together, these results reveal a key role for Reelin in striatal function and demonstrate that Reelin is required for cocaine-related behavioral adaptations. Current studies are assessing the transcriptional consequences of *Reln* knockdown using single-nucleus RNA sequencing approaches to identify molecular mechanisms of Reelin signaling.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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McKnight Foundation Neurobiology of Brain Disorders Award

Title: Temporally specific gene expression and chromatin remodeling programs regulate a conserved Pdyn enhancer

Authors: *R. PHILLIPS[1], E. WAN[1], J. J. TUSCHER[1], D. REID[1], L. IANOV[2,1], J. J. DAY[1]; [1]Univ. of Alabama at Birmingham, Birmingham, AL; [2]Civitan Intl. Res. Ctr., Birmingham, AL

Abstract: Neuronal and behavioral adaptations to novel stimuli are regulated by temporally dynamic waves of transcriptional activity, which shape neuronal function and guide enduring plasticity. Neuronal activation promotes expression of an immediate early gene (IEG) program comprised primarily of activity-dependent transcription factors, which are thought to regulate a second set of late response genes (LRGs). However, while the mechanisms governing IEG activation have been well studied, the molecular interplay between IEGs and LRGs remain poorly characterized. Here, we used transcriptomic and chromatin accessibility profiling to define activity-driven responses in rat striatal neurons. As expected, neuronal depolarization generated robust changes in gene expression, with early changes (1 h) enriched for inducible transcription factors and later changes (4 h) enriched for neuropeptides, synaptic proteins, and ion channels. Remarkably, while depolarization did not induce chromatin remodeling after 1 h, we found broad increases in chromatin accessibility at thousands of sites in the genome at 4 h after neuronal stimulation. These putative regulatory elements were found almost exclusively at non-coding regions of the genome, and harbored consensus motifs for numerous activity-dependent transcription factors such as AP-1. Furthermore, blocking protein synthesis prevented activity-dependent chromatin remodeling, suggesting that IEG proteins are required for this process. Targeted analysis of LRG loci identified a putative enhancer upstream of Pdyn, a gene encoding an opioid neuropeptide implicated in motivated behavior and neuropsychiatric disease states. CRISPR-based functional assays demonstrated that this enhancer is both necessary and sufficient for Pdyn transcription. This regulatory element is also conserved at the human PDYN locus, where its activation is sufficient to drive PDYN transcription in human cells. These results suggest that IEGs participate in chromatin remodeling at enhancers and identify a conserved enhancer that may act as a therapeutic target for brain disorders involving dysregulation of Pdyn.

Disclosures: R. Phillips: None. **E. Wan:** None. **J.J. Tuscher:** None. **D. Reid:** None. **L. Ianov:** None. **J.J. Day:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR374.04/WW18

Topic: H.08. Learning and Memory

Title: Chst9 marks a spatially and transcriptionally unique population of Oprm1-expressing neurons in the nucleus accumbens

Authors: *E. ANDRAKA, R. A. PHILLIPS, III, K. L. BRIDA, E. T. JORGENSEN, J. J. DAY; Neurobio., Univ. of Alabama at Birmingham, Birmingham, AL

Abstract: Opioids produce addictive, analgesic, and euphoric effects via binding at the mu opioid receptor (MOR) in GABAergic neurons. The MOR is encoded by the *Oprm1* gene and is expressed in multiple brain regions that regulate reward and motivation, such as the nucleus accumbens (NAc). *Oprm1* expression in NAc medium spiny neurons (MSNs) mediates opioid place preference, seeking, and consumption. However, recent single nucleus RNA sequencing (snRNA-seq) studies in rat, primate, and human NAc have revealed that multiple discrete subpopulations of NAc neurons express *Oprm1* mRNA, making it unclear which populations mediate diverse behaviors resulting from MOR activation. Using published snRNA-seq datasets from the rat NAc, we identified a novel population of MSNs that express the highest levels of *Oprm1* of any NAc cell type. Differential expression and co-expression analysis revealed that this population was selectively marked by colocalization of the mRNA for *Grm8* (encoding a metabotropic glutamate receptor) and *Chst9* (encoding a carbohydrate sulfotransferase). Notably, this colocalization was also observed in published human and primate snRNA-seq studies, indicating that this unique population may be conserved across species. To validate this observation and characterize spatial localization of this population in the NAc, we performed RNAscope fluorescence in situ hybridization (FISH) studies to detect expression of *Oprm1*, *Grm8*, *Chst9* mRNA along with two well-validated markers of MSNs (*Ppp1r1b* and *Drd1*) in rat coronal brain sections (n = 6, 3M/3F). Image analysis confirmed the expression of *Oprm1* in multiple cell populations, including *Drd1*+ MSNs and *Chst9*- cells. However, *Chst9*+ neurons exhibited the highest expression of *Oprm1*, and formed discrete cellular clusters along the medial and ventral borders of the NAc shell subregion. To define the opioid-induced transcriptional activation profile of this population, we injected rats with saline or fentanyl (n = 8, 4M/4F) and used FISH to probe sections for *Fos*, an immediate early gene expressed in response to neuronal activation. Preliminary results indicate that fentanyl decreases the fraction of *Fos*+ cells in the NAc, but does not alter the fraction of *Chst9*+ cells that are *Fos*+. Together, these results identify a spatially and transcriptionally distinct NAc neuron population characterized by the expression of *Chst9*. The abundant expression of *Oprm1* in this population and the conservation of these cells across species suggests that they may play a conserved functional role in opioid response and identify this subpopulation as a target for further investigation.

Disclosures: E. Andraka: None. **R.A. Phillips:** None. **K.L. Brida:** None. **E.T. Jorgensen:** None. **J.J. Day:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.05/WW19

Topic: H.08. Learning and Memory

Support: NIH NIGMS T32
NIH DA039650
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NIH MH114990

Title: Neuronal activation by cocaine varies across molecularly-defined subpopulations of VTA dopamine neurons

Authors: *N. FITZGERALD[1], E. ANDRAKA[2], R. A. PHILLIPS, III[3], J. J. TUSCHER[4], J. J. DAY[5];
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Abstract: Substance use disorder is a complex neurobiological disease characterized by a loss of control over drug-taking and drug-seeking behaviors. Drugs of abuse increase dopamine (DA) transmission from ventral tegmental area (VTA) dopamine neurons that densely innervate the nucleus accumbens (NAc). While the presence of tyrosine hydroxylase (Th) has long been used to identify DA neurons in this pathway, more recent studies have revealed remarkable heterogeneity among VTA DA neurons, with some neurons co-expressing markers for both dopamine and glutamate transmission. Using single nucleus RNA sequencing to comprehensively profile the VTA, we identified unique markers for these two subpopulations of DA neurons. Slc26a7, a gene that encodes an anion transporter, serves as a selective marker for combinatorial neurons that harbor expression of genes implicated in both glutamate and DA neurotransmission. Likewise, the GTP cyclohydrolase Gch1 was identified as a marker for DA-only neurons. Here, we used multiplexed fluorescence in situ hybridization to examine whether distinct DA neuron subpopulations respond differently to drugs of abuse. We identified unique induction of the neuronal activity marker Fos in Slc26a7+ cells in the VTA 1 hour following cocaine experience. The same response was not observed in Gch1+ cells, suggesting a difference in response to cocaine between these DA neuron populations. Notably, fentanyl administration (or co-administration of fentanyl and cocaine) elevated Fos mRNA in both DA subpopulations. These results suggest that two subpopulations of DAergic cells in the VTA respond to cocaine in unique ways and may in turn drive distinct downstream effects and behavioral responses to cocaine.

Disclosures: **N. Fitzgerald:** None. **E. Andraka:** None. **R.A. Phillips:** None. **J.J. Tuscher:** None. **J.J. Day:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.06/WW20

Topic: H.08. Learning and Memory

Support: NIH R01 NS109965
NIH DP2 NS111134

Title: Functional and molecular dissection of sensory-guided task learning in perirhinal cortex

Authors: *C. A. MCLACHLAN[1,2], D. G. LEE[2,3], K. M. DELGADO[1], O. KWON[1], N. MANJREKAR[1], Z. YAO[4], B. TASIC[4], H. ZENG[4], J. L. CHEN[1,2,3];
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Abstract: Perirhinal cortex (Prh) is involved in learning associations between stimuli and outcomes during task behavior. At a cellular level, it is an open question whether the functional modifications that accompany learning are specific to circuit-elements such as neuronal cell types or regulated by gene programs that span cell types. To address this, we performed chronic two-photon calcium imaging of perirhinal layer 2/3 neurons (200-500 neurons per animal, n = 4 animals) while animals learned a whisker-based, go-no go delayed non-match to sample task. At the conclusion of behavior experiments, spatial transcriptomics was performed on functionally imaged neurons to identify molecularly-defined, Prh-specific cell types and quantify expression of immediate early genes (IEGs) associated with neuronal plasticity. Using generalized linear models, we analyzed the relationship between task-related responses, cell type identity, and IEG expression. While some task-related responses mapped onto putative Prh cell types, other responses were better explained by IEG expression patterns that spanned cell types. To confirm the role of IEGs in task learning and Prh function, we manipulated expression of brain-derived neurotrophic factor (*Bdnf*), a known regulator of task-related IEGs, using a Cre-mediated *Bdnf* conditional knockout (cKO) mouse with targeted gene deletion to Prh. Selective cKO of *Bdnf* in Prh impaired task learning (n=7 cKO animals). Knock out of *Bdnf* altered expression of other IEGs in Prh and disrupted neuronal plasticity. Analysis of population activity (n=4 cKO animals) indicates that reward representations in Prh showed increased stability on a session-to-session basis in *Bdnf* cKO versus control animals. Whereas, stimulus-reward associations emerged over sessions in control animals, those associations failed to form in *Bdnf* cKO animals. This work delineates the specificity in which cell types and gene expression participates in Prh-dependent task learning.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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AFAR #A21105
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NIA T32AG049676

Title: Hippocampal Per1 contributes to time-of-day effects on memory consolidation

Authors: *L. BELLFY, C. W. SMIES, A. R. BERNHARDT, A. SEBASTIAN, S. MURAKAMI, H. M. BOYD, M. J. VON ABO, I. ALBERT, J. L. KWAPIS;
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Abstract: Many biological processes are affected by the circadian system, including memory. Behavioral paradigms, such as the dorsal hippocampus (DH)-dependent Object Location Memory (OLM), show oscillating memory performance across the diurnal cycle. In OLM, mice learn the location of two identical objects during a training session. At test, one object is moved to a novel location and memory is measured as the time spent investigating the novel location compared to the familiar location. We used OLM to gain an understanding of a potential molecular mechanism that underlies the time-of-day memory effects. First, we needed to determine which phase of memory is impacted by the time-of-day effects. To assess acquisition, we tested short-term memory and found that mice were able to acquire memory during day and night, suggesting that nighttime acquisition is intact. To assess memory retrieval, we trained mice at the peak (ZT5) and trough (ZT17) of memory as determined by a long-term memory test across the diurnal cycle, but tested them 36 hours later, so mice trained during the day were tested at night and vice versa. We found the time of memory acquisition, not the time of retrieval, was the driving factor behind whether memory was intact; day-trained mice were able to retrieve the memory at night whereas night-trained mice showed poor retrieval when tested during either the day or night. Together, these results suggest that nighttime memory deficits are due to impaired consolidation. As memory consolidation is transcription-dependent, we performed RNA-seq on DH tissue to identify learning-induced gene changes over the day/night cycle. Notably, the circadian rhythm gene *Period1 (Per1)* was upregulated in response to learning during the day but not night, oscillating in tandem with memory performance. This suggests hippocampal *Per1* may regulate memory across the day/night cycle. To determine if hippocampal *Per1* contributes to memory, we knocked down *Per1* using CRISPR interference (CRISPRi) locally in the DH prior to training at ZT5 which resulted in the mice being unable to learn the training. As *Per1* modulates CREB activity, we also looked at memory allocation and found more neurons allocated to the memory trace during the day (when *Per1* and memory peak) than at night. In conclusion, diurnal oscillations in memory consolidation may be regulated in part by hippocampal *Per1* expression by gatekeeping CREB activity that drives neuronal allocation.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Topic: H.08. Learning and Memory

Support: NIH Grant K99/R00AG056596
NIH Grant R21AG068444
NIH Grant R01AG074041
Whitehall Foundation #2020-05-06
AFAR #A21105

Title: Examining sex differences in memory performance across the day-night cycle

Authors: *G. C. PIFER[1], L. BELLFY[2], J. L. KWAPIS[3];
[1]Biol., Pennsylvania State Univ., State College, PA; [2]208 Mueller Lab., Pennsylvania State Univ., University Park, PA; [3]Penn State Univ., Penn State Univ., University Park, PA

Abstract: The circadian rhythm's "master clock" is the suprachiasmatic nucleus (SCN) of the hypothalamus, but there are a number of satellite clocks within other regions of the brain and body. For instance, the dorsal hippocampus (DH) has diurnal oscillations in activity that may affect a range of functions, including long-term memory consolidation. Long-term memory is defined as memory that persists over a long period of time, which is operationally defined as lasting 24 hours or more following acquisition. Our lab has previously shown that male mice demonstrate better long-term memory performance during the day than at night using the hippocampus-dependent Object Location Memory (OLM) task. Additionally, we have shown that the circadian gene Per1 oscillates in tandem with this memory performance, with the peak of expression occurring during the day. Knocking down Per1 impairs memory formation but has no effect on circadian rhythm or sleep behavior. This demonstrates the possibility of a non-canonical role for Per1 in modulating memory directly within memory-relevant brain regions like the DH. However, as these studies exclusively used male subjects, it is unknown whether the same mechanisms exert diurnal control over memory in females. Here, we tested OLM in a female cohort at various diurnal timepoints, to determine whether memory oscillates in the same manner, with the same peaks and troughs as in male mice. We also measured Per1 levels to determine whether this gene might also regulate diurnal oscillations in memory in females. Finally, we are also testing whether Per1 contributes to age-related memory impairments in female mice, as age-related repression of hippocampal Per1 is known to contribute to memory impairments in old male mice. Together, our work indicates that Per1 is a critical mechanism that operates within satellite clocks, most notably the hippocampus, to regulate memory across the diurnal cycle.

Disclosures: G.C. Pifer: None. **L. Bellfy:** None. **J.L. Kwapis:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.09/WW23

Topic: H.08. Learning and Memory

Support: NIA R00AG056596
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Whitehall #2020-05-06
AFAR #A21105
NIA R01AG074041

Title: The role of HDAC3 on memory competition

Authors: *C. W. SMIES, L. BELLFY, D. S. WRIGHT, S. S. BENNETTS, M. W. URBAN, C. A. BRUNSWICK, J. L. KWAPIS;
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Abstract: Memories are plastic records of experience requiring active maintenance to maintain relevance. While transcription is required for memory formation, it is also required for memory reconsolidation. One mechanism for controlling gene expression is epigenetics, which modulates transcriptional machinery's access to DNA. Histone deacetylation by HDAC3 is one epigenetic mechanism that contributes to the expression of memory-relevant genes, including those required for reconsolidation-based memory updating. Histone deacetylation is associated with decreased gene expression, and we have shown that inhibiting HDAC3 in aged mice enhances memory to the level of performance observed in young mice. Here, to better understand the mechanisms important for memory updating, we used the Objects in Updated Locations (OUL) paradigm. In OUL, mice learn the locations of two identical objects before an update session, in which one object is moved to a novel location. Memory strength is then tested by presenting four identical objects; two in the original locations, one in the updated location, and a fourth in a novel location. At test, mice demonstrate memory for the original and updated locations by preferentially investigating the object in the novel location. Here, we show that aged mice exhibit impaired memory updating even when the original memory is intact, demonstrating a unique aging-related deficit in memory updating. HDAC3 inhibition following the update session rescued aging-induced impairments of reconsolidation-based memory updating. In comparison, post-update HDAC3 inhibition in young mice actually reduced the strength of the original memory, suggesting that the original and the updated information may compete for expression. Together, our work suggests HDAC3 contributes to age-related impairments in memory updating and may help regulate the strength of this information. Current experiments are systematically manipulating the strength of the original and update memories to determine if we can modulate which memory is expressed at test. We are also working to identify transcripts that uniquely support memory formation or updating to selectively target an initial or update memory in future studies and paradigms.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

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Topic: H.08. Learning and Memory

Support: EGR T32 Training Grant
NIH Grant R00AG056595
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Whitehall Grant 2020-05-06
AFAR Grant A21105

Title: Sex-specific epigenetic regulation of context fear memory

Authors: *H. M. BOYD[1], M. W. URBAN[2], J. L. KWAPIS[3];
[1]Biol., Pennsylvania State Univ., University Park, PA; [2]Biol., Pennslyvania State Univ., University Park, PA; [3]Biol., Penn State Univ., University Park, PA

Abstract: Sex-Specific Epigenetic Regulation of Context Fear Memory Hannah Boyd[1,2], Mark Urban[1], Janine Kwapis[1,2]1 Department of Biology, Penn State University2 Center for Eukaryotic Gene Regulation, Penn State University
Post-traumatic stress disorder (PTSD) is known to be more prevalent among women than men, but the mechanisms that underlie this sex difference are unknown. In PTSD, exposure to trauma persistently changes the brain's response to subsequent stress, leading to lasting fear sensitization. Currently, the molecular mechanisms which support the persistence of trauma are unclear, with even less known about sex-specific mechanisms. One possibility is that epigenetic modifications support the lasting effects of trauma. Epigenetic mechanisms, which change gene expression by modifying chromatin structure, are known to drive long-lasting changes to cellular function that may support persistent changes in behavior. In particular, histone acetylation may play a key role, as this epigenetic mechanism is critical for normal fear memory formation. Blocking the repressive enzyme histone deacetylase 3 (HDAC3) in the hippocampus or amygdala, for example, enhances both histone acetylation and fear memory formation. It is less clear whether HDAC3 is also involved in the exaggeration of fear memories acquired following trauma exposure in male or female mice. Here we tested for sex differences in the stress-enhanced fear learning (SEFL) paradigm, in which an acute "trauma" event (10 unsignaled shocks) reliably potentiates subsequent fear learning. We found that two shocks are sufficient to drive SEFL in females, but not males, implying that females may be predisposed to encoding traumatic stimuli more robustly. We then used a dominant-negative, deacetylase-dead point mutant virus to block HDAC3 activity and in turn enhance histone acetylation during this weak trauma event in the amygdala of male and female mice. We found that amygdalar HDAC3 inhibition transformed a weak trauma event into one that established persistent fear sensitization in males. In females, the same manipulation had little effect, likely because the female mice already showed robust stress-enhanced fear learning even with the weak trauma event. Together, this suggests that HDAC3 activity may contribute to stress-enhanced fear learning, possibly by

setting a molecular threshold that enables the severity of fear sensitization observed following acute stress sex-specifically.

Disclosures: H.M. Boyd: None. **M.W. Urban:** None. **J.L. Kwapis:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR374.11/WW25

Topic: H.08. Learning and Memory

Title: High-fat diet produces glucose intolerance, but not memory impairment, in C57Bl/6 mice.

Authors: *P. T. ORR, J. V. GEORGE, A. A. WISLOTSKY, C. CARACHILO;
Univ. of Scranton, Scranton, PA

Abstract: Recent research suggests that high-fat diet (HFD) can affect neural processes, anxiety, and memory In mouse models, HFD-exposure significantly worsened Morris Water Maze and Barnes Maze performance. HFD-exposure also reduced concentrations of vesicular transporters for GABA (VGAT) and glutamate (VGlut). In this experiment, 18 mice (50% female) were randomized to one of three conditions: standard lab chow, HFD, and a sucrose-matched control diet. After 2 weeks, mice in the HFD condition demonstrated glucose intolerance, and all mice were tested in the Open Field and on a Barnes Maze task. Although there were not group-differences on performance in the Open Field or Barnes Maze, degree of glucose intolerance was correlated with escape latency on the final day of the Barnes Maze task ($r = 0.684$, $p = .002$). Following behavioral testing, hippocampus was dissected out, frozen, and probed for GAT1 and VGluT1 via Western blot assay. GAT1 and VGluT1 immunoreactivity did not differ between groups. These results suggest that, while HFD-exposure may lead to changes in memory performance, the specific mechanisms mediating these effects require further investigation.

Disclosures: P.T. Orr: None. **J.V. George:** None. **A.A. Wislotsky:** None. **C. Carachilo:** None.

Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.12/WW26

Topic: H.08. Learning and Memory

Support: NIH Grant 1R01AG055581-01

Title: Isoform-specific effects on synaptic markers in transgenic mice with AMPKβ suppression

Authors: *A. H. MANUEL[1], N. SWIFT[2], X. ZHOU[3], G. R. STEINBERG[4], T. MA[5];
[1]Mol. Biol. and Biochem., Wake Forest Univ., Waxhaw, NC; [2]Dept. of Intrnl. Medicine, Geriatrics, Wake Forest Univ. Sch. of Med., Winston-Salem, NC; [3]Dept. of Intrnl. Medicine, Geriatrics, Wake Forest Sch. Of medicine, Winston-Salem, NC; [4]Div. of Endocrinol. and Metabolism, Departme, Ontario, ON, Canada; [5]Intrnl. Medicine-Geriatrics, Wake Forest Sch. of Med., Winston Salem, NC

Abstract: The AMP-activated protein kinase (AMPK) is a heterotrimeric protein complex critical in maintaining cellular energy homeostasis. It consists of one catalytic subunit, alpha, and two regulatory subunits, beta and gamma. The beta subunit, encoded by the AMPKβ gene, has two isoforms β1 and β2. This study aims to investigate the neuronal function of AMPKβ isoforms, taking advantage of novel transgenic mouse models with isoform-specific suppression of AMPKβ in the brain. Results from behavioral assays suggest isoform-specific roles of AMPKβ in cognitive function. To examine the underlying synaptic mechanisms, we performed biochemical (Western blot) analysis to quantify levels of hippocampal PSD95 and phosphorylated HSF1(Ser121). The results from the Western blot indicate isoform-specific dysregulation of the synaptic markers, which is consistent with the cognitive performance of the transgenic mice.

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Poster

PSTR374. Genes and Molecular Mechanisms of Learning and Memory II

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Program #/Poster #: PSTR374.13/WW27

Topic: H.08. Learning and Memory

Support: NIH grant R03NS128513

Title: Timekeepers of Learning: Unraveling the Molecular Mechanisms Underlying Circadian Regulation of Short Term Olfactory Memory in Drosophila melanogaster

Authors: *M. PENA[1], B. COLLINS[3], J. MYERS[3], G. W. ROMAN[2];
[1]Biol., [2]BioMolecular Sci., Univ. of Mississippi, Oxford, MS; [3]Biol. and Biochem., Univ. of Houston, Houston, TX

Abstract: Circadian clocks are time-keeping systems that synchronize behavior and physiology to optimal times of the day. These behaviors include the ability to form new memories. The rate of olfactory learning in *Drosophila melanogaster* has a circadian rhythm with a peak in

performance in the early to midevening. The mechanisms by which the circadian circuit communicates time-of-day information to downstream neurons, thus influencing behavioral rhythms, remains an area of active investigation. The serotonergic Dorsal Anterior Lateral (DAL) neurons, integral to the central circadian neural circuit, express the central molecular oscillator proteins and exhibit rhythmic expression of these clock proteins. The DAL neurons innervate the alpha/beta posterior neurons of the Mushroom Bodies (MBs). The MBs are critical sites for olfactory associative learning in the *Drosophila* brain. We hypothesize that DAL neurons convey time-of-day information to the MBs through the rhythmic release of serotonin (5-HT). We test this hypothesis by examining the requirement for 5HT1 receptors in supporting the circadian rhythm in olfactory learning. The 5HT1A receptor is expressed in the MB alpha/beta posterior neurons, while the 5HT1B receptor's expression within the MBs is within the alpha'/beta' and gamma lobe neurons. We have found that mutations in *5HT1A* result in loss of the circadian rhythm in the rate of learning, supporting a key prediction of our hypothesis. $5HT1A^{MB09978}$ mutants displayed similar levels of learning compared to wildtype flies, yet these *5HT1A* mutants did not display a significant difference in performance at different times of day ($p > 0.832$). In these experiments, wildtype control flies displayed rhythm in performance ($p < 0.013$). These results suggest a role for the 5HT1A receptor in the modulation of circadian rhythms in olfactory learning. In contrast, the $5HT1B^{MB05181}$ mutants retained a significant difference in performance at different times of the day ($p < 0.036$). These results do not support the role of the 5HT1B receptor in mediating circadian rhythms in olfactory learning. Finally, $rutabaga^{2080}$ adenylyl cyclase mutants performed at a significantly worse level than wildtype flies at all times of day ($p < 0.001$) yet retained a significant rhythm in performance ($p < 0.015$). These results suggest that while Rutabaga signaling is important for short-term olfactory memory, it may not have a direct role in modulating circadian rhythms in learning. Further research is needed to fully understand how circadian clocks and neurotransmitter systems interact to regulate learning and memory processes at certain times of the day.

Disclosures: **M. Pena:** None. **B. Collins:** None. **J. Myers:** None. **G.W. Roman:** None.

Poster

PSTR375. Circuit Tracing

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Program #/Poster #: PSTR375.01/WW28

Topic: I.03. Anatomical Methods

Support: R35 DE030045
R01 DE031477

Title: Central projection of nucleus of tractus solitarius neurons TRAPed by vagus nerve stimulation in mice

Authors: *M. ALI[1], J. MACK[1], Y. KIM[2], M. CHUNG[1];
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Abstract: Electrical stimulation of vagus nerve is clinically useful for treating depression or chronic pain conditions. Vagal stimulation should regulate functions in diverse regions in brain and it is critical to better understand how electrically evoked vagal inputs regulate neural activities across the regions involved in pathophysiology of emotional disorders or chronic pain. Since it is well established that neural inputs through vagus nerve are predominantly transmitted to the nucleus of tractus solitarius (NTS), we determined the direct projections of NTS neurons into different regions of the brain by adopting a targeted recombination in active populations (TRAP) method in mice. We compared the central projections of NTS neurons TRAPped by either the stimulation of cervical vagus nerve (cVNS) or auricular branch of vagus nerve (aVNS). Both cVNS and aVNS induced the expression of Fos-like proteins in NTS, especially in its caudal part (cNTS), which is consistent with previous reports. To elucidate the morphological projection of the Fos-expressing cNTS neurons throughout the brain regions, we injected adeno-associated virus encoding cre-dependent mCherry (AAV-hSyn-DIO-mCherry) in cNTS of Fos^{ERcre} mouse strains. After 3 weeks, intraperitoneal injection of tamoxifen was followed by cVNS or aVNS under anesthesia. Histological assays showed notable differences between cVNS and aVNS in the expression of mCherry in neurons within subdivisions of cNTS. In both cVNS and aVNS, anterogradely labeled mCherry-expressing axonal terminals were observed across different fore brain, mid brain and hind brain areas. The mCherry-expressing terminals were highly enriched in the brain regions implicated in chronic pain, including medullary reticular formation, lateral parabrachial nucleus, medial parabrachial nucleus, ventral posteromedial thalamic nucleus, central amygdala, lateral hypothalamus, and paraventricular hypothalamic nucleus. Moderate to mild anterograde labelings were also observed in other brain regions. The central projections of mCherry+ terminals after aVNS were mostly comparable to the patterns in cVNS. This information provides the roadmap of vagally projecting cNTS neurons with their projections and thus may facilitate studying the modulatory roles of central vagal input in central pain or depression-related circuits.

Disclosures: **M. Ali:** None. **J. Mack:** None. **Y. Kim:** None. **M. Chung:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.02/WW29

Topic: I.03. Anatomical Methods

Support: U19NS107466

Title: Cell types of the medulla based on single cell morphology and transcriptomics

Authors: *J. BAKA[1], E. THOMAS[1], M. ROZSA[1], T. A. FERREIRA[2], M. PROJECT TEAM[2], C. GERFEN[3], L. MCELVAIN[4], K. FANCHER[1], K. SMITH[1], N. DEE[1], B. LEVI[1], B. TASIC[1], J. CHANDRASHEKAR[1], D. KLEINFELD[5], K. SVOBODA[1];
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Abstract: The medulla is involved in a plethora of diverse life-sustaining functions: it's a gateway for somatosensory, visceral and interoceptive information; has pre-motor and motor nuclei for locomotion and orofacial movements and oscillators for breathing, licking, and chewing. Although the role of specific nuclei in the medulla involved in these distinct functions have been studied over decades, little is known about the identity of individual medullary cell types (molecular composition, dendritic and axonal structure etc.), their organization within the medulla or their connectivity. To fill this knowledge gap, we fully reconstructed the long-range projections of hundreds of medulla neurons and classified these neurons into six major, largely non-overlapping, groups based on their main projection areas: cerebellum; medulla/pons; midbrain; thalamus/hypothalamus; striatum; spinal cord projecting. This includes numerous, previously unknown cell types. We used the projection targets to perform retrograde labeling combined with single cell RNAseq (Retro-seq). A joint analysis of morphology, transcriptomic data from Retro-seq and the spatial localization from the whole brain MERFISH dataset (Yao et. al., 2023) was used to link individual morphological types to the transcriptomic taxonomy. For example, these joint morphological and Retro-seq experiments revealed multiple classes of medulla neurons conveying distinct sensory information to the cerebellum. This analysis of medulla cell types is a foundation for understanding how neural signals flow within and out of the medulla.

Disclosures: J. Baka: None. **E. Thomas:** None. **M. Rozsa:** None. **T.A. Ferreira:** None. **M. Project Team:** None. **C. Gerfen:** None. **L. McElvain:** None. **K. Fancher:** None. **K. Smith:** None. **N. Dee:** None. **B. Levi:** None. **B. Tasic:** None. **J. Chandrashekar:** None. **D. Kleinfeld:** None. **K. Svoboda:** None.

Poster

PSTR375. Circuit Tracing

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Program #/Poster #: PSTR375.03/WW30

Topic: I.03. Anatomical Methods

Support: Brain Initiative R34NS122050-01

Title: The comparative organization of single amygdala neuron projection patterns to frontal cortex and striatum in macaques and mice

Authors: *Z. R. ZEISLER, K. A. HESLIN, R. L. CLEM, P. H. RUDEBECK;
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Abstract: Basolateral amygdala (BLA) is involved in a wide range of cognitive processes, from defensive threat responding, to appetitive decision-making, to attention. Such a diverse set of functions is likely due to the wide-ranging projections of BLA across frontal cortex. Yet, little is known about the projection patterns of single BLA neurons to this area. Specifically, the extent to which single BLA neurons branch and project to multiple locations in frontal cortex is unclear, and even less is known about how such branching might vary across species.
Here, we used multiplexed analysis of projections by sequencing (MAPseq), a barcoded virus approach to establish the connectivity of individual BLA neurons in macaques and mice. We were able to profile the connectivity of over 3,000 macaque and over 1,600 mouse BLA neurons to frontal cortex and other subcortical structures. As a first step, we then compared the number of areas that each single BLA neuron projected to in macaques and mice. Here we found that a larger proportion of mouse BLA neurons branched to two or more areas in frontal cortex compared to macaque BLA neurons.
Next, we investigated the network-level features of single BLA neurons by comparing the projections to analogous parts of medial and ventral frontal cortex. In monkeys, BLA neurons that project to dorsal anterior cingulate cortex (dACC) were highly likely to also branch to the ventral frontal cortex, consistent with these areas' shared roles in value-based decision-making. In mice, by contrast, BLA neurons that project to the equivalent of dACC (CG1/CG2) are less likely to also project to ventral areas compared to neurons that project to prelimbic or infralimbic cortex. We also identified a highly specific gradient of BLA connectivity with nucleus accumbens (NAcc) across frontal cortex in macaques that was largely absent in mice. Specifically, mouse BLA neurons projecting to frontal cortex were uniformly and highly likely to connect to NAcc.
Overall, our data show that mouse BLA neurons are more likely to project to multiple parts of frontal cortex and thus likely broadcast information over a wide swathe of cortex. By contrast, individual macaque BLA neurons project to relatively fewer locations in frontal cortex and thus tend to form more segregated networks.

Disclosures: Z.R. Zeisler: None. **K.A. Heslin:** None. **R.L. Clem:** None. **P.H. Rudebeck:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.04/WW31

Topic: I.03. Anatomical Methods

Support: Brain/MINDS: JP15dm0207001

Title: Mapping axonal projections of the marmoset prefrontal cortex

Authors: *A. WATAKABE[1,2], H. SKIBBE[2], K. NAKAE[3], H. ABE[2], N. ICHINOHE[4], M. F. RACHMADI[2], M. TAKAJI[2], H. MIZUKAMI[5], A. WOODWARD[2], R. GONG[2], J. HATA[6], D.

C. VAN ESSEN[7], H. OKANO[8], S. ISHII[9], T. YAMAMORI[10,2];
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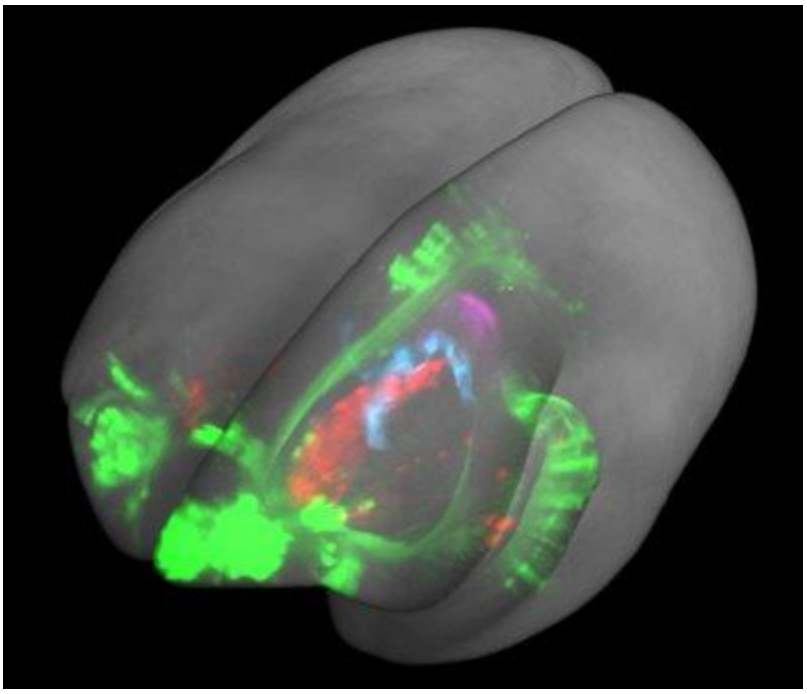
Abstract: Prefrontal cortex (PFC) is greatly expanded in primates compared to other mammals. Advances in imaging and computational methods enable mapping of neural connections with micron-level precision for entire primate brains. To gain insights into the organization of PFC circuits, we used these technologies to perform a connectomic mapping of axonal projections revealed by anterograde and retrograde tracers injected into marmoset PFC (#1). Using this dataset, we found that corticocortical and corticostriatal projections consist of two types, "patchy/columnar" and "diffuse" and that these projections recapitulate PFC gradients in their global and local distribution outside frontal cortex (#2). Here, we show that corticothalamic PFC projections also consist of multiple patches of axonal convergence (patchy projection) plus wide but low-density axonal spread (diffuse projection). Whereas we observed topographic projections from all the PFC subregions toward thalamic MD nuclei, only the dorsolateral PFC areas (e.g., areas 8aD, 8aV and premotor areas) projected to the superior colliculus. The collicular projections were "area-specific" in that area 8aV projects to the intermediate gray layer of the superior colliculus, whereas area 8aD and premotor cortex project to the deep gray layer. These observations have implications for our understanding of the functional organization of marmoset PFC areas.
#1: Our PFC database is open to the public. Visit: https://dataportal.brainminds.jp/marmoset-tracer-injectionPlease also see The Brain/MINDS Marmoset Connectivity Atlas: Skibbe et al. bioRxiv 2022. doi: https://doi.org/10.1101/2022.06.06.494999
#2: Local and long-distance organization of prefrontal cortex circuits in the marmoset brain.Watakabe et al. Neuron. 2023 May 9:S0896-6273(23)00338-0. doi: 10.1016/j.neuron.2023.04.028. Online ahead of print.
<Figure> Cortical (green), Striatal(red), thalamic (blue) and collicular (purple) signals were differentially segmented for display.



Disclosures: **A. Watakabe:** None. **H. Skibbe:** None. **K. Nakae:** None. **H. Abe:** None. **N. Ichinohe:** None. **M.F. Rachmadi:** None. **M. Takaji:** None. **H. Mizukami:** None. **A. Woodward:** None. **R. Gong:** None. **J. Hata:** None. **D.C. Van Essen:** None. **H. Okano:** None. **S. Ishii:** None. **T. Yamamori:** None.

Poster

PSTR375. Circuit Tracing

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Program #/Poster #: PSTR375.05/WW32

Topic: I.03. Anatomical Methods

Support: BRAIN/NIMH/H129 RF1 IR-FG22015 (445110-29248)

Title: New rabies viral resources for multi-scale neural circuit mapping

Authors: *G. WU[1,2], A. BOUIN[3], O. O. KOYUNCU[3], Q. YE[2], K.-Y. KIM[6,7], J. H. C. NGUYEN[3], M. WU[3], L. TONG[2], L. CHEN[2], S. PHAN[6,7], M. R. MACKEY[6,7], R. RAMACHANDRA[6,7], M. H. ELLISMAN[6,7], T. C. HOLMES[4,5], B. L. SEMLER[4,3], X. XU[2,4]; [2]Dept. Anat. & Neurobio., [3]Dept. of Microbiology and Mol. Genet., [4]The Ctr. for Neural Circuit Mapping, [5]Dept. of Physiol. & Biophysics, [1]Univ. of California, Irvine, Irvine, CA; [6]The Natl. Ctr. for Microscopy and Imaging Res., [7]Dept. of Neurosciences, UCSD, La Jolla, CA

Abstract: Over the years, genetically modified viruses have become important tools for studying neural circuit organization and function. Recombinant glycoprotein (G)-deleted rabies virus

vectors have allowed researchers to study monosynaptic, cell-type specific and circuit specific connections. However, existing viral tools are often limited to meso-scale fluorescence imaging applications. Here we developed an array of improved rabies virus vectors that allow for targeted multi-scale imaging, to be made widely available through our service platform at UCI's Center for Neural Circuits Mapping. We created 20 new rabies viral vectors for a broad range of applications across multiple scales including fluorescent proteins targeted to subcellular locations (e.g., nucleus, plasma membrane, pre- and post-synaptic specializations, and mitochondria) and dual-use reporters encoding GFP and ferritin for mesoscale fluorescent imaging and microscale electron microscopy. We demonstrate the discovery power of these tools in our studies on detailed microstructural changes in aging and Alzheimer's disease model mice, in vivo imaging of neuronal activity, and automatic counting of infected cells. These new viral vectors significantly expand the scale and power of rabies virus-mediated neural labeling and circuit mapping across multiple imaging scales.

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Poster

PSTR375. Circuit Tracing

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Topic: I.03. Anatomical Methods

Support: Voelcker Summer Research Fellowship
Trinity University Murchison and Department of Biology
The Brain and Behavior Research Foundation NARSAD Young Investigator Award
Mary E. Groff Foundation
Trinity University Women in STEM Program

Title: Creation of novel techniques for deciphering long range neural circuits

Authors: *A. N. DANG, G. SUN, A. FLANAGAN, M. SARASWATHI, M. HORN, J. CLARK, E. FARRELL, C. WILLIAMS, J. VALADEZ, G. M. BEAUDOIN, III;
Dept. of Biol., Trinity Univ., San Antonio, TX

Abstract: There are a number of methods used to study synapses through selective labeling and control. However, these techniques are often cytotoxic, difficult to implement, or lack the selectivity required to most effectively study microcircuits. We aim to create two novel techniques for studying how the brain is wired - a method for transsynaptic genetic labeling and a method for selectively destroying synapses. For synaptic labeling, we are using the C. elegans

orthologs for the Notch-Delta signaling system, Glp-1 and Lag-2, respectively. Glp-1 was modified by replacing the intracellular portion of the protein with cre-recombinase, a site directed DNA recombination enzyme. It is hypothesized that when a cell expressing the chimeric Glp1-Cre protein comes in close contact with a cell expressing Lag-2, cre-recombinase will be released allowing for cre-dependent expression of a fluorescent molecule. In vitro testing co-plating separately transfected HEK293 cells indicates that the labeling mechanism performs as expected, activating cre-dependent fluorescence in Glp-1-Cre expressing cells based on contact with Lag-2 expressing cells.To selectively destroy synapses, we have developed a chimeric protein, CadPlexin, that will activate repulsion signals through homophilic binding between two cells connected by a synapse. CadPlexin is composed of the extracellular domain of D. melanogaster DE-Cadherin and the transmembrane and intracellular domain of M. musculus Plexin-B2. Cadherins bind to one another homophillically, acting to bind cells with one another. Plexins, when dimerized and activated by their usual ligand, Semaphorin, will cause a downstream signaling cascade that induces cell-cell repulsion. We hypothesize that, when two cells expressing CadPlexin are in close contact (i.e. at a synapse), CadPlexin will selectively destroy the synapse, as the extracellular domains from DE-Cadherin will bind together and mimic semaphorin binding and clustering required to activate plexin signaling. Our results from in vitro experiments suggest that CadPlexin performs as expected, seen through creation of stress fibers in the actin cytoskeleton of Cos7 cells transfected with CadPlexin - a characteristic cytoskeletal change induced through Plexin signaling. Future work is directed towards testing these constructs in primary culture neurons. Together, these techniques will allow us to label synaptically connected neurons and destroy synapses with surgical precision by targeting synapses between some neurons while leaving other synapses intact.

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Poster

PSTR375. Circuit Tracing

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Program #/Poster #: PSTR375.07/WW34

Topic: I.03. Anatomical Methods

Support: AMED Grant JP23wm0525012
JST SPRING Grant JPMJSP2136

Title: Multiplexed mapping of mesoscale projections using fluorescent barcode vectors

Authors: *D. MORIYASU, B. SAHA, H. TAKESHIMA, Y. ISHIDA, S. FUJIMOTO, T. IMAI;
Fac. of Med. Sci., Kyushu Univ., Fukuoka, Japan

Abstract: Brain functions are often generated by the computation across multiple brain areas. To understand the information flow across multiple brain areas, the mesoscopic mapping of inter-areal projections has been widely performed. However, the existing methods have several limitations. Whole-brain imaging combined with single tracer injection visualizes projections from only one area per animal. Moreover, data obtained from multiple animals have to be registered to a standard atlas, making it difficult to evaluate fine topography. More recently, RNA barcode-based tools (e.g., MAPseq) have been developed for multiplexed connectivity mapping. However, this strategy cannot provide detailed morphological information. To overcome these limitations, we developed a multiplexed mesoscopic mapping tool “fluorescent barcode vectors”. We constructed the vectors expressing one or two out of the 7 different fluorescent proteins (XFPs), allowing the multiplexed labeling with up to 28 types (designated “fluorescent barcode vectors”). In the HEK 293T cells transfected with these barcodes, each XFP signal was detected in an all-or-none fashion after linear unmixing of fluorescence signals.We then injected AAVs expressing the fluorescent barcodes into multiple cortical areas. The whole brain slices were imaged with confocal microscopy. The barcodes in neuronal somata were correctly identified at 98.2% by linear classification based on the color distance. Axons were also clearly visualized with different fluorescent barcodes, demonstrating topographic organization in subcortical areas. For the automated identification of fluorescent barcodes, we developed a machine learning-based “barcode reader” that performs pixel classification based on color and morphological information. Despite a model trained on a limited number of datasets, this program detected and identified even dense or fine axonal signals. Our fluorescent barcode vectors with automated barcode reader provide a multiplexed mapping of mesoscopic connectivity without losing any morphological information. Our strategy is powerful for our understanding of the dynamic regulation and dysregulation of mesoscopic connectivity throughout the life cycles and under various disorders.

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Poster

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Topic: I.03. Anatomical Methods

Support: NIH Grant ZIAAG000959

Title: Kremen1-2a-cre knock-in mice: A new tool for studying striosomal spiny projection neurons

Authors: B. SULLIVAN, J. DONG, *H. CAI;
Natl. Inst. on Aging, Bethesda, MD

Abstract: The dorsal striatum is comprised of distinct striosome-matrix compartments that exhibit unique molecular and functional characteristics. Previous research has highlighted the importance of striosomes in regulating mood, decision-making, and reward processing. However, the precise neurochemical organization of striosomes remains less defined, primarily due to limitations in existing mouse models such as Nr4a1-GFP, Pdyn-Cre, and Sepw1-Cre. Our RNA sequencing data has identified Kremen1 as a promising marker for distinguishing striosome-matrix compartments. Therefore, we employed CRISPR/Cas9 technology to develop a new line of Kremen1-2A-Cre knock-in mice. We characterized this mouse strain by crossing it with Ai14 reporter mice, which express red fluorescent protein (RFP) tdTomato in a Cre-dependent manner. Immunohistochemistry using an anti-RFP antibody revealed that the tdTomato signals were localized to the dorsal striatum, specifically within the striosomes. These signals were found to co-localize with the commonly used striosome marker, mu opioid receptor (MOR1), and selectively labeled striosomal spiny projection neurons (SPNs) without labeling interneurons or glial cells in the dorsal striatum. Quantitative analysis of our RNAscope data demonstrated that Kremen1 is expressed in approximately 60% of striosomal SPNs expressing dopamine receptor 1 and 40% of striosomal SPNs expressing dopamine receptor 2. To further investigate the connectivity of striosomal SPNs, we performed stereotaxic injections of AAV-FLEX-SynGFP into the dorsal striatum to label axon terminals with green fluorescent protein (GFP). We quantified the number of GFP puncta in striatal projection targets, including the external globus pallidus and substantia nigra regions. In summary, our findings suggest that the Kremen1-2A-Cre knock-in mouse model is a valuable tool for studying the neuroanatomy and functions of striosomal SPNs. This mouse model provides novel insights into the organization and role of striosomes in the dorsal striatum, shedding light on their potential implications in various neurological processes and diseases.

Disclosures: B. Sullivan: None. **J. Dong:** None. **H. Cai:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.09/WW36

Topic: I.03. Anatomical Methods

Title: Artificial Intelligence-Based Neuronal Soma Detection and Dendritic Tracing in Dense, 3D Neuronal Environment and in Golgi-stained Tissue

Authors: *W. CHOI[1,2], H.-Y. CHANG[2], C.-C. HUANG[2], H. LAI[2], S. MCELROY[2], Q. TRAN[2], L. LUCAS[2];
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Abstract: Techniques and tools for morphological characterization of neurons in 3D have evolved over the years from highly manual to several automated or semi-automated tracing methods. However, many of these automated methods require tedious sample preparation - tissue

clearing, selective neuronal labeling, and high-resolution fluorescent imaging - to achieve the desired analysis outcome. Many thresholding-based methods for automated neuron tracing fail when dense neuron environments are imaged. Meanwhile, for Golgi-stained neurons, the classical standard neuron preparation method for neuroscientists, there are two significant challenges that complicate automated neuronal tracing. First, objects that are out of focus appear as blurry objects on multiple planes. Second, the dark and opaque Golgi stain casts physical shadow from neuronal somas and dendrites, reducing the signal-to-noise ratio.
In this poster, we present a novel way to incorporate artificial intelligence in neuron analysis to achieve improved accuracy and efficiency for neurons imaged in different modalities: 1) Using a modified version of the generalist Cellpose deep learning (DL) algorithm (Stringer et al, 2021), more accurate detection of neuronal somas can be achieved than thresholding-based methods in dense neuronal images. 2) Using machine learning (Random Forest for pixel classification), neuronal somas can be detected, and dendrites traced automatically in Golgi-stained neurons. Once neurons are detected and dendrites traced, a machine learning-based object classification approach is applied to classify neurons based on morphological characteristics for efficient categorization and characterization of neuronal populations. The spatial relationship between neurons (somas/dendrites/spines) to other objects (vesicles/cells) can be automatically calculated using the neuron analysis workflow that is presented in this poster.
In conclusion, this work presents a novel approach utilizing artificial intelligence techniques for the detection and morphological characterization of neurons in 3D. By creating a DL-based image analysis pipeline, accurate detection of neuronal somas in dense environments and automatic tracing of dendrites in Golgi-stained neurons are achieved. This innovative neuron analysis workflow enhances efficiency and enables the categorization and characterization of neuronal populations based on morphological characteristics. Moreover, it facilitates the automatic calculation of spatial relationships between neurons and other objects.

Disclosures: **W. Choi:** A. Employment/Salary (full or part-time):; Leica Microsystems. **H. Chang:** A. Employment/Salary (full or part-time):; Leica Microsystems. **C. Huang:** A. Employment/Salary (full or part-time):; Leica Microsystems. **H. Lai:** A. Employment/Salary (full or part-time):; Leica Microsystems. **S. McElroy:** A. Employment/Salary (full or part-time):; Leica Microsystems. **Q. Tran:** A. Employment/Salary (full or part-time):; Leica Microsystems. **L. Lucas:** A. Employment/Salary (full or part-time):; Leica Microsystems.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

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Program #/Poster #: PSTR375.10/WW37

Topic: I.03. Anatomical Methods

Support: NIH Grant AT011665

Title: Using Manganese Enhanced MRI to Trace and Assess Ascending Vagal Pathways

Authors: X. WANG, J. CAO, ***Z. LIU**;
Biomed. Engin., Univ. of Michigan, Ann Arbor, Ann Arbor, MI

Abstract: The vagus nerve, the key peripheral pathway mediating the bi-directional neural communication between the brain and visceral organs, extends through ascending pathways that interconnect multiple subcortical and cortical areas. Despite its significance, the structural and functional attributes of these ascending vagal pathways have not been fully characterized. Current methodologies, including neural recordings, functional MRI, and viral tracing, have limitations in spatial precision, resolution, and the ability to perform repeatable in vivo studies. To address these limitations, our study employed manganese-enhanced magnetic resonance imaging (MEMRI), for non-invasive, in vivo, and trans-synaptic tracing of the vagus nerve projections to the brainstem and beyond, while assessing their functional dependencies on cervical vagus nerve stimulation using 19 rats and 7-Tesla MRI. Specifically, we administered manganese chloride into the nodose ganglion (NG), and acquired T1-weighted MRI to track the subsequent neuronal uptake and axonal transport of these ions. Our data, collected 12 and 24 hours post-injection, revealed manganese ion uptake by vagal afferent neurons within the NG, and their transport to the nucleus tractus solitarius (NTS) in the brainstem, leading to a contrast enhancement of 19 to 24%. Intriguingly, we observed manganese uptake in regions beyond the NTS, encompassing various brainstem and subcortical nuclei and cerebellar regions. Furthermore, vagus nerve stimulation substantially augmented neural activity and correspondingly enhanced contrast to 40 to 43%. Notably, we also detected temporal and activity-dependent variations. T1-weighted intensity demonstrated a higher contrast enhancement at 12 hours post-injection compared to 24 hours. Vagus nerve stimulation significantly modulated manganese ion uptake, transport, and accumulation. These findings underscore the potential of MEMRI as a non-invasive, repeatable approach for both structural and functional characterization of vagal afferents and their ascending influences on brain activity, offering a promising platform for in-depth exploration of vagal-brain interactions under a multitude of physiological and pathophysiological conditions.

Disclosures: **X. Wang:** None. **J. Cao:** None. **Z. Liu:** None.

Poster

PSTR375. Circuit Tracing

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Program #/Poster #: PSTR375.11/WW38

Topic: I.03. Anatomical Methods

Support: JP23H00395
JP20H00492
JP20H03556
JP22K19481
JP21dm0207117
JP21wm0525005

JP23ama121052
JP23ama121054

Title: Development of an AAV vector for noradrenergic neuron-specific gene expression and application to mesoscale analysis of noradrenergic wiring diagrams in mice.

Authors: *K. SEIRIKI, S. MAEDA, L. KOJIMA, A. KASAI, H. HASHIMOTO;
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Abstract: The locus coeruleus noradrenergic (LC-NA) system is considered to play crucial roles in multiple brain functions including arousal, cognitive and emotional regulations. LC-NA neurons have broad axonal projection throughout the whole brain, but recent technological advances have revealed that individual neurons or neuronal subpopulations send distinct efferent projections to specific brain regions. However, it is not well understood whether and to what extent LC-NA neurons have mutually exclusive or some overlapped projection patterns. To identify their axonal wiring at mesoscale level, we developed an adeno-associated viral (AAV) vector for NA neuron-specific gene expression and conducted whole-brain mapping of LC-NA axons using the serial section whole-brain imaging system. By local injection of the AAV encoding fluorescent protein into the LC, we observed 92% of cells expressing fluorescent proteins were tyrosine hydroxylase-positive neurons. Using an axon-targeting fluorescent protein and retrograde AAV, we found that an LC-NA subpopulation projecting to the ventral hippocampus also sends dense projection to the dorsal hippocampus and collaterals to the wide cortical area. These results suggest that although LC-NA neurons have dense projection in a target-specific manner, they may globally transmit NA and regulate multiple brain regions via collaterals. These findings and the AAV vectors will contribute to more precise understanding of complex organization of LC-NA systems.

Disclosures: K. Seiriki: None. **S. Maeda:** None. **L. Kojima:** None. **A. Kasai:** None. **H. Hashimoto:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.12/WW39

Topic: I.03. Anatomical Methods

Title: Vesicular stomatitis virus based barcoded library for high-throughput synaptic output mapping

Authors: *L. JIN, H. ZHANG, X. GAO;
Lingang Lab., Shanghai, China

Abstract: Decoding neural circuit is an essential way to understand the brain function and the mechanism of neurological disorders. Rabies virus-based barcoded library can map high-

throughput circuits at cellular resolution and a brain-wide scale. However, it's a retrograde tracer for input mapping. The anterograde barcoded virus library is still needed. We established a barcoded virus library based on glycoprotein-deletion vesicular stomatitis virus (VSV). We can package two versions of VSV library: VSVG coated virus for directly anterograde labeling and EnvA coated virus for monosynaptic tracing. By matching the same barcode sequences between source cells and output cells, transcriptomic information can be analyzed based on their connectivity. It can offer high-throughput synaptic output mapping and provide transcriptomic identities of virus-infected cells by using both single-cell RNA-seq and *in situ* sequencing.

Disclosures: L. Jin: None. **H. Zhang:** None. **X. Gao:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.13/WW40

Topic: I.03. Anatomical Methods

Support: NIH Grant 1U01NS126054-01

Title: Measuring Brain-wide multiplexed axon connectivity with RNA-FISH via retrograde AAV and HSV Barcode Viral Vectors.

Authors: *T. KIM[1], C. J. MAGNUS[1,2], V. L. ANDERSON[1,2], S. M. STERNSON[1,2]; [1]UCSD Dept. of Neurosciences, La Jolla, CA; [2]Howard Hughes Med. Institute, UCSD, San Diego, CA

Abstract: To understand the cell type-specific wiring diagrams for the mouse brain, it is necessary to integrate neuronal gene expression information with axon connectivity. Here, we developed retrograde viral tracers based on Adeno-associated virus (AAV) and Herpes simplex virus (HSV) to determine axon projection targets in concert with multiplexed gene expression measurements. We designed axon-infecting AAV and HSV viruses expressing barcode transgenes that can be detected by selective oligonucleotide probes. We can achieve highly multiplexed connectivity maps in individual brains by injecting barcode viral vectors into different brain regions. We also assessed retrograde labeling efficiency and tropism of barcode viral vectors for neuron projections arising from different brain regions in comparison with Cholera toxin subunit B (CTB) retrograde labeling. These viral tools will aid understanding of the axon projection patterns of molecularly defined neuron types in the mouse nervous system.

Disclosures: T. Kim: None. **C.J. Magnus:** None. **V.L. Anderson:** None. **S.M. Sternson:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.14/WW41

Topic:

Support: National Institute of Neurological Disorders and Stroke (NIH Grant R21NS125372)

Title: Non-invasive focal delivery of viral neuronal tracers in the marmoset monkey brain with focused ultrasound

Authors: *T. PARKS[1,2], D. SZUZUPAK[1], S. CHOI[1], D. J. SCHAEFFER[1];
[1]Neurobio., Univ. of Pittsburgh, Pittsburgh, PA; [2]Ctr. for the Neural Basis of Cognition, Pittsburgh, PA

Abstract: Although preclinical neuroscientific modeling species afford the ability for invasive intracranial delivery of neurotropic agents, direct intracranial injections are not readily translatable to clinical therapeutics. Transcranial focused ultrasound (tFUS) has been identified as a technique to circumvent surgical injections altogether by transiently opening the blood-brain barrier (BBB) with selective focus. We recently characterized the ability to focally deliver substances across the BBB in the marmoset, a non-human primate model with similar husbandry requirements to rodents but with cortical topologies more similar to humans. While optimizing for safety and limiting parenchymal damage, we established a reliable method for selectively delivering adeno-associated viral vectors (AAVs) across the BBB in marmoset frontal cortex with focused ultrasound and demonstrate long-range anterograde neuronal tracing. Using a single-element 1.46 MHz transducer, we focally perturbed the BBB (~500 μm^2) in area 8aD of frontal cortex in six adult marmoset monkeys using low intensity focused ultrasound aided by microbubbles. We confirmed BBB opening via a gadolinium-enhanced MRI at 9.4T prior to AAV delivery. Within an hour of opening the BBB, either AAV2 or AAV9 was delivered systemically via tail-vein injection. Four to six weeks later, animals were sacrificed and ex vivo microscopy was performed to confirm the presence of transduced neurons as indicated by EGFP or mCherry fluorescence. Neurons were observed at site of BBB opening with an exiguous distribution of transduced neurons when compared to direct-intracortical injections. The results are compared to direct intracortical injections of anterograde tracers into area 8aD and similar (albeit sparser) long-range connectivity was observed. With evidence of transduced neurons specific to the region of BBB opening as well as long-distance tracing, we establish a framework for which AAVs can be delivered to the marmoset brain noninvasively. This technique will be of utility for the burgeoning marmoset model, with applications for noninvasive delivery of therapeutics, genetic delivery of precursors for techniques like two-photon imaging, or neuronal tracing across the lifespan.

Disclosures: T. Parks: None. **D. Szuzupak:** None. **S. Choi:** None. **D.J. Schaeffer:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.15/WW42

Topic: I.03. Anatomical Methods

Support: UG3MH120094 (WRS)
DP2MH113095 (WRS)

Title: Cell type-specific AAVs to target pyramidal neurons in mammalian cortex

Authors: *B. M. HOOKS[1], A. FRONSTIN[1], T. SHAH[1], J. HE[1], B. PHAN[2], L. BYRNE[4], A. R. PFENNING[3], W. R. STAUFFER[5];
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Abstract: Cell types are the fundamental component of cortical circuitry. Excitatory cortical neurons differ in laminar distribution, dendritic arborization patterns, long-range axonal targets, circuit connectivity, and functional responses. To understand the contribution of specific cell types to cortical processing, a means to target, label, and manipulate cell types across a range of species, including mice and primates, is necessary. Here, we characterize the regional expression, laminar distribution, and long-range projections of several cell type-specific AAVs which label excitatory pyramidal neurons based on enhancer sequences mapped from non-human primate dorsolateral prefrontal cortex. Single enhancer-based AAVs were injected into mouse across multiple cortical areas to test region expression. Whole brains were imaged and expression patterns at the injection site as well as long-range cortical targets were quantified. We compare laminar distribution of fluorophore-expressing neurons for across cortical areas, including granular (S1, V1) and agranular (M1, M2) areas as well as midline wall. Aligning brains to a reference atlas, we further compare projection patterns from these areas to defined cortical regions using fluorescence as a measure of projection strength. These tools will likely be of use for cell type-specific manipulations in rodent, NHP, and other model organisms to address the role of specific cell types in cortical function without complicated transgenic strategies.

Disclosures: B.M. Hooks: None. **A. Fronstin:** None. **T. Shah:** None. **J. He:** None. **B. Phan:** None. **L. Byrne:** Other; Founder/CSO, Avista Theraputics. **A.R. Pfenning:** None. **W.R. Stauffer:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.16/Web Only

Topic: I.03. Anatomical Methods

Support: Shanghai Municipal Science and Technology Major Project Grant 2018SHZDZX05

Title: Pyswcloader: morphology-based single neuron clustering and projectome analysis toolbox

Authors: *T. GAO[1], Z. JIAO[2], Y. MU[1], S. XU[1], X.-H. XU[2], C. XU[1];
[1]Computing and Data Ctr., [2]Inst. of Neuroscience, Chinese Acad. of Sci., Shanghai, China

Abstract: As 3D reconstruction techniques advance, a growing number of datasets of labeled neurons are generated and deposited daily. However, there is still a lack of efficient tools that enable all researchers to easily utilize these data. In this study, we present pyswcloader, a comprehensive toolbox that integrates multiple analysis algorithms with optimized time efficiency. The functions of pyswcloader include (a) neuron morphology and brain region visualization; (b) single-neuron clustering based on a modified Hausdorff match distance; (c) single-neuron morphology parameters calculation; (d) single-neuron projection parameters calculation in the template brain; (e) network analysis based on neuron projection patterns. With pyswcloader, we can differentiate various neuron subtypes and quantify neuron projection characteristics, thereby addressing questions with different research focuses. We validated the clustering algorithm first on our own mouse hypothalamus projectome dataset, as well as two published datasets comprising 77 mouse hypothalamic neurons and ~6400 mouse prefrontal cortex neurons. In all cases, pyswcloader successfully identified distinct neuronal subtypes with both different downstream projection patterns and morphological characteristics, without requiring any manual labeling procedures. Moreover, we conducted projection analysis on our own mouse hypothalamus projectome dataset, uncovering both novel and confirmatory projection patterns connected with existing functional implications. Together, pyswcloader offers an open-source and user-friendly solution, empowering researchers with a versatile tool to exploit single-neuron datasets. It is now published as a Python library readily accessible on PyPi and is continuously updated to ensure its ongoing development and improvement.

Disclosures: T. Gao: None. **Z. Jiao:** None. **Y. Mu:** None. **S. Xu:** None. **X. Xu:** None. **C. Xu:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.17/WW43

Topic: I.03. Anatomical Methods

Support: 2018SHZDZX05

Title: Projectome-defined subtypes and modular intra-hypothalamic subnetworks of peptidergic neurons

Authors: *Z.-L. JIAO[1], T. GAO[1], X. WANG[1], W. ZHANG[1], H. GONG[2], Y. SUN[1], X. XU[1]; [1]Chinese Acad. of Sci. Ctr. for Excellence in Brain Sci. and Intelligence Technol., shanghai, China; [2]HUST-Suzhou Inst. for Brainsmatics, JITRI, Suzhou 215123, China, Suzhou, China

Abstract: The hypothalamus plays a vital role in coordinating essential neuroendocrine, autonomic, and somatomotor responses for survival and reproduction. While previous studies have explored population-level projections of hypothalamic neurons, the specific innervation patterns of individual hypothalamic axons remain unclear. To understand the organization of hypothalamic axon projections,in this study, we used a recently developed axon tracing method based on fluorescence micro-optical sectioning tomography (fMOST) and constructed the single-cell projectomes of 7180 hypothalamic neurons that express specific neuropeptide genes. This provides the largest known single-neuron projectome dataset for the hypothalamus. Our analysis identified 31 distinct subtypes based on projectome-defined characteristics, with many exhibiting long-range axon collateral projections to multiple brain regions. Notably, these subtypes selectively targeted specific subdomains within downstream areas, either unilaterally or bilaterally. Furthermore, we observed that individual peptidergic neuronal types encompassed multiple projectome-defined subtypes, explaining their diverse functional roles. Additionally, by examining intra-hypothalamic axon projections, we uncovered six modular subnetworks characterized by enriched intramodular connections and distinct preferences for downstream targets. This modular organization of the intra-hypothalamic network likely contributes to the coordinated organization of hypothalamic outputs. In summary, our comprehensive projectome analysis reveals the organizational principles governing hypothalamic axon projections, providing a framework for understanding the neural circuit mechanisms underlying the diverse and coordinated functions of the hypothalamus.

Disclosures: Z. Jiao: None. **T. Gao:** None. **X. Wang:** None. **W. Zhang:** None. **H. Gong:** None. **Y. Sun:** None. **X. Xu:** None.

Poster

PSTR375. Circuit Tracing

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR375.18/WW44

Topic: I.03. Anatomical Methods

Support: Glaucoma Research Foundation Shaffer Grant
NIH Grant P30 EY007551
NIH Grant P30 EY002162
All May See Foundation research grant

Title: A large-scale approach to detection of excitatory and inhibitory synapses in the nervous system using parallel computing and deep learning

Authors: V. SALAMA, B. M. MONTGOMERY, ***L. DELLA SANTINA**;
Col. of Optometry, Univ. of Houston, Houston, TX

Abstract: Efficient mapping of neuronal connectivity and characterization of plastic events during development, maintenance or degeneration of neural circuits is limited by our ability to sample large areas of the central nervous system (CNS) and analyze densely packed synaptic layers. We propose a novel approach based on parallel computing and deep learning to quantify synaptic proteins in large portions of the CNS using confocal imaging.
In order to label synaptic proteins, retinal whole mounts were prepared from adult CD1 mice. Individual retinal ganglion cells (RGCs) and their postsynaptic excitatory and inhibitory sites were labeled by biolistic transfection of PSD-95 and Gephyrin tagged with YFP fluorescent protein. Confocal image stacks of individual ON and OFF alpha RGCs were acquired at a voxel size of 0.1x0.1x0.3 microns. The automatic pipeline for detection of synaptic proteins was developed in MATLAB within our open-source program ObjectFinder. A ground truth database of annotated synaptic proteins was created by the consensus of two human experts. Machine learning models were trained using Nvidia Quadro RTX 8000 GPU and MATLAB Deep Learning Toolbox.
Automatic detection of both excitatory and inhibitory proteins was achieved with an accuracy greater than 90% by training ResNet-50 classifier models. A minimum of 8,000 manually annotated synapses per condition was provided as ground truth to ensure model accuracy and generalization across the different labeled synaptic proteins and cell types. Trained models were able to generalize across RGCs of different types. Separate trained models were created to discern between excitatory and inhibitory synaptic proteins.
This approach shows that excitatory and inhibitory synapses can be automatically and efficiently detected in large-scale 3D image stacks using a combination of high-performance computing and deep learning classification of synapse candidates. In synaptic dense neuronal tissues such as the mammalian retina, this approach reduces the current variability of manual and semi-automatic synaptic detection approaches. At the same time it enables survey of large-scale patterns of synaptic rearrangement, such as those undergoing along development and during neurodegenerative diseases.

Disclosures: V. Salama: None. **B.M. Montgomery:** None. **L. Della Santina:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.01/WW45

Topic: I.03. Anatomical Methods

Title: A different interpretation of the DIANA fMRI signal

Authors: *V. PHI VAN[1], S. SEN[3], A. JASANOFF[2];
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[3]Massachussetts Inst. of Technol., Cambridge, MA

Abstract: Direct detection of neural activity by functional magnetic resonance imaging (fMRI) has been a longstanding goal in neuroscience. A recent study argued that it is possible to detect neuroelectrical potentials using a specialized fMRI scanning approach called DIANA. We implemented DIANA in anesthetized rats and measured responses to somatosensory stimulation, reproducing core findings of the original study. We show, however, that neural activity is neither sufficient nor necessary to produce such results. We use a combination of control conditions and simulations to demonstrate that DIANA signals can arise from nonideal aspects of the pulse sequence and specimen that help determine spatiotemporal characteristics of the data. Our analysis emphasizes a need for cautious interpretation and mechanistic evaluation of novel fMRI techniques.

Disclosures: V. Phi van: None. **S. Sen:** None. **A. Jasanoff:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR376.02/WW46

Topic: I.03. Anatomical Methods

Support: PA Department of Health SAP #4100083102 to ACS
CNPQ
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Title: Heterotopic connectivity of callosal dysgenesis in mice and humans

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Abstract: The corpus callosum (CC), the largest brain commissure and the primary white matter pathway for interhemispheric cortical connectivity, was traditionally viewed as a predominantly homotopic structure, connecting mirror areas of the cortex. However, new studies verified that most callosal commissural fibers are heterotopic. Recently, we reported that ~75% of the callosal connections in the brains of mice, marmosets, and humans are heterotopic, having an essential role in determining the global properties of brain networks. In the present study, we leveraged high-resolution diffusion-weighted imaging and graph network modeling to investigate the relationship between heterotopic and homotopic callosal fibers in human subjects and in a spontaneous mouse model of Corpus Callosum Dysgenesis (CCD), a congenital developmental CC malformation that leads to widespread whole-brain reorganization. Our results show that the

CCD brain is more heterotopic than the normotypical brain, with both mouse and human CCD subjects displaying highly variable heterotopicity maps. CCD mice have a clear heterotopicity cluster in the anterior CC, while hypoplasic humans have strongly variable patterns. Graph network-based connectivity profile showed a direct impact of heterotopic connections on CCD brains altering several network-based statistics. Our collective results show that CCD directly alters heterotopic connections and brain connectivity.

Disclosures: D. Szczupak: None. **R. Lent:** None. **F. Tovar-Moll:** None. **A.C. Silva:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.03/WW47

Topic: I.03. Anatomical Methods

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Key R&D Program of Jiangsu Province (BE2022049)
Key-Area R&D Program of Guangdong Province (2018B030333001)

Title: Revealing intracortical microvascular architecture in non-human primate cortex in vivo using 7T MRI

Authors: *J. WANG[1,2], Y. LIU[1], Y. MA[3], L. LIN[1,4], A. PING[1], L. LI[1,4], A. BERMAN[3,5], S. BOLLMANN[6], J. R. POLIMENI[7,8,9], A. W. ROE[1,2,10];
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Abstract: Objective Intracortical blood vessels supply and drain the parenchymal capillary bed, which reflects both functional architecture and shapes local hemodynamic responses exploited in functional MRI (fMRI) (Ohki et al., 2005; O'Herron et al., 2016). Thus, imaging intracortical angioarchitecture is critical to the interpretation of mesoscale fMRI. Previous studies imaged these intracortical vessels in cat (Bolan et al., 2006) and rat (Yu et al., 2016) using small-bore preclinical MRI scanners (≥ 9.4 T); however, there are substantial differences in the functional organization and underlying microvasculature of the cerebral cortex in primates compared with carnivore and rodent species (Schmid et al., 2019; Zhang et al., 2022). To date, no study has measured these vessels *in vivo* in primate. Imaging intracortical vessels in primate is challenging because the vessels have small diameters (tens of microns), the primate cortex is highly folded resulting in multiple vascular orientations, there is limited knowledge about the blood flow velocities needed to optimize the imaging, and large-bore scanners needed for primates lack the strong, fast gradient coils found on small-bore scanners. Methods Using a 7T whole-body human MRI scanner (Siemens Healthcare) and a custom 16-channel RF surface coil (Zhang et al., 2021), we imaged fine-scale vascular architecture in macaque monkeys *in vivo* and non-invasively by optimizing a 2D time-of-flight sequence for high in-plane resolution (64×64 μm^2, 1mm slice thickness and 20 min time of acquisition). Single-slices were positioned on relatively flat patches of V1 and V2/V4 in anaesthetized macaques (n=3, 3-6 kg). Results (1) We detected, for the first time, intracortical vessels; the vessels were approximately one voxel in width, suggesting that they are ≤100 μm in diameter. (2) Detection was reproducible between runs and between different days. (3) Quantitative assessment of vessel distances, densities, and the presence of arterial-venule units were similar to previous histology data (Weber et al., 2008; Adams et al., 2015). (4) Intracortical vessels could be tracked across cortical depth cross multiple acquisitions. (5) Our imaging method includes optimized image acquisition, customized processing and data visualization. Conclusion We show that intracortical vessels can be reliably imaged in primates using ultra-high field 7T MRI. Our results push further the capabilities of MRI technology, which, in combination with fMRI, will provide a powerful approach for studying vascular anatomy and function non-invasively.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.04/WW48

Topic: I.03. Anatomical Methods

Support: Grant 2021ZD0204500

Title: Whole-brain em-connectomics of larval zebrafish with neuronal-type specific multiplex labeling

Authors: *F.-N. LI[1], J.-Z. LIU[2], X. CHEN[2], C. SHI[1], J. LIU[2], J.-B. YUAN[2], L.-L. LI[2], H. HAN[2], J.-L. DU[1];
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Abstract: Mapping the brain micro-connectome is essential to understand the working principle of the brain. However, due to technique challenges, whole-brain micro-reconstruction of vertebrates has not been achieved, and most microscopic reconstructions of a piece of the brain lack cell type identities. In our study, we collected a whole-brain EM dataset with multiple types of neuromodulatory neurons labeled by subcellular-localizing APEX2 in a 6-dpf zebrafish. We reconstructed *Locus coeruleus* noradrenergic neurons (LC-NE)' soma and dendrites and found that both morphologies of dendrites and synaptic inputs are heterogeneous. Some synaptic inputs are clustered as hotspots. Moreover, we found that there are common presynaptic neurons, which innervate several LC-NE neurons and may contribute to synchronous activities of LC-NE neurons. In the future, this dataset will not only help understand the architecture of the brain but also guide functional studies and boost the development of neuromorphic chips.

Disclosures: F. Li: None. **J. Liu:** None. **X. Chen:** None. **C. Shi:** None. **J. Liu:** None. **J. Yuan:** None. **L. Li:** None. **H. Han:** None. **J. Du:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

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Sidney R. Baer, Jr. Foundation
HMS Norman E. Zinberg Fellowship

Title: Disease-informed assessment identifies robust brain correlates of cognition in psychosis and its prodrome

Authors: H. B. WARD[1], A. BEERMANN[2], J. XIE[2], G. YILDIZ[2], K. MANZANAREZ[2], J. ADDINGTON[3], C. E. BEARDEN[4], K. CADENHEAD[5], T. D. CANNON[6], B. CORNBLATT[8], M. KESHAVAN[2], D. H. MATHALON[9], D. O. PERKINS[10], L. SEIDMAN[2], W. S. STONE[2], M. T. TSUANG[5], E. F. WALKER[11], S. WOODS[7], M. J. COLEMAN[12], S. BOUIX[12], D. J. HOLT[13], D. ONGUR[14], A. BREIER[15], M. E. SHENTON[12], S. HECKERS[1], M. A. HALKO[14], K. E. LEWANDOWSKI[14], ***R. O. BRADY, Jr.**[2];
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Abstract: Background: Neurocognitive impairment is a well-known phenomenon in schizophrenia that begins prior to psychosis onset and is believed to reflect progressive disease processes. Connectome-wide association studies (CWAS) have inconsistently linked cognitive performance to resting-state fMRI signals. We hypothesized that reliable brain-behavior associations can be identified in small samples using cognitive instruments tailored to the population. Continuous performance measures such as the Seidman auditory continuous performance task (ACPT), are among the more sensitive tests for distinguishing healthy participants from those with psychosis. We used this task in a CWAS aimed at identifying psychosis-specific circuits of cognitive impairment.
Methods: We used multivariate pattern analysis of whole-connectome data in individuals with psychosis from the Human Connectome Project for Early Psychosis (HCP-EP, n=183) to identify the strongest link between connectivity and ACPT performance. We then tested this cognition-connectivity relationship in data from the North American Prodrome Longitudinal Study (NAPLS2, n=345), a multi-site prospective study of individuals at risk for psychosis.
Results: The ACPT gave rise to robust brain-cognition links across the psychosis spectrum. In our multivariate pattern analysis, the strongest correlate of ACPT performance ($r=0.36$, $p<.001$) was connectivity between a medial prefrontal cortex (MPFC) region and parietal node in the psychosis group. We then tested whether MPFC-parietal connectivity was related to ACPT performance in individuals at risk for psychosis. This connectivity-cognition relationship was present only in at-risk individuals who would later develop psychosis ($r=0.65$, $p=.006$).
Conclusions: CWAS identified reproducible links between connectivity and cognition in multi-site samples. These cognition-connectivity relationships were observed even prior to diagnosis of psychosis. By using a disease-informed approach to identify brain-phenotype relationships, we can find consistent results with meaningful implications.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.06/WW50

Topic: I.03. Anatomical Methods

Support: NIH 1DP2MH132940

Title: Mapping synaptic connectivity at scale by in situ sequencing of barcoded rabies

Authors: A. ZHANG[1], L. JIN[2], S. YAO[3], M. MATSUYAMA[4], C. V. VELTHOVEN[1], H. SULLIVAN[4], M. KELLIS[5], B. TASIC[6], I. R. WICKERSHAM[4], ***X. CHEN**[1];
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Abstract: The connectivity of myriad neuronal types defines the structure of neural circuits and provides the physical foundation for circuit functions. Unraveling the connectivity of single neurons at scale, however, remains challenging, because conventional imaging-based connectivity mapping techniques trade off between resolution and throughput. Here we combine high-throughput *in situ* sequencing with barcoded rabies virus-based tracing to achieve both multiplexed retrograde labeling and transsynaptic labeling. We performed *in situ* sequencing on 3,899,611 cells across three animals, including 7,338 neurons that were labeled with rabies barcodes. By also sequencing a panel of cortical excitatory neuron markers, we robustly identified the transcriptomic types of barcoded neurons that recapitulated known cell types in single-cell RNAseq datasets. We identified long-range projections that were consistent with known connectivity across cell type and cortical areas. We then identified cell types that preferentially synapse onto the same individual post-synaptic neurons. Barcoded rabies-based neuroanatomical approaches complement existing barcode sequencing-based mapping techniques and provide a path for systematically unraveling both local and long-range synaptic connectivity of cell types in large brains.

Disclosures: A. Zhang: None. **L. Jin:** None. **S. Yao:** None. **M. Matsuyama:** None. **C.V. Velthoven:** None. **H. Sullivan:** None. **M. Kellis:** None. **B. Tasic:** None. **I.R. Wickersham:** None. **X. Chen:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.07/WW51

Topic: I.03. Anatomical Methods

Support: R01DC016765
R01DC016915
R01DC017991
P41EB015896
S10OD023637

Title: Laminar Connectomic Analysis of Resting-state Functional MRI

Authors: *P. KOTLARZ, K. LANKINEN, M. HAKONEN, T. TURPIN, J. POLIMENI, J. AHVENINEN;
Athinoula A. Martinos Ctr. for Biomed. Imaging, Charlestown, MA

Abstract: Understanding cortical laminar activity has yielded insights into brain organization. Advances in neuroimaging techniques have enabled noninvasive imaging of intracortical functional activity through high-resolution functional magnetic resonance imaging (fMRI). Although previous fMRI studies have examined connectivity of cortical lamina, no previous studies have applied multilayer graph theory to identify functional differences between cortical depths. Here, we apply a multilayer connectomic framework to understand cortical layer connectivity using resting-state data from thirty subjects, measured using high-resolution 7 Tesla fMRI (1 mm^3 voxel size). Blood oxygen level dependent (BOLD) signals from five cortical depths were used to create layer-by-layer individual and independent matrices alongside one supra-adjacency matrix connecting each layer. Our work shows clear functional differences between cortical layers using graph theory quantifiers. The ability to identify these differences were improved using the multilayer approach compared to single layer connectomics. Notably, the importance of between-layer connections was highlighted by our multilayer approach. Alongside methodological advancements in laminar connectomics, our work provides a specific regional guideline for future functional laminar work. We identified that the temporal, frontal, and occipital brain regions may be best suited for laminar analysis since these regions showed the biggest difference between layers. Furthermore, we found that superficial layers were more important for information transfer while deep layers had a larger role in clustering, potentially playing a role in feedforward/feedback relations in these layers. Our work provides a new methodological framework for functional laminar connectomics while also adding insight into cortical laminar function and locality.

Disclosures: P. Kotlarz: None. **K. Lankinen:** None. **M. Hakonen:** None. **T. Turpin:** None. **J. Polimeni:** None. **J. Ahveninen:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.08/WW52

Topic: I.03. Anatomical Methods

Support: Simons Foundation

Title: Connectome analysis of the brain of a miniature wasp, Megaphragma viggianii

Authors: *K. SHINOMIYA[1], J. WU[1], P. GUNN[1], C. GORDON[1], C.-Y. HO[1], D. NGUYEN-THI[1], A. SHINOMIYA[1], P. STEVENS[1], S. VILLANI[1], H. HESS[2], A. A. POLILOV[3], D. B. CHKLOVSKII[1];
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Abstract: Advancements in electron microscopy have catalyzed substantial progression in connectomics, thereby enabling comprehensive reconstruction and meticulous analyses at both neuronal and synaptic levels. Our project illuminates the complex neuronal structure of the brain of a microscopic parasitic wasp, *Megaphragma viggianii*, while providing anatomical and functional comparisons with other insect nervous systems, especially the fruit fly brain. We reconstructed and analyzed the entire wasp brain by utilizing focused ion beam-aided scanning electron microscopy (FIB-SEM) combined with three-dimensional neuronal segmentation and automated synapse prediction. The brain houses a neuronal network of approximately 9,000 reconstructed neurons, comprising less than 10% of the total neurons in a fruit fly brain. We furthermore predicted more than 200,000 presynapses and over 2 million postsynapses across the entire dataset. Focusing on the sensory pathways of *Megaphragma*, we compared the reconstructed circuits with those of other insects to explore their information processing mechanisms. Our investigation into the motion pathway in the visual system encompassed the T4 and T5 cells and their synaptic partners. We found that various neuron types involved in motion detection are evolutionally conserved across species. This result aligns with our broader observation that the wasp's brain structures, despite significant compactification, remain analogous to larger insect species. The results of this study have demonstrated the feasibility of performing a dense reconstruction of the entire brain within a relatively short period with a small team. These findings underscore the potential of *Megaphragma* as an exceptional model organism for comparative connectomics studies, offering a unique perspective to understand the minimal neuronal functions and circuitry responsible for information processing crucial for animal's survival.

Disclosures: K. Shinomiya: None. **J. Wu:** None. **P. Gunn:** None. **C. Gordon:** None. **C. Ho:** None. **D. Nguyen-Thi:** None. **A. Shinomiya:** None. **P. Stevens:** None. **S. Villani:** None. **H. Hess:** None. **A.A. Polilov:** None. **D.B. Chklovskii:** None.

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PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR376.09/WW53

Topic: I.03. Anatomical Methods

Support: NIH/NINDS 2RF1NS023945-28

Title: Functional architecture of the forebrain cholinergic system: Database and analysis

Authors: *L. ZABORSZKY[1], P. VARSANYI[2];
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Abstract: Basal forebrain (BF) cholinergic neurons via their projection to the cortex influence arousal state, sensory perception, learning, memory and attention. However, the mechanisms by which cholinergic signaling regulates cognitive processes remain elusive due to the complex anatomy of the BF. Patients with Alzheimer's disease and related dementias have a significant decrease of acetylcholine in the cerebral cortex and show pathological changes in cholinergic neurons in the BF. Thus, a complete understanding of its functional organization is warranted. In order to understand the organizational features of the cholinergic projection system we developed a pipeline for spatial registration of vectorial and image data from 73 rat brains that received pairwise conventional retrograde (FB and FG) or virus tracer injections in neocortical areas, (n=106), many of the regions electrophysiologically defined, and cholinergic projection neurons in the BF were mapped in 200 µm series. We created a reference brain from a full series of 50 micron sections of a single brain stained for ChAT and Nissl. The sections were imaged with 2 micron/pixel resolution and aligned in 3D. We created software (Java, ImageJ) that is able to slice this high resolution 3D stack of images in any plane to match sections from the experimental brains. The image registration tool (Java) can communicate directly with the Postgres database containing all the images and mapping. The warping tool is using BSpline-based elastic transformation for registration. We implemented all of the scripts for data visualization in Python within the QGIS framework. Cholinergic neurons in this virtual environment were assessed for spatial correlation in respect to density and to their cortical targets. Using hierarchical clustering of the data, cholinergic projection neurons are organized into 3 main putative networks. Within each of these networks, we found high density spaces that showed systematic correlation of cholinergic projection neurons to pairwise targets that deviated from random distributions, such as between various body parts of the somatosensory and motor cortex; V1/prelimbic or auditory/perirhinal cortex, etc. These results, together with recent data, showing that target-specific groups of cholinergic neurons receive specific combinations of inputs (Chavez and Zaborszky, 2017; Gielow and Zaborszky, 2017) suggest that the architecture of the cholinergic system can support spatially selective modulation of multiple groupings of functionally interconnected cortical areas.

Disclosures: L. Zaborszky: None. **P. Varsanyi:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.10/WW54

Topic: I.03. Anatomical Methods

Support: NHMRC Grant APP1140295

Title: Human Brain Atlas: an in vivo MRI dataset for detailed segmentations

Authors: M. SCHIRA[1], Z. J. ISHERWOOD[2], ***M. S. KASSEM**[3], M. BARTH[4], T. SHAW[4], M. ROBERTS[5], G. PAXINOS[3];
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Abstract: We introduce HumanBrainAtlas, an initiative to construct a highly detailed, open-access atlas of the living human brain that combines high-resolution in vivo MR imaging and detailed segmentations previously possible only in histological preparations. Here, we present and evaluate the first step of this initiative: a comprehensive dataset of two healthy male volunteers reconstructed to a 0.25 mm isotropic resolution for T1w, T2w, and DWI contrasts. Multiple high-resolution acquisitions were collected for each contrast and each participant, followed by averaging using symmetric group-wise normalisation (Advanced Normalisation Tools). The resulting image quality permits structural parcellations rivalling histology-based atlases, while maintaining the advantages of in vivo MRI. For example, components of the thalamus, hypothalamus, and hippocampus are often impossible to identify using standard MRI protocols—can be identified within the present data. Our data are virtually distortion free, fully 3D, and compatible with the existing in vivo Neuroimaging analysis tools. The dataset is suitable for teaching and is publicly available via our website (hba.neura.edu.au), which also provides data processing scripts. Instead of focusing on coordinates in an averaged brain space, our approach focuses on providing an example segmentation at great detail in the high-quality individual brain. This serves as an illustration on what features contrasts and relations can be used to interpret MRI datasets, in research, clinical, and education settings.

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Poster

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Location: WCC Halls A-C

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Program #/Poster #: PSTR376.11/WW55

Topic: I.03. Anatomical Methods

Support: NIH BRAIN Initiative MH117815
HHMI
Simons Foundation
NSF PHY-1734030

Title: Structural properties of the whole-brain connectome of Drosophila

Authors: *A. LIN[1], R. YANG[2], S. DORKENWALD[2], A. MATSLIAH[1], A. STERLING[1], P. SCHLEGEL[3], S.-C. YU[1], C. MCKELLAR[1], M. COSTA[3], K. EICHLER[3], G. JEFFERIS[3], S. SEUNG[2], M. MURTHY[2];
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Abstract: Characterizing the network properties of animal brains may lead to a better understanding of computation and information flow in these complex organs. However, to date, very few whole-brain neuron-level reconstructions have been completed across organisms. The FlyWire project has completed the proofreading of a connectome for a *Drosophila* female brain (FAFB) which contains both hemispheres of the central brain in addition to both optic lobes, totaling roughly 130,000 neurons and over 130 million synapses. Here, we dissect this network and determine its topological properties, examine recurrence by computing the prevalence of two- and three-node network motifs, and compare these topological metrics with wiring diagrams of other animals. We further examine the strength and neurotransmitter composition of motifs. We find that despite its sparsity, the fly brain is robustly interconnected and highly clustered when compared to random null models. We identify a “rich club” population of highly-connected neurons, and examine subsets of these neurons which may act as integrators or broadcasters of signals. We further divided the brain into subnetworks based on 78 anatomically defined brain regions, or neuropils. For each region we computed reciprocity, motif frequency, and other properties, finding significant differences which may correlate with differences in biological function. These results highlight the dense interconnectivity of the connectome of the adult fly, and serve as a foundation for models and experiments comparing neural activity with anatomical structure.

Disclosures: A. Lin: None. **R. Yang:** None. **S. Dorkenwald:** None. **A. Matsliah:** None. **A. Sterling:** None. **P. Schlegel:** None. **S. Yu:** None. **C. McKellar:** None. **M. Costa:** None. **K. Eichler:** None. **G. Jefferis:** None. **S. Seung:** None. **M. Murthy:** None.

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PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.12/WW56

Topic: I.03. Anatomical Methods

Support: SPARC Project
NIH Common Fund, NIH Office of the Director, Award OT2 OD030541

Title: The SPARC Connectivity Knowledge Base of the Autonomic Nervous System

Authors: T. H. GILLESPIE[1], F. T. IMAM[1], M. C. SURLES-ZEIGLER[1], B. DE BONO[2], B. OZYURT[1], J. K. BOLINE[3], S. TAPPAN[4], *J. S. GRETHE[1], M. E. MARTONE[1];
[1]Dept. of Neurosciences, Sch. of Med., Univ. of California San Diego, La Jolla, CA; [2]Whitby et al, LLC, Indianapolis, IN; [3]Informed Minds Inc, Walnut Creek, CA; [4]Rock Maple Science, LLC, Hinesburg, VT

Abstract: The Stimulating Peripheral Activity to Relieve Conditions (SPARC) program is an NIH-funded consortium whose overarching goal is to improve the understanding of how the autonomic nervous system (ANS) interacts with end organs and the central nervous system. A major objective of SPARC is to use this knowledge to develop the next generation of neuromodulator devices as effective therapies for diseases of the nervous system. To assist in that effort, the SPARC Data and Resource Center (DRC) is fielding an infrastructure for sharing data and tools for mapping the ANS. A centerpiece of this infrastructure is the SPARC Connectivity Knowledge base of the Autonomic Nervous System (SCKAN) and associated visual map of ANS circuitry. SCKAN contains knowledge about CNS-ANS-end organ circuitry derived from SPARC data and scientific literature, in a form that supports reasoning. All connections are annotated with the SPARC vocabularies to ensure anatomical structures are used consistently. The SCKAN currently contains two levels of semantic connectivity knowledge: circuit and individual connections. A circuit represents a detailed model of ANS connectivity associated with a particular organ, e.g., bladder or functional circuits, e.g., defensive breathing. These circuits are created through interviews with SPARC investigators, anatomical experts, and the scientific literature. They contain detailed representations of neuron populations giving rise to ANS connections, including mappings of the locations of cell bodies, dendrites, axon segments, and synaptic endings involved in a particular circuit. We also report on updates to the Neuron Phenotype Ontology (NPO) that allow it to serve as the core data model for representing neural connectivity inside of SCKAN. These updates include support for partial orders of location phenotypes and a simplified RDF representation that makes it possible to query neuron types as data instead of just as OWL axioms. To this end, we have a set of predefined query patterns to explore the key contents of SCKAN using the simplified RDF representation. We also developed an intuitive query interface for the knowledge curators to explore and visualize the SCKAN connectivity statements.

Disclosures: **T.H. Gillespie:** None. **F.T. Imam:** None. **M.C. Surles-Zeigler:** None. **B. De Bono:** None. **B. Ozyurt:** None. **J.K. Boline:** None. **S. Tappan:** None. **J.S. Grethe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc. **M.E. Martone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc..

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PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR376.13/WW57

Topic: I.03. Anatomical Methods

Support: SPARC Project
NIH Common Fund, NIH Office of the Director, Award OT2 OD030541

Title: Populating a knowledge base of ANS connectivity using a natural language processing-based pipeline

Authors: *S. J. TAPPAN[1], B. OZYURT[2], F. IMAM[2], T. H. GILLESPIE[2], M. SURLES-ZEIGLER[2], D. DEL PIANO[3], Z. SINNEMA[3], A. RAPUANO[3], A. PINTO[3], S. DALLOUL[3], J. K. BOLINE[4], J. GRETHE[2], M. E. MARTONE[2];
[1]Rock Maple Sci., Hinesburg, VT; [2]UCSD, La Jolla, CA; [3]MetaCell, Cambridge, MA; [4]Informed Minds Inc, Walnut Creek, CA

Abstract: The SPARC Connectivity Knowledge Base of the Autonomic Nervous system (SCKAN) is a semantic store housing a comprehensive knowledge base of autonomic nervous system (ANS) nerve to end organ connectivity derived from data and scientific literature. To facilitate extracting this knowledge from the literature and maintaining relevance over time, we developed a Natural Language Processing (NLP) pipeline, which identifies connectivity relationships between unique anatomical structures within a sentence from the scientific literature. The heart of the system is a domain specific discriminatively pretrained transformer deep learning model (Bio-ELECTRA) based relation extraction system trained in an active learning manner. The pipeline (run monthly) first identifies sentences in published literature via SVM-based classifier with anatomical regions of interest from the SPARC vocabulary. Then the sentences with connectivity relations are selected by the relation extraction classifier, evaluated to ascertain relevance for addition to SCKAN through a number of criteria, and composed into connectivity statements subsequently validated by anatomical experts. This manual distillation of knowledge is complex, labor-intensive and can be prone to human error. To address these challenges, we have developed SCKAN Composer, a web application authoring interface that permits display, inspection, and knowledge formulation for SCKAN additions, as well as the ability to query and augment knowledge information within SCKAN. Composer breaks down this complex operation into a series of steps that can be assigned to teams with specific permissions and roles. Detailed representations of neuron populations giving rise to ANS connections, including mappings of the locations of cell bodies and processes are mapped in terms of the anatomical structures where they pass through or terminate. Because all connections are annotated with SPARC vocabulary, it allows us to support computational reasoning to create a queryable visual atlas of ANS circuitry through the SPARC Portal, supporting the major goal of SPARC in using this knowledge to develop the next generation of bioelectronic devices that serve as effective disease therapies.

Disclosures: **S.J. Tappan:** None. **B. Ozyurt:** None. **F. Imam:** None. **T.H. Gillespie:** None. **M. Surles-Zeigler:** None. **D. Del Piano:** None. **Z. Sinnema:** None. **A. Rapuano:** None. **A. Pinto:** None. **S. Dalloul:** None. **J.K. Boline:** None. **J. Grethe:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc. **M.E. Martone:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SciCrunch Inc..

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PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR376.14/WW58

Topic: I.03. Anatomical Methods

Support: NIH NINDS R35 NS097265
NIH NINDS U19 NS123717
AHA Predoctoral Fellowship 906077

Title: Imaging, Reconstruction and Analysis of Whole Mouse Brain Vascular and Lymphatic Network

Authors: *X. JI[1], S. HUANG[1], B. FRIEDMAN[2], M. MORENO[1], L. ZHAO[3], Y.-K. HONG[3], D. KLEINFELD[1];
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Abstract: Brain vasculature is a critical life-maintaining system spanning multiple length scales. To systematically understanding the role of vasculature structure in maintaining brain homeostasis and regulating blood flow, we developed experimental and computational pipelines to construct the first whole mouse brain microvascular connectome at sub-micrometer resolution. Analysis reveals a common network topology that leads to shared robustness against damage, while region-specific network geometry matches resting metabolism rate to maintain a universal tissue oxygen tension across the brain. To further complete the brain vascular connectome with systematic sources (arteries) and drains (veins), as well as explore the connections between brain vasculature and the lymphatic system surrounding the brain, whole mouse brain within the skull should be imaged with sub-micrometer resolution. Toward this goal, we are developing a considerably advanced serial two-photon microscope with concurrent optical ablation to iteratively image and optically remove heterogeneous biological tissues. Amplified femtosecond laser pulses are simultaneously spatiotemporally focused for precise and efficient skull and tissue removal. We will apply this technique to map the connectivity of the brain vascular and lymphatic network and construct the vascular connectome for cerebral blood flow simulation.

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PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.15/WW59

Topic: I.03. Anatomical Methods

Title: Live cell morphological tracing of neural networks

Authors: *C. ZHOU, S. SEMAN, S. P. CARMODY, O. A. SHEMESH;
Neurobio., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Tracing the morphology of neurons is critical for understanding their connectivity and physiology. To view the morphology of neurons, the Brainbow technique was developed, through a stochastic expression of colorful proteins. This technology enabled the tracing of neurons in fixed brain slices or fixed cultured neurons. Despite the multiple advantages provided by Brainbow, it is challenging to image neuron morphology *in-vivo*, due to light scattering, low brightness of colorful proteins and the mixing of multiple colors within a diffraction limited spot. To image neuron morphology in live neurons, we have generated a live cell morphological-tracing technique, through combinatorial expression of targeted, spectrally and temporally diverse, fluorescent proteins. The technique can be used for tracing the processes of densely packed and live neurons. In addition, the technique allows to trace densely packed neurons while imaging their spiking activity using calcium sensors, therefore linking between the morphology and the physiology of neural networks.

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PSTR376. Connectomics: Other Nervous Systems

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Topic: I.03. Anatomical Methods

Support: NIH Grant 1S10OD023602-01A1
NIH Grant 1U19NS104648
Simons Collaboration on the Global Brain

Title: Fast imaging of millimeter-scale areas with beam deflection transmission electron microscopy

Authors: *Z. ZHENG[1], C. OWN[2], A. A. WANNER[1], R. KOENE[2], E. HAMMERSCHMITH[1], W. SILVERSMITH[1], N. KEMNITZ[1], D. W. TANK[1], H. SEUNG[1];
[1]Princeton Univ., Princeton, NJ; [2]Voxa, Seattle, WA

Abstract: We have achieved a three fold increase in the speed of transmission electron microscopy (TEM) by using a beam deflecting mechanism to enable highly efficient acquisition of multiple image subtiles for each motion of the mechanical stage. For millimeter-scale areas, acquiring a supertile of nine subtiles at each stage position doubles the duty cycle of imaging to more than 30%, yielding a net average imaging rate of 0.3 gigapixels per second. A rectangular supertile can maximally consist of 30 subtiles, arranged in a 6 x 5 grid. Each subtile has a size of 36 megapixels (6,000 x 6,000), with a pixel resolution of 4 nm. Taking into account subtile overlap, the stitched supertile covers a 100 µm x 100 µm area per stage position. Beam deflection can be utilized for high magnifications up to 100,000x with a pixel size of 0.09 nm. Additionally, beam deflection can be combined with the previously reported GridTape technology (Phelps et al. 2021, Yin et al. 2020) for automated imaging in retrofit JEOL TEMs, making it a cost-effective means to substantially enhance imaging throughput. An imaging pipeline with four beam deflection TEMs has been built in Princeton. To demonstrate the feasibility of the imaging pipeline, a proof-of-principle dataset of hippocampus CA3 region (1 mm x 1 mm x 80 µm) has been acquired with the beam deflection TEMs.

Disclosures: Z. Zheng: None. **C. Own:** A. Employment/Salary (full or part-time):; Voxa. **A.A. Wanner:** A. Employment/Salary (full or part-time):; ariadne.ai. **R. Koene:** A. Employment/Salary (full or part-time):; Voxa. **E. Hammerschmith:** None. **W. Silversmith:** None. **N. Kemnitz:** A. Employment/Salary (full or part-time):; Zetta. **D.W. Tank:** None. **H. Seung:** A. Employment/Salary (full or part-time):; Zetta.

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PSTR376. Connectomics: Other Nervous Systems

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Program #/Poster #: PSTR376.17/WW61

Topic: I.03. Anatomical Methods

Support: NIH SPARC Grant 75N98022C00018
NIH Grant OT2 OD025340
US Dept of Veterans Affairs 1IS1BX004384

Title: New Insights into the Anatomy of the Cervical and Thoracic Vagus Nerves via Gross Dissection, Histology, and Imaging

Authors: *L. M. LUNASCO[1,2,3], B. BRUNSMAN[2], K. WORKMAN[2], S. BOKHARI[2], N. NUZOV[3], A. M. BLITZ[4], M. MARKLEY[4], D. A. HERZKA[4], C. A. FLASK[4], N. A. PELOT[5], A.

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[2]Dept. of Anat., [3]Dept. of Biomed. Engin., [1]Case Western Reserve Univ., Cleveland, OH; [4]Dept. of Radiology, Univ. Hosp. Cleveland Med. Ctr. and Case Western Reserve Univ., Cleveland, OH; [5]Dept. of Biomed. Engin., Duke Univ., Durham, NC; [6]Advanced Platform Technol. Center, Rehabil. Res. and Develop., Louis Stokes Cleveland Dept. of Veteran Affairs Med. Ctr., Cleveland, OH

Abstract: Background:Vagus nerve stimulation (VNS) is a promising therapeutic modality that is FDA approved for drug-resistant epilepsy and depression, with many more indications in development. However, its use remains complicated by off-target side effects. To improve the design of new VNS electrodes and inform on ideal targeting strategies, a better understanding of vagal anatomy, including variation between people, is needed. To help solve these issues and provide an improved understanding of organ-level anatomy of the vagus nerve, we developed a dissection technique that is combined with multiple imaging modalities including magnetic resonance imaging (MRI), microCT, and histology.
Methods:Cadavers (n=8) were MRI scanned using a 3D constructive interference in steady state (CISS) sequence in a 3 Tesla scanner prior to dissection. The cervical and thoracic vagus nerves were then dissected from their origin at the medulla to their distal targets. Branches were identified and marked with custom paints and the distance from major anatomical landmarks to the main trunk where branches originated were measured. The VN was then removed from the body, fixed to an engraved acrylic grid, stained with phosphotungstic acid, and scanned with a microCT to confirm branches lacking positive identification on gross examination. Samples were then processed for histology (H&E) to further characterize the tissue composition.
Results:We have identified the VN on MRI from its medullary origin to the carina, although tracing the VN in full continuity as it descends through the neck and thorax and identifying many of its branches remain elusive. Anatomic dissections reveal that variation in the position of the VN relative to surrounding vascular structures as well as shared epineurium with nearby nerves, like the hypoglossal nerve and sympathetic trunk, are common and these variants cause uncertainty in VN identification on MRI. The nature of these connections remains unclear despite connections exceeding 1 cm in some cases. We have also identified a consistent branch from the cervical VN supplying the thymus with histologic confirmation. There were 2-6 connections between the vagus nerve and the carotid artery with unknown function. Finally, our dissections only revealed a single cervical cardiac branch unlike the classic vagus schema that includes both superior and inferior cervical cardiac branches of the vagus nerve.
Conclusions:Normal anatomical variation of the VN is not well understood, yet vital for identifying it on imaging. CISS MRI allows visualization of the VN in cadavers and represents a potential method for identifying the VN in patients.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.18/WW62

Topic: I.03. Anatomical Methods

Support: NIH SPARC Grant 75N98022C00018
NIH Grant OT2 OD025340
US Dept of Veterans Affairs 1IS1BX004384
National Defense Science & Engineering Graduate (NDSEG) Fellowship Program

Title: Not All Who Wander Are Lost: New Insights into Cardiac and Diaphragmatic Branches of the Human Vagus Nerve

Authors: *B. BRUNSMAN[1,2], L. LUNASCO[2,3], K. WORKMAN[2], S. BOKHARI[2], N. NUZOV[3], A. UPADHYE[3], J. CHIN[3], A. SHUNMUGAVEL[3], J. COLEMAN[3], N. PELOT[4], A. J. SHOFFSTALL[3], A. CROFTON[2];
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Abstract: The vagus nerve (VN) is the longest cranial nerve and wanders extensively through the body. It originates in the medulla and treks through the neck and thorax, until the two sides ultimately connect and terminate in the abdomen. Vagus nerve stimulation (VNS) alleviates symptoms of a wide variety of disorders but off-target side effects remain a challenge due to the lack of clarity on the end organs supplied by the branches of the VN. Further elucidating the anatomy of the VN will enable rational design of new VNS electrodes and proper placement of these electrodes to maximize on-target effects while limiting side effects. The specific VN branches supplying each target organ are inadequately characterized in the literature. Additionally, few studies describe the extensive variation in vagal anatomy between individuals. To address these knowledge gaps, we have developed a cadaveric dissection approach that allows for visualization of the entire VN and its branches from origin to termination. Our method allows the entire VN to be removed as a single unit for ex situ microCT and histologic imaging. In addition, we have developed a method for marking the VN and its branches in relation to major anatomical landmarks using custom paints with enhanced adherence and longevity on tissue. This marking facilitates ex situ co-registration of the nerve's orientation and its branches during and after subsequent microscopic analyses, which enables confirmation of small branches (<2 mm) and reconstruction of the nerve relative to key anatomical landmarks and organs. This method can be applied to all peripheral nerves, enabling fascicular-level characterization of the entire peripheral nervous system. Our preliminary data in 10 cadavers reveals significant variability in cardiac innervation arising from the vagus nerve. In addition to cardiac branches arising directly from the VN, many branches of the esophageal plexus travel to the pericardium and may represent VN fibers. Also, prior to traversing the esophageal plexus, the vagal trunks give off multiple branches that travel to the diaphragm, with most branches traveling to the right hemidiaphragm. The functional significance of these fibers remains uncertain, but suggests that C3, C4, and C5 spinal nerve fibers may hitchhike with the VN to reach the diaphragm or the VN may contribute to the innervation of the diaphragm. Future studies are needed to elucidate the significance of the cardiac branches arising from the esophageal plexus and the diaphragmatic

branches of the VN. A better understanding of this exploratory data could be used for site specific VNS that would decrease off target side effects and lead to better patient outcomes.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.19/WW63

Topic: I.03. Anatomical Methods

Support: NIH SPARC OT2 OD025340
NIH SPARC 75N98022C00018

Title: Computational modeling of vagus nerve stimulation: translation, 3D morphology, and efficient optimization for selective stimulation

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Abstract: Background: Electrical stimulation of the vagus nerve can treat a wide range of diseases, from epilepsy to rheumatoid arthritis to heart failure. However, mechanisms remain unclear and this poses challenges for informed design of therapies and selection of stimulation parameters. We are implementing computational models to inform parameter selection for vagus nerve stimulation (VNS).
Methods: We modeled populations of rat, pig, and human vagus nerves, and we compared thresholds for activation across individuals and species. We advanced the models of human VNS using microCT-based 3D morphology of the nerves. We implemented a GPU-based surrogate model of mammalian myelinated axons to decrease run time and enable rapid optimization of stimulation parameters for selective activation.
Results: Activation thresholds for pig and human VNS were ~10-100x higher and varied more between individuals than for rat VNS. Due to the inter-individual variability, the ratio of activation thresholds for small myelinated fibers to scale between species spanned ~15-40x for rats to pigs, ~25-100x for rats to humans, and ~1-5x for pigs to humans.
We compared activation thresholds between models of human VNS using microCT-based 3D nerve morphology (3DMs) versus 2D extrusion models (2DEMs). The 2DEM and 3DM input-output curves were generally consistent, especially once the nerves were deformed to conform to the circular cuff. However, the spatial patterns of activation differed between the 2DEMs and 3DMs, indicating that 3DMs may be required for accurate design of selective stimulation

therapies.
To enable efficient design, we implemented and parameterized a simplified cable model of a mammalian peripheral axon for GPU. The thresholds of this surrogate model were within ~5% of those predicted by the original biophysical model, but required ~8000x less compute time. Within a couple of minutes, we could thus optimize the current amplitudes for a six-contact cuff for selective activation of target fascicles.
Conclusions: Our computational models provide important insights for translating VNS therapies to clinical application, including the spatial patterns of fiber activation across individuals and species, as well as novel tools to design parameters for selective stimulation.

Disclosures: M. Hussain: None. **D. Marshall:** None. **E. Musselman:** None. **A. Shoffstall:** None. **W.M. Grill:** None. **N. Pelot:** None.

Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.20/WW64

Topic: I.03. Anatomical Methods

Support: NIH SPARC Grant 75N98022C00018
NIH Grant OT2 OD025340
US Dept of Veterans Affairs 1IS1BX004384
National Defense Science & Engineering Graduate (NDSEG) Fellowship Program

Title: Co-registration of 3D optical tracking and MRI for mapping the pathways of the human vagus nerve

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Abstract: The human vagus nerve (cranial nerve X) is the longest nerve in the autonomic nervous system, innervating many vital organs. The vast reach of the vagus is leveraged for therapeutic purposes through vagus nerve stimulation (VNS) to treat epilepsy, depression, obesity, and stroke sequelae. However, the gross anatomy of the vagus nerve in humans is complex, highly variable between individuals, and insufficiently characterized. Visualizing the nerve's trajectory, including location of branches and measurements of distances, will inform surgical access for new VNS implant locations, points of intervention for non-invasive

stimulation, and preservation of neural pathways during surgical procedures. First, we segmented the skeletal and vascular systems from high-resolution MRI of human cadavers to establish anatomical landmarks. Then, after dissection, we used a 3D optical tracking stylus to trace the entire pathway of the vagus nerve from brainstem to abdomen in the context of standardized anatomical landmarks; we annotated each branch with its name and innervated organ. Finally, we co-registered the 3D tracing data to MRI using the common skeletal and vascular landmarks. The co-registered 3D tracing and MR data provide visualization of the pathway of the vagus nerve and its branches to scale in an individual overlaid on their unique anatomy. We quantified the distances between branches using the 3D tracing data, which varied substantially between people. Similarly, the number of branches to each organ differed considerably, as well as between the left and right cervical-thoracic segments within a given individual. The human vagus nerve appears as a single connected neural complex, with extensive right-left and anterior-posterior connections in the distal thorax and abdomen, as opposed to distinct left and right sided nerves which is currently the norm in literature. Extension of this preliminary quantification of nerve trajectory and branching across 50 cadavers will reveal the range of anatomical variation of vagal pathways between people and discover any consistent branching locations. VNS of single branches has been shown to relieve organ-specific symptoms and reduce side effects relative to cervical VNS, but understanding the arrangement of branches and their surgical or percutaneous accessibility are the leading obstacles. The anatomical 3D model will be used to devise techniques to access the newly targeted branches.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.21/WW65

Topic: I.03. Anatomical Methods

Support: 75N98022C00018
OT2 OD025340
1IS1BX004384

Title: Mapping the fascicular pathways of the human vagus nerve using microCT

Authors: *A. SHUNMUGAVEL[1,3], A. UPADHYE[1,3], J. CHIN[1,3], C. KOLLURU[1], D. WILSON[1], M. JENKINS[1], N. PELOT[4], A. R. CROFTON[2], A. J. SHOFFSTALL[1,3]; [1]Biomed. Engin., [2]Dept. of Anat., Case Western Reserve Univ., Cleveland, OH; [3]Advanced Platform Technol. Center, Rehabil. Res. and Development, Louis Stokes Cleveland Dept. of Veteran Affairs Med. Ctr., Cleveland, OH; [4]Dept. of Biomed. Engin., Duke Univ., Durham, NC

Abstract: Vagus nerve stimulation (VNS) is a neuromodulation therapy for the treatment of refractory epilepsy and depression. VNS has been approved by the FDA as a therapy for adults and children 4y and older. Despite its efficacy, the technique is not completely devoid of side effects. VNS is occasionally correlated with therapy-associated cough and throat pain due to activation of efferent motor fibers in the vagus nerve (VN). It is presumed that the heterogeneity in neuroanatomy and fascicular organization of the vagus nerve impose limitations on achieving selective activation of afferent VN fibers which are putatively responsible for the therapeutic effects of VNS in epilepsy. Therefore, a better understanding of the microanatomy and fascicular organization of the vagus nerve will help to optimize electrode designs and determine optimal electrode placement to improve VNS efficacy. To better visualize and map the fascicular anatomy using microCT (μCT), we first used osmium tetroxide (OsO_4), a routinely used electron microscopy stain for myelinated nerve fibers. However, lipid components outside the perineurium produced imaging artifacts and the relatively larger size of the nerve block (25 cm in length) hindered the full and even permeability of OsO_4 into the nerves. Here, we optimized the staining technique for the vagus nerve using phosphotungstic acid (PTA) in place of OsO_4. Vagus nerves harvested from the human cadavers of the REVA project were glued onto an engraved acrylic grid and stained with 3% vol/vol PTA overnight. Stained nerves were imaged in blocks of 1.5 cm with Quantum GX2 μCT (Perkin Elmer, Waltham, MA, USA) and each block of scan comprised an overlap of 0.3 cm tissue. Blocks of images were reconstructed and stitched together following a pairwise stitching algorithm to generate a full-length 3D image of the VN. PTA staining significantly increased the perineurial and fascicular contrast of the vagus nerve, while not introducing additional lipid staining artifacts. The objective of μCT part of the REVA project is to reconstruct the vagus nerve and its ramifications *ex vivo* which demands a high resolution/contrast image of the vagus nerve. Our preliminary results with PTA staining demonstrate an optimum visualization of the perineurial boundaries and fascicular details and meet the image quality requirement for mapping the fascicular pathways of VN.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Topic: I.03. Anatomical Methods

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US Dept. of Veterans Affairs 1IS1BX004384

Title: A stitching and segmentation pipeline for large-scale micro-computed tomography images of human vagus nerve

Authors: *J. ZHANG[1], O. BUYUKCELIK[1], A. UPADHYE[1], J. CHIN[1], C. KOLLURU[1], D. WILSON[1], M. JENKINS[2], N. PELOT[3], A. SHOFFSTALL[1];
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Abstract: *Objective.* Vagus nerve stimulation (VNS), which activates the primary pathway of the parasympathetic autonomic system, holds great potential for neuromodulation therapy for various conditions. However, current VNS practices modulate the entire nerve without specific targeting of fibers, which can lead to off-target stimulation and side effects. Mapping the neuroanatomy of the human VN at high resolution has the potential to enhance the design of more effective VNS devices and techniques. Micro-computed tomography (microCT) enables detailed visualization of fascicular structure within the VN over a large field of view. However, existing workflows and tools are inefficient and lack scalability for handling the vast amount of imaging data generated by microCT, particularly for larger sample sizes. To overcome these limitations, we propose an enhanced microCT image processing pipeline that prioritizes efficiency and scalability for stitching and segmentation tasks.*Method.* For stitching, we employed a montage approach with the open-source Python image processing library Insight Toolkit (ITK). For segmentation, we utilized the Segment Anything Model (SAM), a cutting-edge neural network model developed by Meta AI. SAM is the world's first generalized segmentation model, enabling zero-shot prediction without specialized training. Our workflow also incorporates human-in-the-loop options for visualization and validation.*Results.* Overall, our new pipeline showed high scalability and faster processing time. In comparison to traditional pairwise stitching, our workflow reduced stitching time by an average of 17-fold and up to 20-fold for larger testing datasets, all while delivering accurate stitching outcomes. For the segmentation task, our zero-shot approach showed results comparable to a custom segmentation neural network (2D U-Net) trained on manually labeled data. The new segmentation approach achieved Dice scores of 0.87 for fascicle and 0.93 for epineurium on a set of example segmentations with an inference speed of 0.3 s per image. By integrating SAM into our pipeline, we decreased the need for labor-intensive annotation and ensured high throughput. *Discussion/conclusion.* Our proposed pipeline offers a time-efficient and scalable solution for stitching and segmentation tasks in neuroanatomical analysis. It minimizes manual labor and training data requirements, enabling rapid quantification of fascicular structure in large microCT datasets. This advancement has the potential to enhance our understanding of the human VN's neuroanatomical structure and aid in the development of next-generation VNS techniques.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR376.23/WW67

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Institutes of Health (NIH) OD026585 (LAH)
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (LAH)

Title: Nerve fiber organization and clustering in lumbosacral ventral roots of rhesus macaques depend on the presence of autonomic fibers

Authors: *L. HAVTON[1], P. M. BARTMEYER[1], G. BORTOLANCA CHIAROTTO[2], Y. SIM[1], E. CELIK[1], N. BISCOLA[1];
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Abstract: Neuromodulation devices and treatments are being extensively investigated for different research groups. It is known that peripheral nervous system (PNS) electrical stimulation may augment autonomic function after spinal cord injury or in neurodegenerative disorders. However, information on nerve fiber type, myelination, and spatial distribution of fibers within the PNS is not well understood. More studies are needed as its contents may influence response to electrical stimulation. L6-S3 ventral roots (VRs) in rhesus macaques (n=9) were used as a model to map preganglionic parasympathetic, γ-motor, α-motor fibers, and C-fibers. Tissues were embedded in plastic resin followed by sectioning and toluidine blue staining for light microscopy (LM). Ultrathin sections were also prepared for transmission electron microscopy (TEM). Entire ventral roots were imaged by LM and TEM, and montages were analyzed after digital segmentation of all myelinated and unmyelinated fibers to obtain comprehensive ground truth data. Fibers underwent size correction based on fiber dispersion angles, and 2-dimensional distribution maps were used for cluster analysis based on fiber size and myelination. There was extensive variability in the relative composition of nerve fiber types and degree of fiber heterogeneity between different L6-S3 VR levels within and across different animals. The L7-S2 VRs showed more dominance for the presence of preganglionic parasympathetic fibers. A correlation between the proportion of autonomic fibers and the degree of nerve fiber clustering was detected, and unmyelinated fibers had a strong tendency to cluster with small myelinated fibers within the size range for autonomic preganglionic fibers. We conclude that nerve fiber variability and cluster heterogeneity between VR vary extensively between individual lumbosacral VRs and need to be taken into consideration for the refinement of neuromodulation strategies.

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Poster

PSTR376. Connectomics: Other Nervous Systems

Location: WCC Halls A-C

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Program #/Poster #: PSTR376.24/WW68

Topic: C.11. Spinal Cord Injury and Plasticity

Support: National Institutes of Health (NIH) OD026585 (LAH),
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National Institutes of Health (NIH) OD028183 (MPW)
Dr. Miriam and Sheldon G. Adelson Medical Research Foundation (LAH)

Title: Laterality and sexual dimorphism in the cervical segment of the human vagus nerve

Authors: *N. BISCOLA[1], P. M. BARTMEYER[1], E. STERN[1], P. MIHAYLOV[3], M. WARD[4], T. L. POWLEY[5], L. HAVTON[2];
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Abstract: Neuromodulation by vagus nerve stimulation (VNS) of the left-sided cervical nerve segment may be used as adjunctive treatment of medication-refractory epilepsy and clinical depression. However, the mechanisms of action are not well understood, and implementation of VNS treatment protocols are limited by off-target effects. An improved understanding of the human vagus nerve fascicular and sub-fascicular organization is needed for the development of refined stimulation protocols and VNS strategies. We performed light microscopic (LM) and transmission electron microscopic (TEM) mapping of the human cervical vagus nerve procured from deceased transplant organ-donors (n=27). Nerve tissues were embedded in plastic resin, sectioned, stained with toluidine blue, and studied by LM. Ultrathin sections were contrasted and evaluated by TEM. Studies of laterality, including paired-analyses, showed a higher number of fascicles ($p<0.01$) as well as larger combined cross-sectional fiber area ($p<0.001$) and endoneurium area ($p<0.001$) on the right side compared to the corresponding left-sided cervical vagus segment. Studies of sexual dimorphism showed a significantly higher total number of fascicles in the cervical vagus nerve segment for women compared to men ($p<0.05$). Separate analysis of the right- and left-sided cervical vagus segments also showed a higher fascicle count on both sides for women compared to men ($p<0.05$). Ultrastructural studies with high resolution TEM imaging, montage formation of entire fascicles, and digital segmentation of all myelinated and unmyelinated fibers within individual fascicles showed extensive variability between fascicles with regards to fiber type compositions based on size and myelination criteria. The vast majority of fibers were unmyelinated. A high degree of clustering of subsets of fiber types was identified within individual fascicles. Assisted by an action potential interpreter tool, nerve fiber morphology was used to predict conduction properties and expected compound nerve action potentials with findings supportive of the notion that nerve fiber populations within individual fascicles of the human cervical vagus nerve show extensive functional heterogeneity.

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Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.01/WW69

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

Support: NSF Grant 1931249

Title: Calibrating Bayesian Decoders of Neural Spiking Activity

Authors: *Z. TAJIK MANSOURI[1], G. WEI[5], X. WANG[2], I. H. STEVENSON[1,3,4];
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Abstract: Accurately decoding external variables from observations of neural activity is a major challenge in systems neuroscience, and Bayesian decoders, that provide probabilistic estimates, are some of the most accurate and widely used. Here we show how, in many common settings, traditional Bayesian decoders, based on the Poisson Generalized Linear Model (GLM), are overconfident. That is, the uncertainty estimates for the decoded stimulus or movement variables are much too precise. Here we compare Poisson and Negative Binomial GLMs and we show how Bayesian decoding with latent variables, which account for low-dimensional shared variability in the observations, can improve calibration, although additional correction for overconfidence is still needed. We examine: 1) decoding stimulus direction from spike recordings in the primary visual cortex, 2) decoding movement direction from recordings in the primary motor cortex, 3) decoding natural images from multi-region recordings, and 4) decoding position from hippocampal recordings. We find that considering latent factors can improve both accuracy and uncertainty estimation in each of these cases. Additionally, we test whether some external variables, such as speed for the movement task or stimulus contrast for the visual task are related to decoder uncertainty. Properly calibrated Bayesian decoders, that accurately reflect confidence levels, may alter theoretical results on probabilistic population coding and lead to improvements in brain-machine interfaces.

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Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.02/Web Only

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

Support: RF1MH117155
R01NS109553

Title: A High Resolution Fiber-Length-Connectome of the Human Cortex

Authors: *C. LIU[1], B. ROSEN[2], E. HALGREN[1];
[1]UCSD Dept. of Neurosciences, La Jolla, CA; [2]Dept. of Neurosci., Washington Univ. Sch. of Med., St Louis, MO

Abstract: The freely available Human Connectome Project (HCP) diffusion MRI (dMRI) dataset and advances in dMRI tractography methods within recent years have made the analysis of *in vivo* structural connectivity increasingly feasible and accurate, leading to the creation of full-cortex connectomes and analyses of properties of major white matter fiber bundles. However, connectomes have concentrated on the strength of connections while leaving under-explored length of fibers connecting regions, which is also an important property of structural connectivity. Having a baseline full-cortex length-connectome is valuable because it can serve as a control for comparisons against populations with conditions that alter the cerebral structure, such as alcoholism and autism. Furthermore, an accurate corticocortical distance matrix is essential for mathematical modeling of cortico-cortical interactions that are related to conduction speed, such as cortico-cortical evoked potentials and coherence, and is a meaningful improvement over Euclidean or geodesic alternative distance matrices. Currently, the most comprehensive length-connectome based on the HCP dMRI dataset provides the mean streamline lengths for the ~65,000 connections between the 360 HCP cortical parcels. However, mean inter-parcel lengths poorly represent the distances between nearby parcels, and no information is available regarding intra-parcel distances. Furthermore, this length-connectome was a byproduct of the strength-connectome, and potential sources of error were not rigorously excluded in estimating distance. To fulfill this need for a high-resolution baseline length-connectome, we used probabilistic tractography and the HCP dMRI dataset to generate cortico-cortical length-connectomes at the vertex level (~32k vertices, ~500M connections per hemisphere; 1500M total). We generated distributions of individual streamline lengths and then extracted the most reasonable lengths from them, accounting for the variance that exists across tractography-generated streamlines due to both the stochastic nature of probabilistic tractography and algorithmic limitations including gyral bias and distance bias. Additionally, due to the high-resolution of the surface we were not able to get connections between all vertices, even with a high number of samples. To fill in lengths for missing connections, we used a recursive nearest neighbors interpolation algorithm to estimate values based on existing surrounding connections. Our goal is to provide a freely-available database of vertex-level cortico-cortical distances as well as the software that we used to generate this database.

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Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

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Program #/Poster #: PSTR377.03/WW70

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

Support: Wellcome Trust (216324)
Simons Foundation
NIH Grant U19NS12371601

Title: Atlas of intrinsic timescales in the mouse brain

Authors: *Y. SHI[1], R. ZERAATI[2], T. INTERNATIONAL BRAIN LABORATORY[3], A. LEVINA[2], T. ENGEL[1];
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Abstract: Neural activity fluctuates over a wide range of timescales, which vary systematically across the forebrain. Differences in intrinsic timescales have been related to functional specialization and hierarchical organization of cortical areas and have been hypothesized to originate from the anatomical connectivity in the cortex. However, the distribution of timescales and their relationship to anatomical structure have not been characterized beyond the forebrain. We report a cellular-resolution brain-wide map of intrinsic timescales in the mouse brain and quantify the link between timescales, connectivity, and gene expression profiles.
We measured timescales in the spontaneous spiking activity of single neurons using a dataset of brain-wide Neuropixels recordings in mice, which consists of 504 insertions covering 245 brain regions in 326 sessions from 11 laboratories. Spatial locations of neurons recorded in individual experiments are registered into a common reference brain.
We quantified timescales by the exponential decay rate of spike-count autocorrelation in 19,289 single neurons. Most brain regions showed a broad distribution of timescales. The average timescales varied across brain regions ranging from tens of milliseconds to several seconds. The midbrain and hindbrain showed three-fold longer timescales than the cortex and thalamus. The median timescales of cortical and thalamic regions were weakly correlated with their anatomical hierarchy scores.
We then quantified timescales of communication between 10,159 pairs of simultaneously recorded regions, measured by the exponential decay rate of cross-correlation between their population spiking activity. The communication timescales among cortical and thalamic areas showed a weak correlation with the strength of mesoscale anatomical connectivity.
Finally, we tested the relationship between brain-wide heterogenous timescales and molecular signatures of cell types in gene expression profiles. We used spatial expression profiles of 4,345 genes from the Allen Gene Expression Atlas to predict the local intrinsic timescales in 200um3 voxels across the entire brain volume. The gene-expression profiles accounted for 6% of the variance in the spatial distribution of timescales, significantly more than the variance explained by the brain-region parcellation. Thus, spatial gene-expression profiles partially explain fine-scale variability of timescales within brain regions.
In summary, we provide a comprehensive brain-wide survey of intrinsic timescales and show that gene-expression profile and anatomical connectivity contribute to the spatial distribution of timescales.

Disclosures: Y. Shi: None. **R. Zeraati:** None. **T. International Brain Laboratory:** None. **A. Levina:** None. **T. Engel:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.04/WW71

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

Support: DE-SC0021110

Title: Constructing a hierarchical Kalman filter for extraction of adaptive neural population dynamics

Authors: *D. PÉREZ-RIVERA[1], S. SCHIERECK[2], C. M. CONSTANTINOPLE[3], C. SAVIN[3]; [1]New York Univ. Ctr. For Neural Sci., New York, NY; [2]New York Univ., [3]Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Cognition requires integrating and using information at multiple timescales. For example, during a value-based decision making task, neurons in the Orbitofrontal Cortex (OFC) are known to reflect the value of offered rewards on individual trials, while also adapting to changes in reward distributions over trials. However, how populations of neurons represent cognitive variables on multiple timescales is unclear. We constructed a hierarchical model to extract these signatures separately from rat OFC neurons during decision-making in a temporal-wagering task. This hierarchical model describes neural activity using latent state space models which capture fast within trial dynamics that are themselves modulated on the slower time scale of trials by another set of low dimensional latents. We used Expectation-Maximization to learn the parameters of this hierarchical model and extract the corresponding latents at the two timescales. We found that the model provides a robust characterization of single neuron responses, with slow latents that reflect rats' inferred reward states over trials, and fast latents that reflect individual reward offers. These results provide a computational framework for extracting dynamical signatures of cognitive computations spanning multiple timescales. Effectively, model fits reflect intermediate latents that evolve according to within trial dynamics including offered rewards, while contextual adaptations are represented on longer timescales (over trials). This framework characterizes cognitive computations across multiple time scales through the lens of circuit dynamics.

Disclosures: D. Pérez-Rivera: None. **S. Schiereck:** None. **C.M. Constantinople:** None. **C. Savin:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.05/WW72

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

Title: Large-scale functional connectome fingerprinting for generalization and transfer learning in neuroimaging

Authors: *M. OGG, L. M. KITCHELL;
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Abstract: Functional MRI currently supports a limited application space stemming from modest dataset sizes, large interindividual variability and heterogeneity among scanning protocols. These constraints prevent fMRI researchers from taking advantage of modern deep-learning tools that have revolutionized other fields such as NLP, speech transcription, and image recognition. To address these issues, we scaled up functional connectome fingerprinting as a neural network pre-training task, drawing inspiration from speaker recognition research, to learn a generalizable representation of brain function. We hypothesized that such a pre-training approach would distill useful information from a large number of participants and arrive at a representation of brain function that could serve as a useful starting place for new downstream tasks and smaller datasets. This pre-training approach performs exceptionally well at neural fingerprinting on a previously unseen scale, across many popular public fMRI datasets (internal validation folds: MPI-Leipzig, NKI-Rockland, OASIS-3, and HCP). We show that this representation can also generalize to support accurate neural fingerprinting for completely new datasets not used in training. Finally, we demonstrate that the representation learned by the network encodes features related to individual variability and supports transfer learning to new tasks. These results open the door for a new generation of clinical applications based on functional imaging data.

Disclosures: M. Ogg: None. **L.M. Kitchell:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.06/WW73

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

Support: NSF Grant SMA-1734795

Title: Skeleton coupling: a method for improving community detection performance in spike train data

Authors: B. KILIC[1,2], ***S. MULDOON**[1];
[1]State Univ. of New York, Univ. at Buffalo, Buffalo, NY; [2]Dept. of Biomed. Engin., Lerner Res. Institute, Cleveland Clin., Cleveland, OH

Abstract: Dynamic community detection (DCD) in temporal networks is a complicated task that involves the selection of an algorithm and its associated parameters. In functional temporal

networks derived from neuronal spike train data, communities are expected to be transient, and it is common for the network to contain multiple singleton communities. Here, we compare the performance of different DCD algorithms on functional temporal networks built from synthetic neuronal time series data with known community structure. We find that, for these networks, DCD algorithms that utilize interlayer links to perform community carryover between layers outperform other methods, but still perform poorly on synthetic data with transient and singleton communities. We therefore define a novel method for defining interlayer links in temporal networks called skeleton coupling that is specifically designed to enhance the linkage of communities in the network throughout time based on the topological properties of the community history. We show that integrating skeleton coupling with current DCD methods improves algorithm performance in synthetic neuronal time series data. The use of skeleton coupling to perform DCD will therefore allow for more accurate and interpretable results of community evolution in real-world neuronal data with transient structure and singleton communities.

Disclosures: B. Kilic: None. **S. Muldoon:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.07/WW74

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

Support: MH111177

Title: Learning induced changes in the schizophrenia connectome: Graph theoretic applications

Authors: *C. C. ABEL II[1], J. KOPCHIK[1], D. BHATT[2], P. THOMAS[2], D. KHATIB[2], U. RAJAN[2], C. ZAJAC-BENITEZ[2], L. HADDAD[2], A. AMIRSADRI[2], J. A. STANLEY[1], V. A. DIWADKAR[1];
[1]Dept. of Psychiatry, [2]Wayne State Univ., Detroit, MI

Abstract: Associative learning and memory modulate network connectomics in the healthy brain (Takehara-Nishiuchi, 2022), but this is aberrant in schizophrenia (SCZ)(Baajour et al., 2020). Disordered network topology during learning has been documented in SCZ (using a graph theoretic measure of betweenness centrality, BC)(Meram et al., 2023; Rubinov and Sporns, 2010). Learning is possible due to the adaptiveness of networks to change; however, BC has not yet been used to characterize *changes* in the connectome over the course of learning. Thus, in exploratory analyses, we investigated learning-induced connectomic alterations in a sample (n=88) of SCZ patients (n=49) and healthy controls (HC). fMRI data (Siemens 3T) were collected using an established associative learning paradigm (participants learned associations between memoranda) (Diwadkar et al., 2008) administered over eight successive time cycles. Each cycle involved four task conditions (Encoding, Post-Encoding Rest, Retrieval, Post-

Retrieval Rest). We investigated condition-specific connectomic changes over the eight cycles. For each participant, in each of the four conditions and at each of the eight time cycles, the full uFC matrix (undirected graph) was estimated from time series (246 bilateral regions/nodes)(Fan et al., 2016). Then, within each graph, we estimated the BC of each node before rank ordering them. Finally, for each node, we used linear regression to estimate the relationship between time (eight time points) and rank order (RO) separately in each group and condition. Nodes with significant effects (p_{FDR}<.01) are projected to cortical surfaces (Figure 1). Changes in RO of BC (ΔBC_{RO}) were unique to each group. Notably, in the Encoding and Retrieval conditions (each of which demanded explicit task-directed processing), learning induced changes (ΔBC_{RO}) in the SCZ connectome were observed in fewer nodes. These effects suggest a loss of task-induced flexibility in the SCZ brain during learning, consistent with SCZ being characterized by a loss of synaptic plasticity (Stephan et al., 2006; Robison et al., 2020).

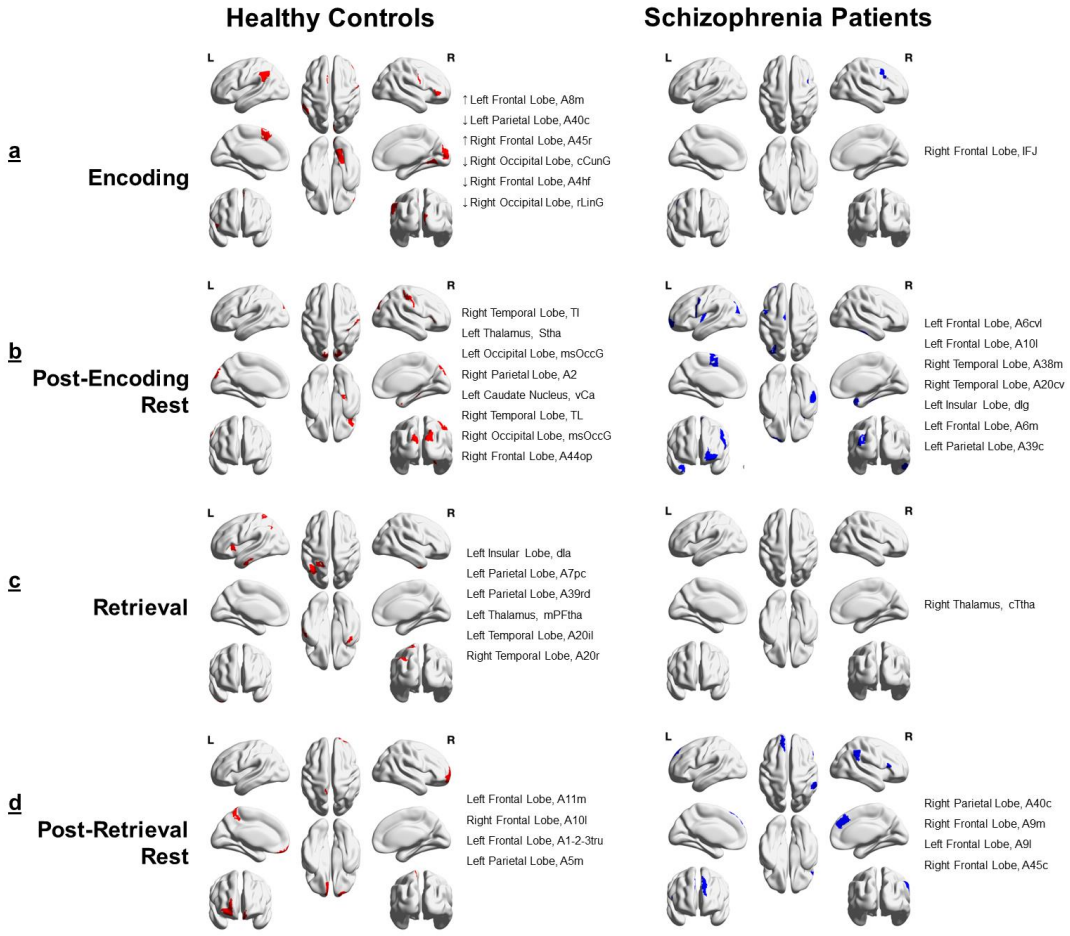

Figure 1: Within task condition changes in rank ordered betweenness centrality over eight time cycles of an associative learning task
Projected to cortical surfaces (and listed alongside) are regions displaying significant (p_{FDR}<.01) changes in rank ordered betweenness centrality (ΔBC_{RO}) within task condition (a-d) across eight time cycles of an associative learning task. Healthy controls (left) and patients with schizophrenia (right) had unique sets of regions displaying ΔBC_{RO} in each task condition. Additionally, patients with schizophrenia had fewer regions displaying ΔBC_{RO} during encoding (a) and retrieval (b) across the eight time cycles.

Disclosures: **C.C. Abel II:** None. **J. Kopchik:** None. **D. Bhatt:** None. **P. Thomas:** None. **D. Khatib:** None. **U. Rajan:** None. **C. Zajac-Benitez:** None. **L. Haddad:** None. **A. Amirsadri:** None. **J.A. Stanley:** None. **V.A. Diwadkar:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.08/WW75

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

Support: Emory Neuromodulation and Technology Innovation Center (ENTICe)
NSF NCS 1835364
NIH BRAIN/NIDA RF1 DA05567
Simons-Emory International Consortium on Motor Control
Emory Neurosurgery Catalyst Grant

Title: Dynamical mechanisms of flexible pattern generation in spinal neural populations

Authors: *L. N. WIMALASENA[1], C. PANDARINATH[1,2], N. AU YONG[2];
[1]Biomed. Engin., Emory Univ. / Georgia Tech., Atlanta, GA; [2]Neurosurg., Emory Univ., Atlanta, GA

Abstract: Recent investigations into the coordinated activity of neuronal populations in motor circuits have revealed low-dimensional dynamical features hypothesized to support the generation of patterned motor output. However, such studies have typically relied heavily on simplifications-including trial-averaging and temporal smoothing-and are often analyzing cortical neural activity far removed from motoneuronal activity, the ultimate output of the motor system. Thus, it is not clear whether the uncovered dynamical descriptions are only useful for intuition-building, or whether they accurately capture the underlying mechanisms that allow motor circuits to achieve the temporal precision of motor control. Here, we investigate spinal interneuronal activity-the neural population in closest proximity to motoneuronal output-to determine the degree and precision with which spinal population dynamics shape muscle activity. We analyzed lumbar intermediate zone interneuronal population recordings along with simultaneous bilateral, intramuscular EMG recordings from two decerebrated T11 spinalized cats performing air-stepping. We computed firing rates of lumber interneurons in 10-ms bins and applied AutoLFADS, an unsupervised deep learning method to infer latent dynamics, for data from multiple sessions. AutoLFADS provided de-noised firing rate estimates for sorted single-unit activity, allowing us to analyze the relationship between spinal population dynamics and muscle activity on a single gait-cycle basis. Spinal population dynamics were highly predictive of multi-muscle activity on a moment-by-moment basis for individual gait cycles. However, the reverse was not true: the spinal activity could not be completely predicted from muscle activity, suggesting that spinal activity contained additional features not directly related to muscle output. We hypothesized that these additional features may contain timing mechanisms that handle the

precise alternation of flexor and extensor muscle activations to generate stable gait. Specifically, we investigated the relation between the spinal population activity and the ipsilateral extensor muscle burst duration, which ranged from 200- to 800-ms for individual steps and varied in correspondence with the length of the gait cycle. We discovered oscillatory dynamics within the spinal population activity that occur during extensor bursts, and found that the amount of time that the spinal population state oscillated was highly predictive of single-gait cycle variations in extensor burst duration (cat1: 304 cycles, r = 0.95; cat2: 148 cycles, r = 0.78), precise to within tens of milliseconds.

Disclosures: L.N. Wimalasena: None. **C. Pandarinath:** F. Consulting Fees (e.g., advisory boards); Synchron, Meta (Reality Labs). **N. Au Yong:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.09/WW76

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

Title: Dynamic functional structural connectivity learning ICA identified significant functional connectivity in structurally related network for schizophrenia

Authors: M. FOULADIVANDA[1], L. WU[1], *V. CALHOUN[2];
[1]Tri-institute Translational Res. Ctr. for Neuroimaging Data (TReNDs Center), Atlanta, GA;
[2]Georgia Inst. of Technol., Decatur, GA

Abstract: The dynamics of human brain functional networks are tightly linked to structural connectivity, but much is still unknown about the relationship between the two. Particularly, in subjects with specific functional disorders as schizophrenia which impacts structural and functional architecture. In this work, we used multimodal images to jointly inform the estimation of dynamic intrinsic connectivity networks (ICNs) in healthy controls and schizophrenia patients. Resting state functional magnetic resonance imaging (rs-fMRI) and diffusion weighted MRI (dMRI) including 149 schizophrenia patients and 162 controls were used. After preprocessing of all data, structural connectivity was estimated by whole brain tractography on dMRI images. To define ICNs, we propose a dynamic functional-structural connectivity learning independent component analysis (ICA) model (dynamic_fsICA) based on spatially constrained ICA (CICA). A multi-objective optimization framework was used to jointly maximize independence and similarity to the spatial prior. Additionally, we included a functional-structural learning objective by minimizing the distance of the dynamic functional information constrained with structural connectivity. Dynamic information was assessed by segmenting time courses to windows and then clustering them to a small number of functional states. The estimated ICNs showed more coherence and focality and the timecourses fit the data better, Figure (1). Moreover, we analyzed group differences on computed functional connectivity using linear regression model considering age, gender, diagnosis, site, and motion effects. Results showed more significant connectivity in

regions related to subcortical, cerebellum, and cognitive control whereas sensory regions (auditory, visual and, sensory motor) showed decreases. Results suggest that multimodal analysis improves identification of the intrinsic brain activity in healthy and diagnosis conditions.

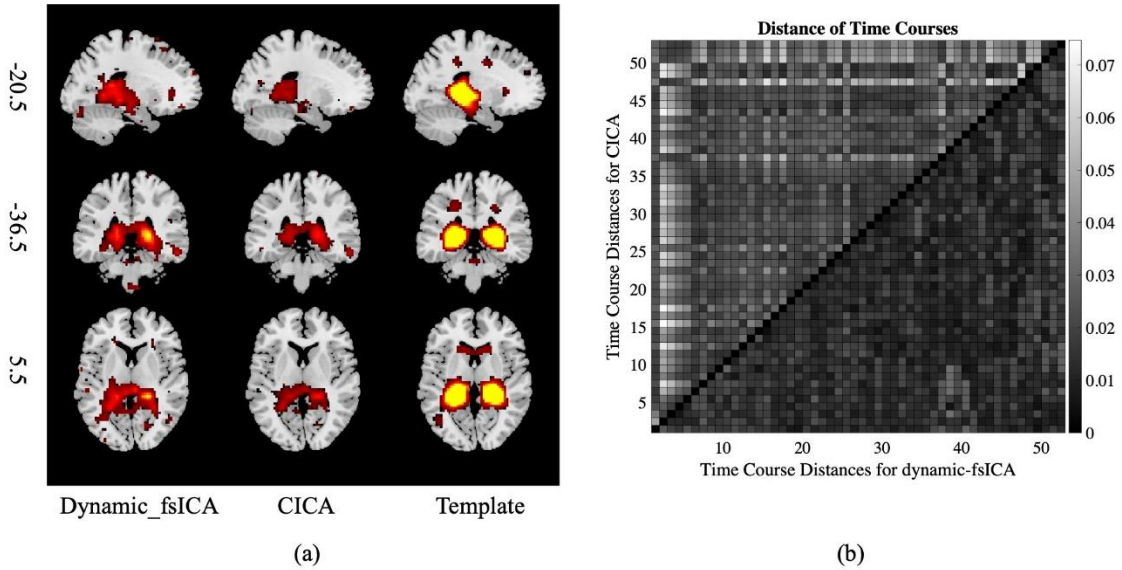

Figure 1. (a). Shows estimated ICNs using Dynamic_fsICA, CICA and Template and (b) compared distance of the ICNs time courses for both methods.

Disclosures: **M. Fouladivanda:** None. **L. Wu:** None. **V. Calhoun:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.10/WW77

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

Title: Diagnosis of schizophrenia using a novel resting-state fMRI marker of regional interactions in the brain network

Authors: *A. WILLIAMS, L. SANCHEZ, S. SARMA;
Biomed. Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Schizophrenia is a complex mental disorder characterized by abnormal neural connectivity patterns and differences in cortical volumes. The gold standard for diagnosis requires structured interviews and visual examination of magnetic resonance imaging (MRI) by medical professionals. The high level of training and experience required for accurate diagnosis

limits access to care from many patients with this debilitating mental illness. To improve equitability and accessibility of treatment, we investigated a potential quantitative method of diagnosing schizophrenia using resting-state functional magnetic resonance imaging (rsfMRI). We constructed personalized dynamic network models (DNMs) to examine a novel biomarker, the "sink index," which characterizes how each node, representing a specific cortical region of interest (cROI), in the brain network is influenced by the remaining nodes. Under this paradigm, sources are regions that exert a significant influence but are not themselves influenced, while sinks represent regions that are heavily influenced by other regions but do not exert influence on others. We hypothesized that cROI associated with schizophrenia would be detected as sinks and present a different distribution of sink indices (real numbers ranging from 0 to 2) compared to healthy brain regions.
Subjects included in this preliminary analysis were taken from the DecNef rsfMRI open dataset and divided into two cohorts (4 healthy subjects and 4 schizophrenia) and selected to ensure matching demographics. Both cohorts were composed of right-handed females in a three-year age window with all rsfMRI recorded in the same facility. We examined seventy (70) cROI (as labelled by the Desikan-Killiany Atlas). The distributions of the Sink Indices were then compared between the two cohorts by cROI.
Our findings revealed significant differences in influence within specific cROI between healthy controls and schizophrenia patients ($p<0.001$), including the frontal pole (1.18±0.37 vs. 0.88±0.24), orbitofrontal cortex (0.67±0.3 vs. 0.53±0.23), parahippocampal gyrus (1.28±0.24 vs. 1.43±0.26), and rostral middle frontal gyrus (1.55±0.29 vs. 1.35±0.25). All of the identified regions are known to play crucial roles in both positive and negative symptoms present in patients with schizophrenia. Our results provide further evidence supporting the hypothesis that aberrant network connectivity patterns contribute to the pathophysiology of this psychiatric disorder. The application of such models to large-scale rsfMRI datasets offers a promising avenue for identifying biomarkers for schizophrenia and other related disorders.

Disclosures: A. Williams: None. **L. Sanchez:** None. **S. Sarma:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.11/WW78

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

Support: NIH/NINDS Brain Initiative Grant 1UF1NS115779

Title: Metastable dynamics in gustatory cortex during taste mixture-based perceptual decision-making

Authors: *L. LANG[1], J. BLACKWELL[2], Y. ZHENG[1], G. LA CAMERA[1], A. FONTANINI[1]; [1]Neurobio. & Behavior, Stony Brook Univ., Stony Brook, NY; [2]Neurosci., Brown Univ., Providence, RI

Abstract: Neural activity in gustatory cortex (GC) is *metastable*—that is, characterized by abrupt transitions between ensemble-coordinated firing rates called *states*. Previous work has shown that metastable dynamics underlie GC's crucial role in mediating taste-based decision-making. For example, metastable states coding for taste, abstract cues (that is, taste's predictive value), and decision variables have been found in the GC of mice performing a taste-based decision-making task. The dynamics of these states support sequential encoding of sensory, cue, and action information over time. However, it is still unclear exactly what properties of metastable states allow for representation of decision-making task-relevant variables, and how this coding framework applies when the taste stimulus is a mixture and varies along a continuous gradient rather than a categorical scale. Here we investigate metastability in the GC of mice performing a sucrose/salt binary taste mixture-based decision-making task. This task requires animals to (1) sample a sucrose/salt mixture (sucrose/salt: 0/100, 25/75, 35/65, 45/55, 55/45, 65/35, 75/25, 100/0) from a central spout, (2) wait for a delay period, and then (3) lick one of two lateral spouts for a water reward based on the dominant stimulus in the mixture (i.e., sucrose>NaCl --> lick left; NaCl<sucrose --> lick right). Hidden Markov Models (HMMs) were used to extract the metastable states from GC ensembles recorded using high-density Neuropixels probes in mice performing this task. Linear classification analysis revealed that metastable state duration (rather than binary occurrence, as has been used in the past) contains information about the stimulus concentration and about the animal's directional choice. We assigned a label to each state based on whether its mean duration vs. concentration profile was best fit by a line or a step function (or neither). Distributions of state onset times indicate that the linear-coding states tend to precede the step-coding states, which is consistent with concentration-based/sensory coding preceding binarized/decision coding in this task. Future work will explore how these dynamics may change with discrimination learning and explain them in terms of circuit/network-level modifications by using a clustered spiking neural network model.

Disclosures: L. Lang: None. **J. Blackwell:** None. **Y. Zheng:** None. **G. La Camera:** None. **A. Fontanini:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.12/WW79

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

Support: Brain/MINDS grant 19dm0207093h0001
Grant-in-Aid for Scientific Research 23H03700

Title: Quantifying macroscopic post-saccadic traveling waves with graph-based algorithm

Authors: *K. HO[1], C. CHEN[2], H. ONOE[3], T. ISA[1,2,3];
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the Advanced Study of Human Biol. (ASHBi), [3]Human Brain Res. Center, Grad. Sch. of Med., Kyoto Univ., Kyoto City, Japan

Abstract: Traveling waves (TW) are temporal fluctuation of local field potential (LFP) that propagate spacially across the cortical surface. In the past years, many methods based on Hilbert transform and circular-linear correlation have been developed to identify and quantify TWs, successfully relating TWs to various brain functions. However, these methods all assume single wave with known and stable spaciotemporal structure, such as planar or rotational, in the recorded area. These assumptions render them not applicable to our hemispheric multi-channel (~100) electrocorticography (ECoG) on marmosets that covers large brain region and simultaneously records multiple TWs of complex patterns. To solve this problem, we developed an algorithm based on existing methods and graph theory. In brief, normalized LFP of neighboring electrode pairs were time-shifted and compared, to determine if there was a causal relationship. The causal relationship was represented by edges in a directed phase gradient (PG) graph, whose nodes represent electrodes. Root nodes with only outgoing edges were identified as wave source, together with nodes they have a path to, forming induced subgraphs of the PG graph which defines the spacial scope of a TW at a moment. Across time, by concatenating subgraphs with the same root node, the spaciotemporal scope of a TW can be quantified. We then benchmarked this algorithm with simulated data, and the results showed high spacial and temporal accuracy under moderate noise level. In ECoG data from 2 marmosets performing visually-guided saccade task, we observed an increased beta-band power (~30 Hz) in occipital areas during 0~100 ms after saccade offset. Visual inspection confirmed this increased beta power results from TWs from the primary visual cortex. Using the graph-based algorithm, we successfully extracted and quantified these TWs. These TWs are large in spacial scope, traveling across higher visual areas and even to temporal and parietal areas. These TWs are more prominent after saccades to contra-lateral targets, shown by larger coverage, longer duration, and higher amplitude compared to those after ipsi-lateral saccades. The traveling speed of these TWs is 800~1600 mm/s, consistent with the conduction speed of myelinated axons, which are suggested to be substrates for macroscopic TWs. Taken together, our graph-based algorithm makes it possible to identify and quantify multiple simultaneously occurring TWs of complex patterns in large scale recording systems, which may provide insight into how information is processed across brain regions.

Disclosures: **K. Ho:** None. **C. Chen:** None. **H. Onoe:** None. **T. Isa:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.13/WW80

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

Title: Identification of Recurrent Dynamics in Distributed Neural Populations

Authors: *R. OSUNA OROZCO, S. R. SANTACRUZ;
Biomed. Engin., Univ. of Texas at Austin, Austin, TX

Abstract: Interactions between anatomically distributed neural populations are characterized by complex nonlinear and nonstationary dynamics. The advent of modern recording techniques for tracking neural activity at high spatial and temporal resolution over broad anatomical areas has enabled exploration of these complex dynamical patterns. However, there is still a need for methods that are widely applicable and robust that can capture these complex dynamics while remaining adequately constrained by observed data and offering interpretability. As a data driven approach, time-varying vector autoregressive methods can account for nonlinear and nonstationary dynamics through local linear approximations but scaling them for large number of recorded dynamical variables and extracting interpretable insights from them remains challenging. Dimensionality reduction approaches have been used extensively to enable scaling of systems-based approaches while emerging techniques such as switching linear dynamical systems can augment interpretability by approximating the dynamics by a small number of recurrent linear systems. This contribution explores the interplay between dimensionality reduction and time-varying dynamics and offers an unsupervised approach for identification of recurrent linear dynamics including estimates of parameter uncertainty for the identified linear systems. To this end, we use the time-varying autoregression with low rank tensors (TVART) method on simulated neural mass model data as well as local field potential (LFP) data from a non-human primate animal subject. For the simulated data, the recurrence structure of the dynamics that arises from exploration of the attractor space in a multistable system can be recovered by our approach even in the presence of high noise. Moreover, for simulations based on human connectivity data we demonstrate that dynamics for parameters that allow for the coexistence of low and high activity states require higher rank approximations than those which only exhibit a repertoire of high activity states. Finally, we uncover frequency dependent recurrent structure in LFP recordings by examining the time series of short time band limited spectral power from a non-human primate at rest and during trained task execution. We observed robust variation in the dynamics that were concurrent with the task behavior across frequency bands as well as frequency dependent variation at rest. We also describe the interplay between rank reduction and temporal variation resolution in reducing the prediction error across experimental conditions.

Disclosures: R. Osuna Orozco: None. **S.R. Santacruz:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.14/WW81

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

Support: Allen Institute

Title: A computational approach to uncover neuromodulator dynamics in experiments with hyperspectral fiber photometry

Authors: *R. GALA, S. SUNIL, K. PODGORSKI, U. SÜMBÜL;
Allen Inst., Seattle, WA

Abstract: Neuromodulators can act in a concerted fashion to influence circuit dynamics in behaving animals. It has been a challenge to study this because of difficulties in measuring multiple neuromodulators simultaneously. We address this problem by building a fiber photometry system that records spectrally resolved emission with up to 5 excitation wavelengths, enabling multiplexed measurements of fluorescent indicators in vivo. Analyzing the resulting measurements requires denoising and separation of spectrally mixed signals into source components. To this end, we propose a computational approach consisting of a generative model and a denoising neural network. The generative model uses simulated neuromodulator activity, indicator properties, and experimental imaging parameters to construct simulated observations. The denoising neural network is trained to recover the underlying neuromodulator activity, while also reconstructing the simulated observations. This trained network can then be fine-tuned using only a reconstruction loss on experimental measurements, towards inferring latent neuromodulator activity. We demonstrate an end-to-end proof-of-concept of this approach.

Disclosures: **R. Gala:** None. **S. Sunil:** None. **K. Podgorski:** None. **U. Sümbül:** None.

Poster

PSTR377. Network Computations: Data Analytics and Statistics

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR377.15/WW82

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

Support: NIH IRP FUNDING

Title: : increased sensitivity in identifying language related functional connectivity networks using jackknife vs traditional fc static analyses.

Authors: J. LIANG[1], N. SINAII[2], S. ACKER[1], A. KUROSU[1], ***N. BIASSOU**[3];
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Abstract: Increased sensitivity in identifying language related functional connectivity networks using Jackknife vs Traditional FC static analyses
Static functional connectivity (FC) is used extensively in the analysis of task-based functional magnetic resonance imaging (tbfMRI). The FC networks are calculated using the Spearman correlation of (Blood-Oxygen-Level-Dependent) time series data signal from the interested brain regions to identify statistically significant networks. A computational approach, known as

Jackknife correlations, determines FC by systemically eliminating each time point and recalculating the impact on the remaining network correlations. A comparison of the FC networks generated by these two methods is unknown. We compared the language-related FC networks identified using the traditional static method versus the Jackknife method for an auditory language comprehension task. We conducted surface-based analyses for static FCs of auditory language comprehension networks for 172 young healthy adults (mean 27.6 ±3.8 years, range 22-37 years) obtained from the Human Connectome Project (HCP). We matched subjects for years of education (Avg mean 14.9 ±1.7 years), sex (50% female), and handedness (11% left-handed) as defined by the Revised Edinburgh Handedness Inventory. The language tasks included vocabulary comprehension with math tasks as the baseline control. We used AFNI to preprocess and motion-correct the fMRI imaging data. We superimposed Desikan-Killiany parcellations to extract the corresponding time-series for each region. We identified static FCs using two static analysis methods: (i) Static nonparametric Spearman rank correlation coefficients; (ii) Jackknife Pearson correlation. Both methods corrected for multiplicity using the Bonferroni corrections to establish significant FCs. We included robust and reproducible networks using common network connections activated in at least 85% of subjects. Traditional static analyses identified 75 FC networks during the language tasks. Jackknife analyses identified 100% of these same networks. In addition, Jackknife analyses identified 12 more language related networks (4 left, 3 right, and 5 inter-hemispheric networks; $p<0.001$) that were not identified by the traditional static analyses. Jackknife method demonstrates relatively higher sensitivity in identifying language related functional connectivity networks. We think that the use of Jackknife correlation approaches may lead to more robust neurocomputational modeling of language-related changes in functional connectivity networks secondary to CNS injury as measured by tbfMRI.

Disclosures: **J. Liang:** None. **N. Sinaii:** None. **S. Acker:** None. **A. Kurosu:** None. **N. Biassou:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.01/WW83

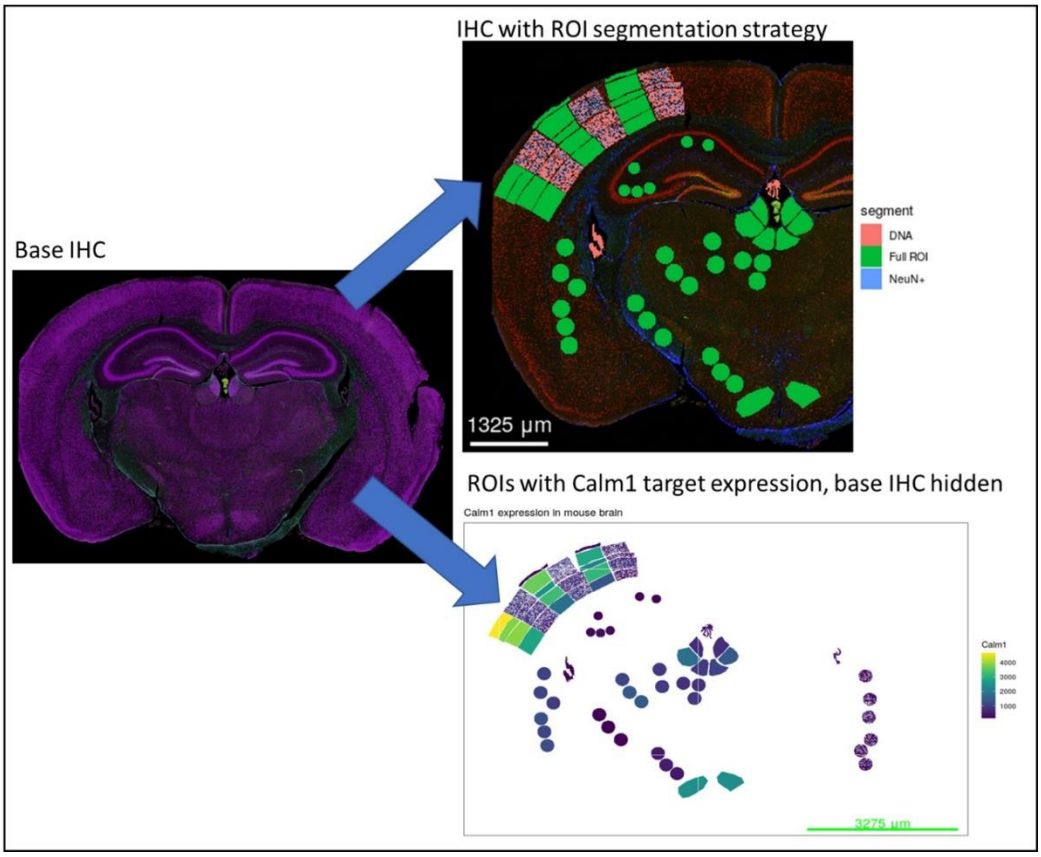
Topic: I.07. Data Analysis and Statistics

Title: Integrated visualizations of expression and imaging data in spatial biology using the SpatialOmicsOverlay R package

Authors: M. GRISWOLD, ***M. E. G. VANDENBERG**, A. HECK, C. WILLIAMS, N. ORTOGERO, R. BOYKIN, J. BEECHEM;
NanoString Technologies, Seattle, WA

Abstract: Integrating gene expression and rich imaging data in multi-omic experiments is a critical component to any study in the spatial biology field. Generating visualizations is key for

data sharing and communication, as well as understanding experimental design and biological findings. To overcome the challenges associated with representing integrated spatial imaging with transcriptomics, we developed the R package SpatialOmicsOverlay (SOO), freely available on Bioconductor. It is designed to work with the GeoMx® Digital Spatial Profiler (DSP) platform but is not limited to this use case. The DSP profiles expression in user-defined regions of interest (ROIs) of nearly any shape or design, which can be further segmented on immunohistochemistry signal. Flexibility and sophistication in ROI selection strategies yield complex experimental design. We developed the SOO package to contain features that integrate expression and imaging data effectively. Built on R's ggplot, SOO expands standard plotting with custom functions for scale bar, axis orientation, image display (colors, channels, thresholding), and ROI coloring by any user annotation or expression level of any of >21,000 RNA targets. Using this package, we analyzed transcriptomes on four 5-µm thick FFPE brain coronal sections from C57BL/6 male mice (see image below) using the Mouse Whole Transcriptome Atlas. We used a complex profiling strategy from 21 distinct areas of the mouse brain. Some areas were split into neuronal antigen-positive (NeuN+) and DNA autofluorescence or neuropil segments (including glial cells in neuropil segments), for a total of 360 segments across 297 ROIs. We focused on cortical layers and employed differential expression analyses to identify hundreds of spatially variable genes across layers and between NeuN+ and NeuN- segments, any of which can be plotted, as shown for Calm1. Our results demonstrate how the SOO package enables intuitive visualizations to represent complex spatial biology experiments of the brain. For research use only, not for use in diagnostics.



Disclosures: **M. Griswold:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M.E.G. Vandenberg:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Heck:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **C. Williams:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **N. Ortogero:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **R. Boykin:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.02/XX1

Topic: I.07. Data Analysis and Statistics

Support: NIH U01NS118300

Title: Self-supervised machine learning for computational adaptive optics in widefield microscopy of the brain

Authors: *I. KANG[1], Q. ZHANG[1], S. X. YU[4], N. JI[1,2,3,5];
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Abstract: Optical microscopy studies biological structures at subcellular resolution noninvasively. However, sample heterogeneity, such as in the intact brain of living animals, can lead to optical aberrations, reducing imaging resolution and contrast. Typically, additional hardware is used to measure and correct these aberrations, which adds complexity and cost to the microscope. In this study, we introduce CoCoA, a novel machine learning algorithm designed to jointly estimate wavefront aberration and extract 3D structure, thereby eliminating the

requirement for additional hardware. A self-supervised machine learning method, CoCoA does not require external datasets for training. We evaluated CoCoA by applying it to widefield imaging of fixed mouse brain neurons and comparing it to direct-wavefront-sensing-based adaptive optics. We also quantified the performance limits of CoCoA in terms of signal-to-noise ratio and signal-to-background ratio. Finally, we successfully demonstrated the first *in vivo* widefield imaging of the mouse brain using machine-learning-based adaptive optics.

Disclosures: I. Kang: None. **Q. Zhang:** None. **S.X. Yu:** None. **N. Ji:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

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Program #/Poster #: PSTR378.03/XX2

Topic: I.07. Data Analysis and Statistics

Title: A Multi-Omics Cell Segmentation Pipeline Using RNA Spots and High-Plex Protein Neuro Images

Authors: *A. WARDHANI, L. WU, W. LEUNG, M. KORUKONDA, D. ROSS, S. BONNETT, A. ROSENBLOOM, J. BEECHEM;
NanoString Technologies, Seattle, WA

Abstract: Brain cell segmentation is an important and challenging task in single-cell analysis. The variations in cellular density, morphology, and staining patterns and the lack of reliable membrane labeling reagents pose a challenge. The most common brain cell-segmentation method is to use nuclei, followed by fixed dilation to capture cytoplasmic areas. This arbitrary dilation ignores the cell shape and structure, thus incorrectly combining projections from neighboring cells. Regions that are highly packed with condensed chromatin, combined with weak membrane staining, make cell segmentation challenging. Low-quality tissue can further decrease the staining quality. Currently, ground truth and existing methods that detect projections from neurons and glial cells are extremely limited. As a result, in spatial transcriptomic research, transcripts located along these projections are often excluded.
In multi-omics profiling using CosMx™ Spatial Molecular Imaging proteomics data can be acquired with cycles of re-staining and clearing using photo-cleavable markers. This provides an unlimited source of morphology data that can be used to enhance cell boundary signals. In addition, when membrane staining is missing, we can leverage transcriptomics information to infer cell locations. In areas where RNA spots are dense, the spatial heatmap generated from the readout density can mirror the morphology of cells, therefore can be treated as an auxiliary morphology channel. Protein images expressing similar morphology are combined into a single channel using maximum intensity projection. The combined protein channels and the RNA raw spot heatmap are fed into a deep-learning segmentation module.
Our algorithm uses Cellpose to detect cell soma and edge-enhancing filters and connected component analysis to segment the projections into individual components. The original 2-

channel model architecture is expanded to a higher-plex model using transfer learning. The new network is initialized with the original 2-channel model parameters to reduce the training burden and ensure good initial segmentation performance. The network is fine-tuned to optimize the parameters for the remainder channels. Parameters are finalized once they meet a specified learning rate and minimize specific cost functions. Thus, we use curated segmentation results from existing custom cell segmentation algorithm using the 2-channel model as the initial ground truth. The use of both high-plex proteomics images and raw spot density from transcriptomics data provides a rich description of cell boundaries and enables the detection of partially stained or missing cells in morphology images.

Disclosures: **A. Wardhani:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **L. Wu:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **W. Leung:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **M. Korukonda:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **D. Ross:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **S. Bonnett:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **A. Rosenbloom:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies. **J. Beechem:** A. Employment/Salary (full or part-time):; NanoString Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NanoString Technologies.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

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Program #/Poster #: PSTR378.04/XX3

Topic: I.07. Data Analysis and Statistics

Support: NIH 1U24MH130919-01

Title: Modeling Single Cell Omics Technologies within a multi-center consortium: building consensus through minimal metadata

Authors: P. BAKER[1], T. FLISS[2], M. GIGLIO[3], S. NADENDLA[4], ***P. RAY**[2], C. L. THOMPSON[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Allen Inst. For Brain Sci., Seattle, WA; [3]Neurosci. Multi-omic Archive (NeMO), Baltimore, MD; [4]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: The BICCN (doi:10.1101/2022.10.26.513573) and BICAN generate large, high-quality datasets for cell type classification using a range of single-cell transcriptomic and epigenomic techniques (doi:10.1038/s41586-021-03950-0).These protocols vary in ways relevant to provenance and annotation, from tissue preparation and library generation to QC metrics. This presents a challenge for annotating datasets with detailed provenance and metadata necessary to satisfy FAIR principles for data sharing. Ideally, there would be a resource of record for techniques that provides standardized definitions and protocols that maps related samples and processes across these methods to permit, facilitate, and enhance data interoperability.
To meet these needs, we created an application ontology (BICANO) that describes entities to help scientists communicate about experiments and results. Though mostly focused on omics, BICANO includes imaging and electrophysiological methods used in BICAN, allowing us to link related entities and map data across techniques and modalities. We have documented more than 60 methods across BICAN/BICCN, and our submitters have deposited over 90 versioned protocols at protocols.io.
BICANO provides a structure that allows linking between experimental types, hierarchical classifications of techniques, and groupings. This provides a hierarchy, which enables searching or browsing; class annotations that provide linkages between techniques, assays, and tools; and well-defined, reusable, metadata elements for annotating datasets. In addition, we model each technique as a complete process, including biological tissue modeling for specimens, and data artifacts for experimental outputs. Metadata schemas are validated via a Metadata Review Process, which, along with our Vocabulary Services technologies and tools (LinkML, PURL), provides a central service for harmonization of terms and definitions cross-consortium and ensures consistent data flow through the ecosystem. This provides researchers and the community a suite of tools for data integration and supports the BICAN data ecosystem.
BICANO provides a framework for rigorous documentation, harmonization, and organization of experimental techniques. This provides scientific value for reproducibility, technological value through implementation in knowledge graphs, and organizational value through accurately indexing data and metadata. Future directions include harmonizing our efforts with related consortia (e.g. HuBMAP, SCORCH), ensuring interoperability of data models with global efforts (HCA), and extending a framework for a knowledge graph.

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Poster

PSTR378. Software Tools: Microscopy and Imaging

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Program #/Poster #: PSTR378.05/XX4

Topic: I.07. Data Analysis and Statistics

Support: NIMH Grant R42MH124566

Title: Characterization of pre and post synaptic proteins using expansion microscopy and deep machine learning

Authors: *M. HEAL[1], A. WILSON[1], N. J. O'CONNOR[2], N. ROUSSEL[1], B. EASTWOOD[1], A. RODRIGUEZ[2], P. ANGSTMAN[2], J. GLASER[2];
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Abstract: Expansion microscopy (ExM) has several advantages for ultrastructural analysis of complex brain morphology: visualizing nanoscale objects at nanoscale resolution; overcoming limitations of diffraction while still using light microscopes; and abounding possibilities for repeated hybridization, labeling, and multi-channel imaging. The morphological characteristics of neurons (axons, dendrites, spines, boutons, etc.) using traditional histological processing methods can often be readily reconstructed and analyzed automatically. However, due to the physical and isotropic separation of tissue biomolecules done with expansion protocols, ExM image stacks are characterized by a much more granular appearance, with fluorophores dispersed inside neuronal processes and dendritic spines at varying densities. Because of this, existing methods for algorithmically distinguishing foreground from background are limited with ExM data. To overcome these challenges, we are employing novel machine learning techniques in neuron reconstruction and analysis software tool, Neurolucida 360. With a U-Net architecture, trained with manually labelled image patches of ExM image data, we can segment 3-dimensional ExM image stacks in a way that results in a more rapid and accurate morphological reconstruction of cellular processes and spines. For thorough spatial investigation of nanoscale populations that are highly visible in expanded tissues (e.g., mRNAs and proteins) - and their proximity or colocalization to larger cellular morphologies - we've also implemented robust machine learning-based methods for puncta detection and analysis in addition to image registration techniques that enable alignment of multiple labeling and imaging rounds. Together, the AI-driven approaches in this software serve as a powerful toolkit for researchers across neuroscience disciplines to investigate the elaborate spatial transcriptome and proteome in the central nervous system - with neuromorphological context and nanoscale precision.

Disclosures: **M. Heal:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A. Wilson:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N.J. O'Connor:** A. Employment/Salary (full or part-time):; MBF Bioscience. **N. Roussel:** A. Employment/Salary (full or part-time):; MBF Bioscience. **B. Eastwood:** A. Employment/Salary (full or part-time):; MBF Bioscience. **A. Rodriguez:** A. Employment/Salary (full or part-time):; MBF Bioscience. **P. Angstman:** A. Employment/Salary (full or part-time):; MBF Bioscience. **J. Glaser:** A. Employment/Salary (full or part-time):; MBF Bioscience.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.06/XX5

Topic: I.07. Data Analysis and Statistics

Support: CIFAR-Temerty Innovation Catalyst Grant
University of Toronto - Baden Havard Endowment fund
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Title: An artificial intelligence-driven magnetic resonance imaging synthesis framework

Authors: *T. LATYPOV[1], M. TAWFIK[1], D. JOERGENS[2], P. SRISAIKAEW[2], P. ALCAIDE LEON[3], B. FINE[4], F. RUDZICZ[5], M. HODAIE[1];
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Abstract: ***Introduction***: Brain Magnetic Resonance Imaging (MRI) is essential for deriving vital information about brain structures and diagnosing neurological disorders. However, variations in MRI acquisition parameters across scanners impede image comparisons, especially between different facilities, often necessitating repeat sessions. Our proposal explores artificial intelligence (AI) for MRI data synthesis, employing pre-existing T1-weighted images, which are the most prevalent, to generate the often-missing Diffusion Tensor Imaging (DTI) and contrast-enhanced T1 sequences (T1c). This AI-driven approach aims to circumvent redundant MRI sessions, enhancing efficiency in patient care.
Methods: We used 3D T1 and DWI data from the Amsterdam Open MRI Collection dataset (n=928) as well as T1 and T1c data from Brain Tumour Segmentation Challenge 2021 (n=1435) dataset. DWI data was processed and fractional anisotropy (FA) was extracted using the "Tractoflow" pipeline. We used a 3D U-Net consisting of 4 contracting blocks, 4 expanding blocks and one convolutional block trained in a supervised fashion using the combination of multi-scale structural similarity index (SSIM) loss and L1 loss to generate synthetic FA images and synthetic T1c images using contrast-free T1 data as an input. Reconstruction accuracy was confirmed for the whole image using voxel-wise correlation and SSIM. In addition, Johns Hopkins University (JHU) White matter atlas regions and a two one-sided T-test (TOST) were used for regional FA similarity assessment.
Results: We trained 2 models synthesizing FA and T1c volumes respectively from contrast-free T1 data. Testing subsets of FA and T1c data showed high similarity between real and synthesized images (SSIM> 0.93, R sq >0.96). Regional intensity values of FA within all major 48 white matter structures from the JHU White Matter Atlas were significantly similar across all testing subjects (p corrected <0.0001). These measures confirmed the capability of frameworks to produce realistic FA and T1c data from the commonly acquired T1 sequence.
Conclusions: The approaches proposed in this study show high performance and will have valuable and immediate impacts on the way patients undergo brain imaging. Importantly, imaging data synthesis can significantly reduce the acquisition time and cost of MR procedures, making it more efficient, and preventing unnecessary repeated imaging, thereby improving the patient experience, and avoiding negative outcomes.

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Poster

PSTR378. Software Tools: Microscopy and Imaging

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Topic: I.07. Data Analysis and Statistics

Support: NSF Grant 1513126
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NSF Grant 1926990
Florida High Tech Corridor Grant 20-10
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Title: Deep learning-based estimates of global neuron counts from low magnification images

Authors: *H. MORERA[1], P. DAVE[1], G. DENHAM[2], S. ALAHMARI[3], J. RINCON[2], D. GOLDGOF[1], L. O. HALL[1], P. R. MOUTON[2];
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Abstract: Changes in the total number of neurons, glial cells and other stained structures in specific brain and spinal cord regions play a critical role in understanding, preventing and treating a wide range of neurological conditions. We have shown that deep learning (DL)-based methods combined with systematic-random sampling and unbiased stereology probes can make accurate, reproducible and efficient estimates of global numbers of immunostained neurons and microglial cells in tissue sections from neocortex (NCTX) and hippocampus of mouse brains. Here we extend this work to include stereology-based assessments of total numbers of Nissl-stained cresyl violet (CV) neurons in NCTX using only local [low magnification (log-mag), 20x] images. This approach uses a standard curve built by training a convolutional neural network (CNN) with 20x images of CV-stained neurons in NCTX of male and female mouse brains aged 7 to 14 months (n=14). Each low-mag image from training cases is labeled by the corresponding global counts of stereology-based total neurons at high magnification (100x) for that case then the cases were sorted into two classes of high (n=7) and low (n=7) mean total number of neurons in NCTX ($p < 0.05$). Age- and sex-matched mouse brains (n=14) not included in the training set were used as test cases (unknowns). Following input of low-mag images from each test case, the DL model assigned test cases to either global class (high or low) if over half the images from that case fell into that class. This DL approach correctly predicted the global stereology-based class of CV neurons in NCTX for ~80% of the cases, i.e., 11 of 14 correct (78.6%) using only the local 20x images from each test case. The time required for classifying each test case was less than 1 minute and there was 100% reproducibility (Test-Retest). These findings show for the first time that a CNN-trained model can reliably predict statistically different global counts of neurons in NCTX using only input of local images from test cases, without high magnification cell counts or cell-level segmentation. This novel approach has the strong potential to accelerate progress of neuroscience research and drug testing in neurodegenerative, neuroinflammatory and

other neurological conditions. Ongoing efforts include enhancing accuracy with further fine-tuning of the model; improving resolution with more classes; expanding the method to tissue sections from human and non-human primate brains; and developing a cloud-based approach using local images uploaded from an ordinary mobile phone.

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Poster

PSTR378. Software Tools: Microscopy and Imaging

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Topic: I.07. Data Analysis and Statistics

Support: Gift from Mathworks
NIH BRAIN Initiative
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Stanford Wu Tsai Neuroscience Institute

Title: Fast, scalable, and statistically robust cell extraction from large-scale neural calcium imaging datasets

Authors: H. INAN[1], **F. DINC**[2], O. HERNANDEZ[1], C. SCHMUCKERMAIR[5], S. HAZIZA[3], Y. ZHANG[6], O. HAZON[3], T. TASCI[1], B. O. AHANONU[8], J. LECOQ[4], M. J. WAGNER[1], M. ERDOGDU[9], *M. J. SCHNITZER[7];
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Abstract: State-of-the-art fluorescence Ca^{2+} imaging studies that monitor large-scale neural dynamics can produce video datasets ~10 terabytes or more in total size. Processing such data volumes requires automated, general-purpose and fast computational methods for cell identification that are robust to a wide variety of noise sources. We present EXTRACT, an algorithm that is based on robust estimation theory and uses graphical processing units (GPUs) to extract neural dynamics in computing times up to 10-times faster than imaging durations [1,2]. Owing to its agnosticism about the statistical distributions of noise that may be present in the Ca^{2+} imaging data, and its lack of assumptions about Ca^{2+} signaling waveforms or background fluorescence, EXTRACT can be broadly applied to data from a wide range of Ca^{2+} imaging formats, including one- or two-photon fluorescence Ca^{2+} imaging data from either head-fixed or freely behaving animals, without requiring any algorithmic changes. The algorithm is also applicable to high-speed voltage-imaging movies of neural spiking. We validated EXTRACT on simulated and experimental data and processed 199 public datasets from the Allen Institute Brain

Observatory in one day. EXTRACT outperforms prior state-of-the-art cell extraction algorithms regarding both speed and accuracy using a single GPU, and its capability to use multiple GPUs at once makes EXTRACT a scalable approach that can analyze the dynamics of tens of thousands of cells in close to real-time. Runtime comparisons between EXTRACT and prior cell extraction algorithms show that EXTRACT processes two-photon Ca^{2+} imaging datasets about 10-fold faster than previous methods. The scalability of EXTRACT to large datasets makes it the only existing cell extraction algorithm that can process neural activity videos as large as 10 TB without sacrificing speed or accuracy. Showcasing its superiority over past cell extraction methods at removing noise contaminants, neural activity traces from EXTRACT also allow more accurate decoding of animal behavior. Overall, EXTRACT provides neuroscientists with a powerful computational tool matched to the present challenges of neural Ca^{2+} imaging studies in behaving animals. The userbase for EXTRACT is currently estimated to be hundreds of neuroscience labs around the globe. Upon request, tutorial sessions are available to new users on an approximately biweekly basis.
[1] doi.org/10.1101/2021.03.24.436279. [2] https://github.com/schnitzer-lab/EXTRACT-public

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Poster

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Topic: I.07. Data Analysis and Statistics

Support: Zebrafish Center for Disease Modeling (ZCDM), Korea
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Title: Statistically unbiased prediction enables accurate denoising of voltage imaging data

Authors: *S. HAN[1], M. EOM[1], P. PARK[2], G. KIM[1], E.-S. CHO[1], J. SIM[1], K.-H. LEE[3], S. KIM[4], H. TIAN[2], U. L. BÖHM[5], E. LOWET[6], H.-A. TSENG[6], J. CHOI[1], S. E. LUCIA[1], S.

RYU[4], M. ROZSA[7], K. SVOBODA[7], S. CHANG[4], P. KIM[1], X. HAN[6], K. D. PIATKEVICH[8], M. CHOI[4], C.-H. KIM[3], A. E. COHEN[2], J.-B. CHANG[1], Y.-G. YOON[1];
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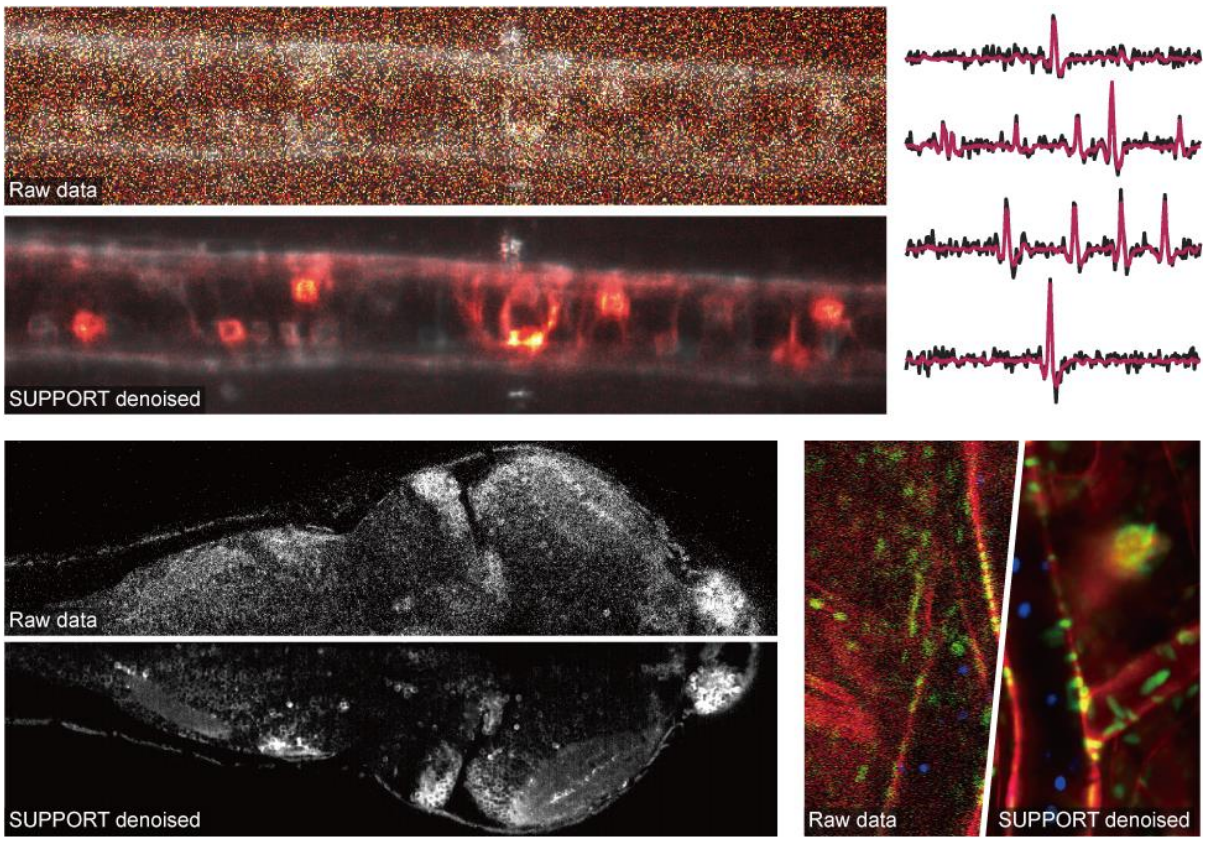
Abstract: We propose SUPPORT (Statistically Unbiased Prediction utilizing sPatiOtempoRal information in imaging daTa), a self-supervised denoising method for functional imaging data that is robust to fast dynamics in the scene compared to the imaging speed. SUPPORT is based on the insight that a pixel value in functional imaging data is highly dependent on its spatiotemporal neighboring pixels, even when its temporally adjacent frames alone fail to provide useful information for statistical prediction. By learning and utilizing the spatiotemporal dependence among the pixels, SUPPORT can accurately remove Poisson Gaussian noise in voltage imaging data in which the existence of the action potential in a time frame cannot be inferred from the information in other frames. We demonstrate the capability of SUPPORT using diverse voltage imaging datasets acquired using Voltron1, Voltron2, paQuasAr3-s, QuasAr6a, zArchon1, SomArchon, and BeRST1. The analysis of the voltage imaging data with simultaneous electrophysiological recording shows that our method preserves the shape of the spike while reducing the statistical variance in the signal. We also show that SUPPORT can be used for denoising time-lapse fluorescence microscopy images of Caenorhabditis elegans (C. elegans), in which the imaging speed is not faster than the worm's locomotion, as well as static volumetric images of Penicillium and mouse embryos. SUPPORT is exceptionally compelling for denoising voltage imaging and time-lapse imaging data, and is even effective for denoising calcium imaging data. Finally, we developed software with a graphical user interface (GUI) for running SUPPORT to make it available to the wider community. Code for Pytorch implementation of SUPPORT is available online at Github repository (https://github.com/NICALab/SUPPORT).



Disclosures: **S. Han:** None. **M. Eom:** None. **P. Park:** None. **G. Kim:** None. **E. Cho:** None. **J. Sim:** None. **K. Lee:** None. **S. Kim:** None. **H. Tian:** None. **U.L. Böhm:** None. **E. Lowet:** None. **H. Tseng:** None. **J. Choi:** None. **S.E. Lucia:** None. **S. Ryu:** None. **M. Rozsa:** None. **K. Svoboda:** None. **S. Chang:** None. **P. Kim:** None. **X. Han:** None. **K.D. Piatkevich:** None. **M. Choi:** None. **C. Kim:** None. **A.E. Cohen:** None. **J. Chang:** None. **Y. Yoon:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.10/XX8

Topic: I.07. Data Analysis and Statistics

Support: NIH UF1MH128339: Open-Access AAV Toolbox for Basal Ganglia Cell Types and Circuits

Title: A cloud based data processing pipeline for whole brain images from the SmartSPIM Lightsheet Microscope

Authors: *N. LUSK[1], C. LAITON[2], M. TAORMINA[3], D. TOGLIA[4], E. PETERSON[3], J. ROHDE[5], J. WATERS[3], S. WAY[6], S. DE VRIES[2], C. ARSHADI[4], J. CHANDRASHEKAR[4], K. SVOBODA[7], D. FENG[2], S. SESHAMANI[2], S. YAO[6], B. TASIC[6];
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Abstract: Light sheet fluorescent microscopy (LSFM) has steadily become a prominent technique within the field of neuroscience, providing fast acquisition of high-resolution isotropic 3D images. However, a current drawback to LSFM is the proliferation in data, with individual imaging sessions in the Gigabyte to Terabyte range, depending on acquisition resolution. This presents both technical and computational difficulties necessitating strategic solutions for storage, processing, and visualization. To address this, we have developed a completely cloud based pipeline for whole-brain nuclei and cell-body quantification. The inputs to this pipeline are whole mouse brains imaged at near isotropic (1.8μm, 1.8μm, 2.0μm) resolution using SmartSPIM light sheet microscope. Leveraging Amazon S3 storage, Code Ocean computational research platform, and Cellfinder segmentation, we have developed a highly parallelized process for fusion, registration, segmentation, and quantification of LSFM images. All data is registered to the Allen Institute's common coordinate framework (CCFv3) allowing for direct comparative analysis across datasets. Along with quantitative information, both full resolution and CCFv3 aligned images are stored in a multi-scale OME-Zarr format for visualization using the cloud-based viewer, Neuroglancer. Importantly, all data processed through this pipeline is publicly available providing a powerful resource for the research community.

Disclosures: N. Lusk: None. **C. Laiton:** None. **M. Taormina:** None. **D. Toglia:** None. **E. Peterson:** None. **J. Rohde:** None. **J. Waters:** None. **S. Way:** None. **S. de Vries:** None. **C. Arshadi:** None. **J. Chandrashekar:** None. **K. Svoboda:** None. **D. Feng:** None. **S. Seshamani:** None. **S. Yao:** None. **B. Tasic:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.11/XX9

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant 1R24MH117295-01A1
NIH Grant 2P41EB019936-06A1

Title: Appraising and enhancing reexecutability, transparency, and portability of published neuroimaging results

Authors: *H.-I. IOANAS[1], A. MACDONALD[2], Y. O. HALCHENKO[3];
[1]Dartmouth Col. Psychological & Brain Sci., Hanover, NH; [2]Dartmouth Col., Dartmouth Col.,

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Abstract: The value of experimental research articles is inextricably contingent on data analysis results which substantiate their natural language claims.
However, the intricacy of data analysis procedures, alongside their high reliance on extrinsic tools, makes them fragile with respect to re-use and may endanger their value as a repository of procedural knowledge.
It is therefore of crucial importance for all constituent instructions to be not only recorded and accessible, but also to represent the encapsulated domain and operational knowledge as automatically executable code, in order to reliably support re-execution.
In this study, we examine a peer-reviewed neuroimaging experiment, which already publishes automated data analysis instructions, in light of its reexecution reliability.
We document a number of prominent difficulties with de novo article generation, arising from the rapid evolution of extrinsic tools, and from nondeterministic data analysis procedures.
To compensate for these difficulties, we formulate a novel reexecution standard which leverages mutable-state dependency management, environment isolation, as well as emerging technologies for provenance tracking.
This novel standard consists in a general purpose resource topology with well-defined entry points, and is illustrated by a reference implementation which can fully re-execute the original article.
We further leverage this technological advancement to produce a fine-grained reproducibility assessment at the article level.
This assessment encompasses inline statistical summaries (e.g. F and p values), figures, as well as the relationship between these values and the qualitative statements they underpin.
The reproducibility analysis demonstrates that article reexecution in our novel standard showcases high accuracy (coherence in statistical summaries between our regenerated article and the original article reexecution process, Fig.1), and very high precision (coherence in statistical summaries between multiple de novo reproductions).

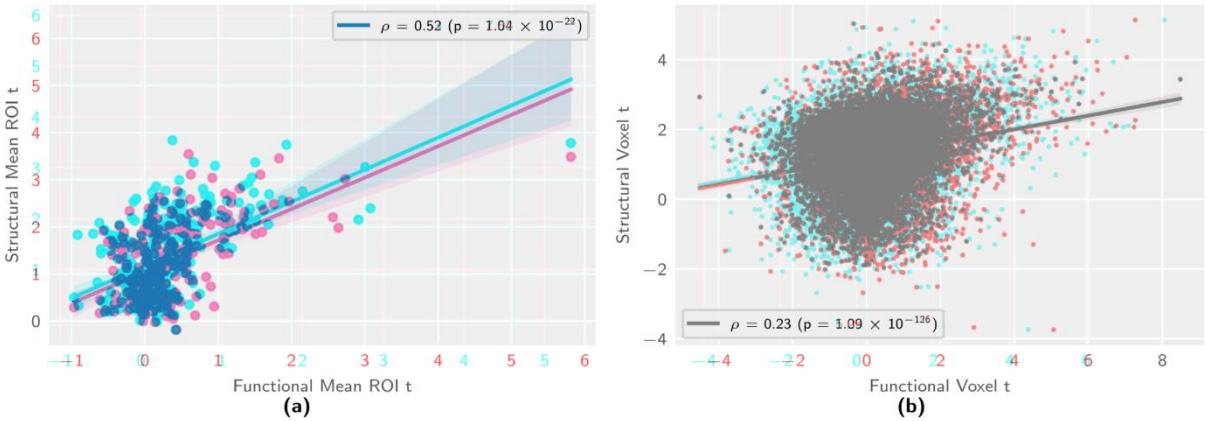

Figure 1: The novel reexecution environment produces highly stable statistical parameters, even in view of non-deterministic processing characteristics at both the parcellation (a) and voxel levels (b). Depicted are hue-shifted overlay differential images of individual figures between article reexecutions, using the original workflow (red), and the novel reexecution standard (blue).

Disclosures: **H. Ioanas:** None. **A. Macdonald:** None. **Y.O. Halchenko:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.12/XX10

Topic: I.07. Data Analysis and Statistics

Support: IARPA Contract 2020-20081800401

Title: Automated Functional Co-Registration of the IARPA MICrONS "Minnie65" Dataset using Symmetrical Diffeomorphic Registration

Authors: J. JOYCE[1], S. PAPADOPOULOS[2], *D. XENES[1], C. BISHOP[1], P. FAHEY[2], F. COLLMAN[3], N. DA COSTA[3], A. TOLIAS[2], P. RIVLIN[1], B. WESTER[1];
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Abstract: Under the IARPA MICrONS Program, several large-scale connectomics datasets were generated, including the Minnie dataset which covers a cubic millimeter of mouse visual cortex. This dataset contains functional activity for 75,000 neurons and a reconstruction of a serial EM imaging volume that consists of over 200,000 cells. While these two image volumes are coincident within the visual cortex, there are non-linear deformations that make volumetric co-registration and matching of neurons challenging using both manual and automated methods, especially given the scale. However, co-registration of structural and functional data is critical to enabling researchers to better model and explain the organization and behavior of excitatory neurons within and across cortical areas. Thus, our team has developed a new automated and scalable technique to register functional units to their structural cell counterparts for this and other petascale image volumes. Our method involves extending the global transformation developed by the Allen Institute of Brain Sciences to account for local, non-linear deformations in electron microscopy and two-photon imagery. We first increase the signal-to-noise ratio in the structural photon stacks by applying a Meijiring filter and skeletonization step to both blood vessels and cells such that the structures are distinguishable and separated. Then, a local diffeomorphic transformation is computed using the symmetrical diffeomorphic registration algorithm from the DIPY Python package. The transformation is calculated for the entire volume, and the lowest residual for each excitatory nuclei is selected as the primary match.Following the completion of our new co-registration workflow, our team has generated 36,544 total neuron matches with high confidence between the functional and structural datasets in the Minnie65 dataset. This expands the number of viable functionally-matched neurons in Minnie65 by roughly 24,000 on top of the 12,054 matches that were manually verified by the MICrONS performers. The automated set of matches generated by our workflow is available in the Minnie65 CAVE annotation framework, which includes the corresponding IDs from the structural and functional datasets and the residual distances between them.

Disclosures: **J. Joyce:** None. **S. Papadopoulos:** None. **D. Xenes:** None. **C. Bishop:** None. **P. Fahey:** None. **F. Collman:** None. **N. da Costa:** None. **A. Tolias:** None. **P. Rivlin:** None. **B. Wester:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.13/Web Only

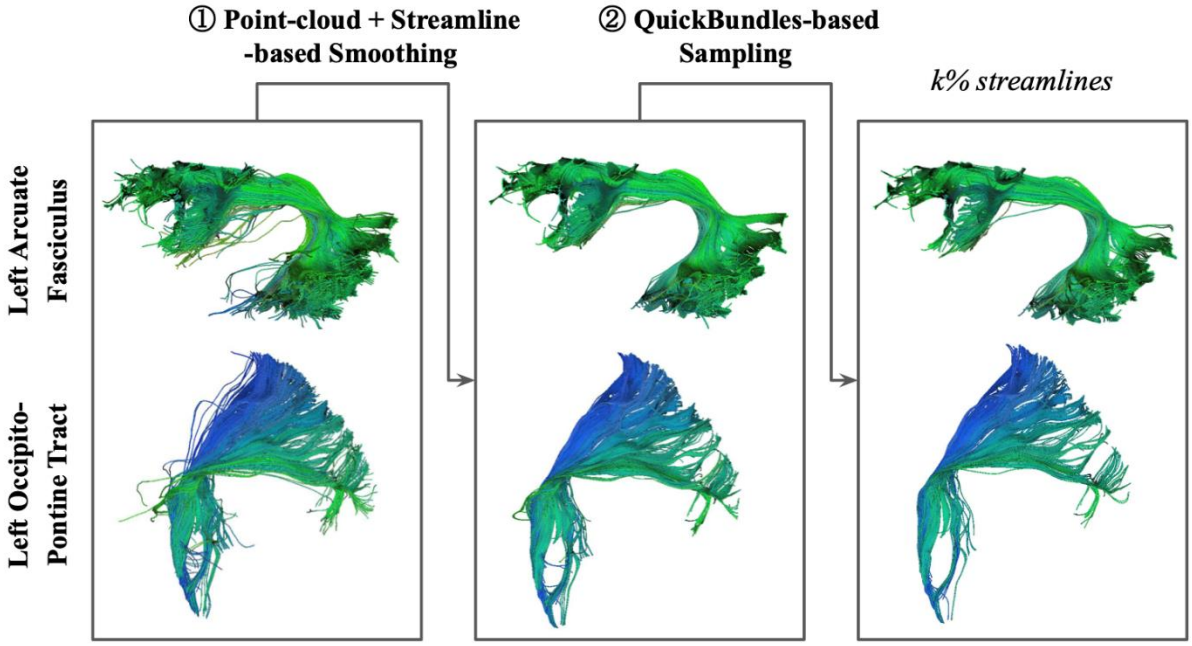
Topic: I.07. Data Analysis and Statistics

Support: NIA grant RF1AG057892

Title: Bundlecleaner: point-cloud based denoising and subsampling of tractography data

Authors: *Y. FENG[1], B. Q. CHANDIO[1], A. A. JOSHI[2], P. M. THOMPSON[1];
[1]USC, Marina Del Rey, CA; [2]Ming Hsieh Dept. of Electrical and Computer Engin. - Systems, USC, Los Angeles, CA

Abstract: Tractography data generated from 3D diffusion MRI allows for in-vivo visualization of white matter tracts in the brain. However, a high level of noise in the tractograms can affect downstream applications such as tractometry, where detected group differences may be due to artifacts. Post-hoc streamline filtering methods, such as FiberNeat and FINTA, have been proposed to remove false positive streamlines. Even so, spurious streamlines may be structurally plausible if they are evaluated individually, but they may not be aligned with the overall bundle shape. It may therefore be beneficial to evaluate streamline geometry at the bundle level. In addition, “valid” streamlines may still contain noise that can be addressed with smoothing in addition to filtering.
We propose BundleCleaner, a brain tractography analysis framework that can not only filter but also denoise and subsample fiber bundles, based on both global bundle shape and local streamline features. BundleCleaner is composed of 4 steps: 1) Streamline resampling and initial cluster-based pruning; 2) Point-cloud based smoothing regularized with a robust point-cloud Laplacian; 3) Streamline-based smoothing using the Savitzky-Golay filter to ensure that sequential information is preserved; and 4) final pruning and subsampling as needed. Pruning in steps 1 and 4 uses QuickBundles, a streamline-based clustering algorithm, to remove unwanted clusters based on a distance threshold. In step 4, streamlines are evenly subsampled based on the remaining clusters to better preserve the original bundle shape. Examples of one left arcuate fasciculus and occipito-pontine tract cleaned using BundleCleaner are shown in Figure 1. A simple command line interface is available at https://github.com/wendyfyx/BundleCleaner.



Disclosures: **Y. Feng:** None. **B.Q. Chandio:** None. **A.A. Joshi:** None. **P.M. Thompson:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.14/XX11

Topic: I.07. Data Analysis and Statistics

Support: R01NS127524

Title: Computational framework for diffusion MRI data preprocessing for predictive biomarkers and therapies in post-traumatic epilepsy.

Authors: ***A. SHARMA**[1], A. BENNETT[1], C. ALBA[1], M.-H. CUI[2], P. G. SALETTI[2], S. L. MOSHE[2], W. LIU[2], R. FLEYSHER[2], C. A. BRANCH[2], A. S. GALANOPOULOU[2], D. DUNCAN[1];
[1]USC, Los Angeles, CA; [2]Albert Einstein Col. of Med., New York City, NY

Abstract: Post-traumatic epilepsy (PTE) following traumatic brain injury (TBI) lack predictive biomarkers, hindering the development of preventive treatments. The Translational Platform for Epilepsy Therapy and Biomarker Discovery Study, which includes data from multiple sites including, Albert Einstein College of Medicine, UCLA, and University of Melbourne, aims to establish an effective model for screening new treatments and identifying biomarkers in a rodent TBI model, with a focus on diffusion magnetic resonance imaging (dMRI). The present study introduces an automated computational framework for preprocessing dMRI data, employing

advanced algorithms to ensure data quality and reliability for the Translational Platform study. The pipeline, utilizing several software packages such as MRTRIX, QIT, and FSL, encompasses multiple preprocessing stages and quality control (QC) metrics. Diffusion-weighted imaging (DWI) volumes are standardized and normalized mitigating intensity variations. The pipeline systematically executes denoising, Gibbs ringing removal, corrections for eddy currents and inhomogeneity distortions, brain masking, and bias field correction. Additionally, a QC module generates and visualizes metrics such as motion and susceptibility distortions and identifies outliers. The results (Fig 1) demonstrate successful artifact removal (top) and generated visualizations of QC metrics (bottom). This automated pipeline streamlines dMRI data processing, employing artifact corrections and QC metrics, ensuring data integrity. Essential for the Translational Platform, it fosters robust imaging metric extraction, critical for biomarker identification and PTE therapy development. The results focused on data from the Albert Einstein College of Medicine, primarily to demonstrate the capabilities of the pipeline. However, the study has collected similar data across other sites. This framework will enable researchers to carry out cross-site comparisons, facilitating a more comprehensive analysis and accounting for potential variations and biases.

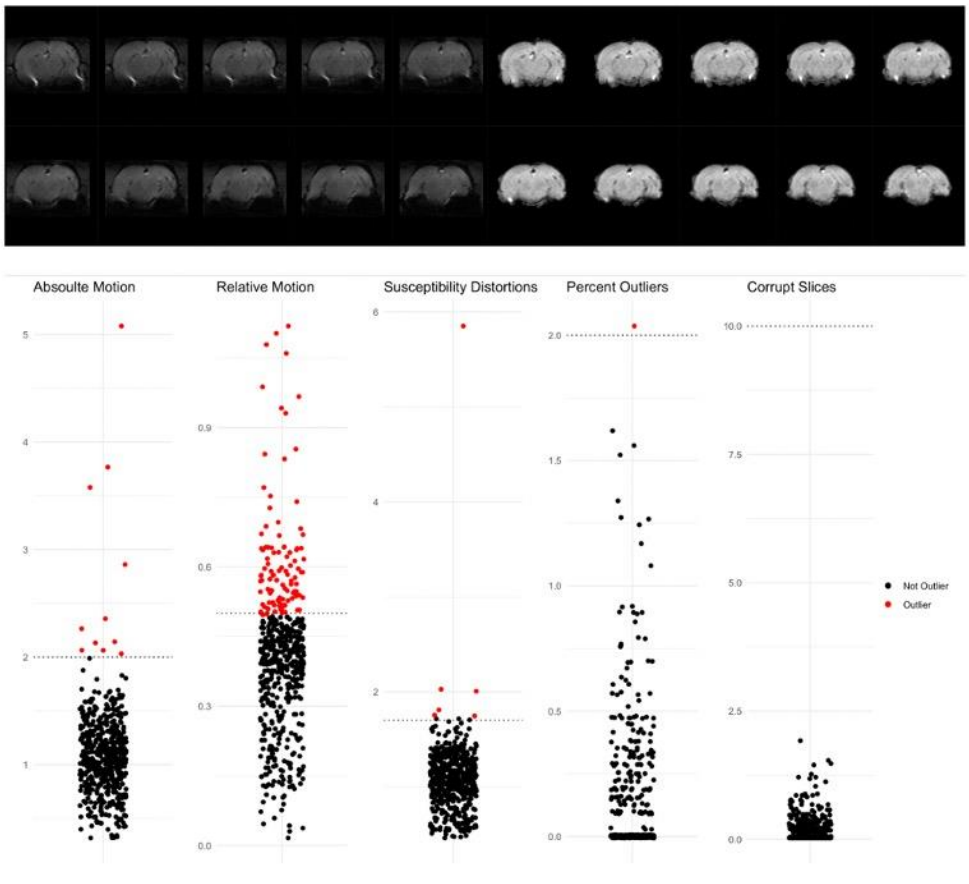


Figure 1. Top: Preprocessing results; raw image (left), preprocessed (right). Bottom: visualization of QC metrics. Dotted line indicates outlier threshold and red dots represent outliers.

Disclosures: **A. Sharma:** None. **A. Bennett:** None. **C. Alba:** None. **M. Cui:** None. **P.G. Saletti:** None. **S.L. Moshe:** None. **W. Liu:** None. **R. Fleysher:** None. **C.A. Branch:** None. **A.S. Galanopoulou:** None. **D. Duncan:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.15/XX12

Topic: I.07. Data Analysis and Statistics

Support: R01MH12668

Title: Big-data electron microscopy for novel community hypotheses: measuring and retrieving knowledge

Authors: M. E. WIMBISH[1], N. GUITTARI[1], P. K. RIVLIN[1], M. HINTON[1], J. MATELSKY[3], V. ROSE[1], J. RIVERA[1], K. CHUNG[3], T. JOSEPH[4], ***E. C. JOHNSON**[2], B. WESTER[5], W. GRAY-RONCAL[1];
[1]Johns Hopkins Univ. Applied Physics Lab., Laurel, MD; [2]Johns Hopkins Univ. Applied Physics Lab., Columbia, MD; [3]Johns Hopkins Univ., Baltimore, MD; [4]Univ. of Maryland, Baltimore County, Baltimore, MD; [5]Johns Hopkins Univ. APL, Laurel, MD

Abstract: Recent developments and innovation in Electron Microscopy (EM) and X-Ray Microtomography (XRM) imaging technologies have led to an increase in available spatial resolution, image acquisition rate, and scale of structural neural data. Data throughput is increasing, along with the availability of datasets and annotations from different species and experiments. This presents an opportunity for new secondary analyses by the connectomics community, but the variability in standards used for gathering, labeling, and organizing data may limit meaningful comparisons. To help address this barrier, BENCHMARK has pursued techniques to streamline the storage and management of data through the standardization of metadata and common software tools, including tools for validating connectomics metadata. This included forming a community Working Group to generate standards for connectomics image and experimental metadata and annotation metadata. These are being incorporated into portals tied to BossDB, the BRAIN Initiative data archive for EM and XRM connectomics. The team has developed tools for querying and analyzing EM and XRM datasets, and is making these resources accessible to the connectomics community. We also provide a package of Jupyter notebooks and documentation to provide accessible starting points for developing and testing new hypotheses using the BENCHMARK standard for connectomic analyses. Overall, BENCHMARK aims to facilitate meta-analyses and knowledge sharing to enable secondary data analyses and comparative connectomics.

Disclosures: **M.E. Wimbish:** None. **N. Guittari:** None. **P.K. Rivlin:** None. **M. Hinton:** None. **J. Matelsky:** None. **V. Rose:** None. **J. Rivera:** None. **K. Chung:** None. **T. Joseph:** None. **E.C. Johnson:** None. **B. Wester:** None. **W. Gray-Roncal:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.16/XX13

Topic: I.07. Data Analysis and Statistics

Support: NIEHS Contract GS-00F-173CA-75N96021F00109

Title: Fipha: an open-source fiber photometry analysis platform in r/shiny

Authors: *M. F. BRIDGE[1], L. WILSON[2], K. STEVANOVIC[2], A. LETSINGER[2], S. MCBRIDE[1], H. CUNNY[3], K. R. SHOCKLEY[4], J. D. CUSHMAN[2];
[1]DLH, Durham, NC; [2]Neurobio. Lab., [3]Office of Program Operations, [4]Biostatistics and Computat. Biol. Br., Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: Fiber photometry is a widely used technique in modern behavioral neuroscience, employing genetically encoded fluorescent sensors to monitor neural activity and neurotransmitter release in awake-behaving animals. With its low cost and ease of implementation, it has become a valuable tool for the behavioral neuroscientist. However, analyzing such data can be laborious and time-consuming, especially considering that experimental setups may often vary based on the specific brain region and type of sensor being used. Consequently, many research laboratories have developed their own bespoke analysis pipelines that are not easily applicable to outside data. Here we describe a collaborative project that has been undertaken to develop a general-purpose fiber photometry analysis application in R called FiPhA (Fiber Photometry Analysis). This application utilizes the Shiny framework, providing interactive visualization, quality control, and batch processing functionalities to an otherwise tedious stage of data analysis. Simplifying a range of tasks, the program includes visualizations of event-triggered averages, event filtering, and spectral analysis capabilities. Furthermore, it is able to work with data derived from multiple photometry systems, including those that are spectrally resolved, camera-based, and involve lock-in demodulation methods. A user-friendly interface provides a highly configurable experience for researchers without necessitating knowledge of the R programming language itself.

Disclosures: **M.F. Bridge:** None. **L. Wilson:** None. **K. Stevanovic:** None. **A. Letsinger:** None. **S. McBride:** None. **H. Cunny:** None. **K.R. Shockley:** None. **J.D. Cushman:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.17/XX14

Topic: I.07. Data Analysis and Statistics

Support: NIH Grant P41EB015922
NIH Grant U54EB020406
NIH Grant R01NS115845
NIH Grant R25HD105583
Michael J. Fox Foundation's PPMI – award #8283.03

Title: Increasing data accessibility through augmented reality and web technology - Schol-AR

Authors: *T. ARD[1], M. S. BIENKOWSKI[1,2], J. STANIS[1], C. O'DRISCOLL[1], S.-L. LIEW[3,4], A. W. TOGA[1];
[1]USC Stevens Neuroimaging and Informatics Inst., Los Angeles, CA; [2]USC Zilkha Neurogenetic Inst., Los Angeles, CA; [3]USC, USC, Los Angeles, CA; [4]USC Chan Div. of Occup. Sci. and Occup. Therapy, Los Angeles, CA

Abstract: The multidisciplinary field of neuroscientific research utilizes many forms of data, such as MRI scans, 3D surfaces, image stacks, and more. While these forms of data are typically digitally viewed throughout the course of research with interactive software, they are reduced to 2D static figures for publication due to the limitations of print. Many efforts have been made to address this widely acknowledged limitation, perhaps most notably being the practice of providing digital supplementary materials alongside manuscripts. Unfortunately, while this technique is broadly established, median views and downloads of supplementary materials have been measured below .04% that of their principal articles[1], indicating these materials do not substantially impact the way the general readership views scientific information. Ultimately, the enduring discrepancy between the digital tools we use to conduct research and the printable mediums we use to communicate it hinders the effective communication of the complex information that underlies modern neuroscientific research. Here, we demonstrate advances to the Schol-AR framework, a software tool that enables the inclusion of data directly into scientific articles, posters, and other mediums as mobile augmented reality and browser-integrated augmentations. The goal of this framework is to improve the accessibility of scientific data in articles and other mediums, supporting both more thorough reporting of data as well as more comprehensive digital representations[2]. Here, we demonstrate how the framework's capabilities can now directly support improved augmentation of volumetric data, video information, and 3D models as well as improved implementation of direct links which open both an article and its associated data simultaneously. All augmentation capabilities function within standard PDF documents and are openly accessible to both authors and readers. Overall, our software framework continues to support the inclusion and viewing of data in scientific articles, ultimately aiming to improve the state of scientific communication.
1. Flanagin, A. *et al*. Editorial Evaluation, Peer Review, and Publication of Research Reports With and Without Supplementary Online Content. *JAMA* 319, 410 (2018).

2. Ard, T. *et al*. Integrating Data Directly into Publications with Augmented Reality and Web-Based Technologies - Schol-AR. *Nature Scientific Data* 298 (2022).

Disclosures: **T. Ard:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ardist Inc. **M.S. Bienkowski:** None. **J. Stanis:** None. **C. O'Driscoll:** None. **S. Liew:** None. **A.W. Toga:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

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Program #/Poster #: PSTR378.18/XX15

Topic: I.07. Data Analysis and Statistics

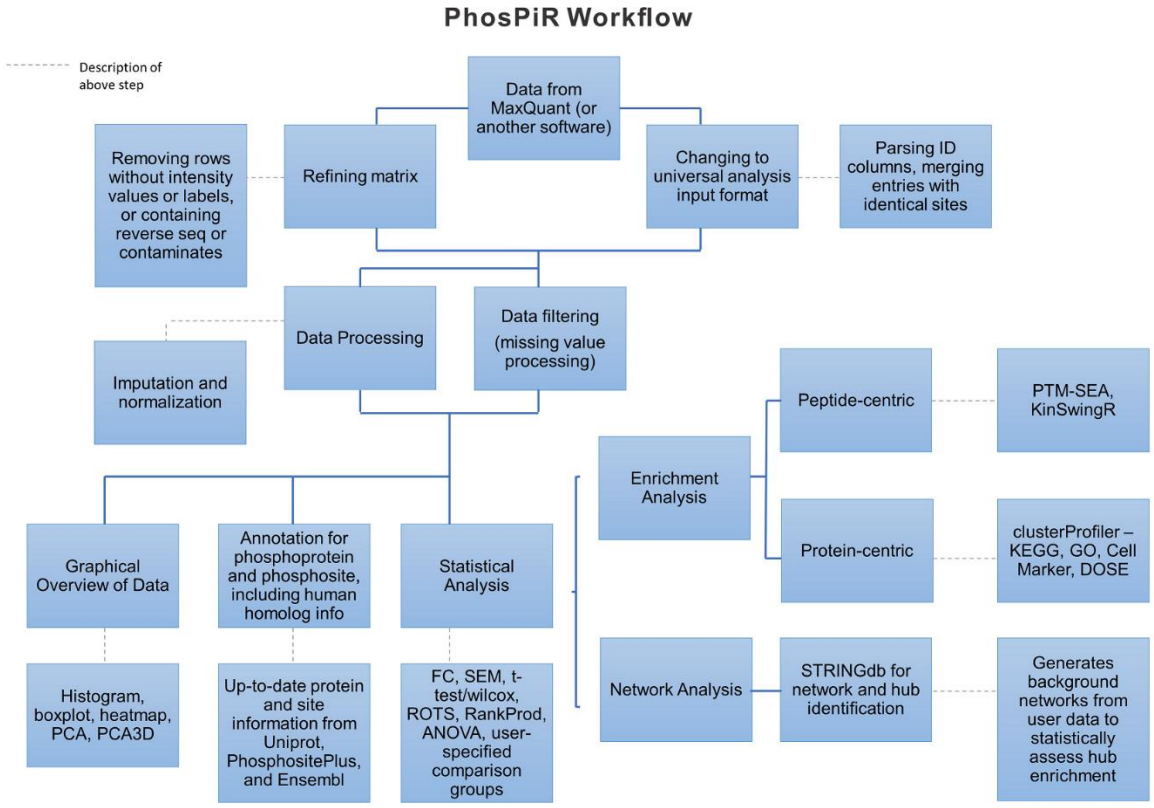
Support: Business Finland Grants (1817/31/2015 and 1545/31/2019)
Michael J Fox Foundation Grant 008489
Academy of Finland Grant 26080953
Academy of Finland Grants (310561 and 335611)
MATTI graduate school funding

Title: Phospir: an automated pipeline for proteomics and phosphoproteomics analysis

Authors: *Y. HONG[1,3,4], D. FLINKMAN[3,5], T. SUOMI[1,4], S. PIETILÄ[1,4], L. ELO[1,4], P. JAMES[3,5], E. COFFEY[3,4,2];
[1]Univ. of Turku, TURKU, Finland; [2]Univ. of Turku, Turku, Finland; [3]Åbo Akademi Univ., Turku, Finland; [4]Turku Biosci. Ctr., Turku, Finland; [5]Lund Univ., Lund, Sweden

Abstract: Phosphoproteomics, the large-scale study of proteins' phosphorylated derivatives via mass spectrometry (MS), provides essential insights for understanding disease biology and advancing drug discovery. The process of transforming this raw data into biologically meaningful interpretations, however, can be an arduous task requiring multiple analysis platforms that are not adapted for automated high-dimensional data analysis. We present PhosPiR, an integrated pipeline developed to streamline this process. PhosPiR employs an ensemble of R packages for comprehensive phosphoproteomics (and proteomics) data analysis, and its functionality is enhanced by the seamless integration with extant databases and knowledge resources. In a single run, PhosPiR performs data clean-up, rapid data overview, diverse statistical testing, differential expression analysis, phospho-site annotation, cross-species annotation and translation, multi-level enrichment analyses, proteome-wide kinase activity mapping, substrate mapping, and network hub analysis. The output includes tables, information files, and visual formats such as heatmaps, box plots, volcano plots, and circos plots. On average, the pipeline generates over 100 result files and figures from a single dataset, without counting annotation files. PhosPiR is designed to facilitate proteome-wide data mining of pathophysiological mechanisms without requiring programming knowledge from the user. As a demonstration of its utility, PhosPiR was applied to identify regulators of the sleep/wake cycle

and sleep deprivation stress in synaptic terminal preparations from a mouse brain. This identifies known and novel regulators, thereby validating the pipeline's utility while at the same time contributing to discovery. We anticipate that PhosPiR will provide researchers with limited programming expertise with a user-friendly means to conduct comprehensive functional prediction analysis on their proteomics and phosphoproteomics data with robust statistical support.



Disclosures: **Y. Hong:** A. Employment/Salary (full or part-time):; Åbo Akademi University, University of Turku, MATTI graduate school. **D. Flinkman:** A. Employment/Salary (full or part-time):; Åbo Akademi University. **T. Suomi:** None. **S. Pietilä:** None. **L. Elo:** 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.; Academy of Finland. **P. James:** None. **E. Coffey:** 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.; Business Finland, Michael J Fox Foundation, Academy of Finland.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.19/XX16

Topic: B.01. Transmitters, Transporters, and Other Signaling Molecules

Support: NINDS IRP

Title: A MATLAB toolbox for analyzing calcium imaging data in vitro and in vivo

Authors: *N. S. DESAI, C. ZHONG, R. KIM, D. A. TALMAGE, L. W. ROLE;
NINDS, Bethesda, MD

Abstract: Calcium imaging of neuronal populations has long been an important tool in neuroscience. Over the years, the uses and potential of this tool have expanded dramatically - mostly because of advances in hardware, but also because of advances in analysis. The analytical advances have included the introduction of sophisticated mathematical techniques, such as non-negative matrix factorization (NNF) and the Wasserstein metric, and a variety of open-source and commercial software projects designed to exploit the sophisticated techniques and incorporating the full range of programming environments and languages. Today, the result is a profusion of options: different methodologies for detecting activity; some efforts focused on cell identification, others on characterizing population activity; some requiring great technical sophistication from users, others with more modest requirements. Into this space, we offer - as an option - software we developed in our laboratory to aid in our analysis of population calcium data collected in vitro (brain slices) and in vivo (microendoscopic imaging). In developing this software, we were guided by three requirements: easy-to-use software, built around a simple graphical user interface and that makes minimal technical demands of users (i.e., no coding); a complete analysis workflow compatible with multiple sources of data and that encompasses cell detection, population characterization, and figure preparation; and "future proofing," by which we mean software that can easily be expanded to incorporate new techniques or to exploit new software products. The typical workflow consists of: (1) reading data saved as TIFF, AVI, MPEG, or other standard formats; (2) detecting active neurons using constrained NNF, with guidance on parameter choice; (3) quality control, both manual and automated; (4) analysis of population characteristics, including pairwise correlations and Earth Mover's Distance; (5) export of analysis numbers to the generic CSV format; and (6) figure preparation of commonly-employed parameters and using easily-modified formats (e.g., scalable vector graphics, SVG). We built our software as a MATLAB toolbox, but we will also create a non-proprietary standalone version.

Disclosures: **N.S. Desai:** None. **C. Zhong:** None. **R. Kim:** None. **D.A. Talmage:** None. **L.W. Role:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.20/XX17

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

Support: RF1MH130391
U01NS128537
R01GM139850

Title: Seavue: a high-throughput image analysis pipeline for standardized extraction of time-series fluorescence signals from genetically encoded biosensors

Authors: *A. MOGHADASI[1], S. ZUNIGA[1], J. LEE[2], S. WAIT[2], A. BERNDT[1];
[1]Bioengineering, [2]Mol. Engin. and Sci., Univ. of Washington, Seattle, WA

Abstract: The development of computational tools to streamline and automate the analysis of in-vitro cell images is a crucial step to account for the growing use of fluorescent microscopy to study various dynamic neurological processes over multiple time-spans. Current methods for data extraction are characterized by lengthy and ambiguous procedures that vary among scientists. As a result, image analysis often lacks reproducibility.
To solve this problem, we generated a versatile and standardized image analysis pipeline to accelerate this process. SEAVue integrates and combines advanced image processing algorithms and machine learning techniques with a highly accessible and user-friendly interface placed on Google Colaboratory. SEAVue consists of a segmentation component, compatible with Omnipose and Cellpose - deep-learning methods with potential for specialized cell-type segmentation. This flexibility provides an adaptable and customizable system for a wide range of imaging datasets. Supplemented with a cell identification component, we aim to account for the temporal connectivity of cells across time-lapse fluorescence images. This component consists of unsupervised training of cell visual features that are cell-specific and carry that information across frames. Ultimately these components yield an exhaustive list of Regions of Interest (ROIs) that define well-segmented and fully-connected cells across an image dataset. As a result, this pipeline combines methods for efficient data extraction that allows for high-confidence data analysis and visualization.
To validate the performance of SEAVue we generated time-resolved image datasets from mammalian cells expressing genetically encoded fluorescent biosensors for calcium, oxidative stress, and neuromodulators. Our pipeline reliably increased identified ROI numbers with accurate segmentation and proper account for cell movement across frames. Furthermore, our analysis suggests more consistent measurements for the intrinsic properties of biosensors such as expression, response amplitudes, and kinetics.
SEAVue aims to present a standardized, user-friendly method for the analysis of in-vitro cell images that allows for high-confidence data extraction and analysis. It supports batch processing of large datasets, adaptability to different datasets, and reduced manual efforts. SEAVue is an open source tool, accessible through a cloud-based service, and without the need for extensive computational resources. These features would facilitate widespread use, allow for efficient data analysis, and promote reproducibility.

Disclosures: **A. Moghadasi:** None. **S. Zuniga:** None. **J. Lee:** None. **S. Wait:** None. **A. Berndt:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.21/XX18

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

Support: NIH R24-MH-114793

Title: The Brain Image Library

Authors: M. KENNEY[1], G. HOOD[1], A. WETZEL[1], I. CAO-BERG[1], L. TUITE[1], I. VASYLIEVA[3], A. WATSON[3], *A. ROPELEWSKI[2,4];
[1]Pittsburgh Supercomputing Ctr., [2]Carnegie Mellon Univ., Pittsburgh, PA; [3]Ctr. for Biol. Imaging, Univ. of Pittsburgh, Pittsburgh, PA; [4]Pittsburgh Supercomputing Ctr., Pittsburgh, PA

Abstract: With the growing size and complexity of microscopy and neuroimaging datasets, it has become essential for investigators to have easy access to resources that allow them to interact with and compute on these datasets. Average dataset size has increased and handling that size and amount of data presents unique challenges. In response to this need, the Brain Image Library (BIL) aims to overcome barriers to data reuse and secondary analysis by offering computational resources and visualization tools that directly access large datasets. While there are several storage options for brain microscopy datasets, the Brain Image Library stands out by providing users with state-of-the-art resources, enabling them to easily search for and interact with imaging datasets without the need to download them.

The Brain Image Library serves as a repository dedicated to facilitating access to brain microscopy data for the neuroscience community. Its primary objective is to provide persistent and centralized access to valuable brain data, enabling researchers to reanalyze and explore the data for novel research purposes. BIL is comprised of over 7,000 datasets, each ranging from gigabytes to hundreds of terabytes in size. With petabytes of primary imaging data, BIL currently houses more than 133 million public files that are readily accessible for reuse. Researchers can find data from various imaging methods such as confocal microscopy, two-photon microscopy, lightsheet microscopy, fluorescent MOST, injection tracing, cell counting, Brainbow, Spatial FISH (e.g., MERFISH), multi-modal techniques (e.g., Patch-seq), and many others. By encompassing a broad spectrum of imaging modalities, researchers have access to a wide range of data that may suit their specific research needs.

The Brain Image Library offers an integrated Analysis Ecosystem consisting of a computational cluster and a VM system equipped with modern GPUs that facilitates exploration, visualization, and seamless access to its data without the need for downloading. To support computationally intensive tasks, BIL provides direct access to its data on the Bridges-2 Platform, a high-performance computing system. This integration allows researchers to leverage these computational resources and tools available along with the BIL data. Among the resources provided, researchers can utilize Open OnDemand, which enables computation on BIL data using popular environments such as JupyterLab or RStudio. Additionally, remote desktop applications are accessible, providing a user-friendly interface for interacting with the data.

Disclosures: **M. Kenney:** None. **G. Hood:** None. **A. Wetzel:** None. **I. Cao-Berg:** None. **L. Tuite:** None. **I. Vasylieva:** None. **A. Watson:** None. **A. Ropelewski:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.22/XX19

Topic: E.02. Cerebellum

Support: Wellcome Trust-DBT India Alliance Intermediate Fellowship 500040/Z/09/Z
Wellcome Trust-DBT India Alliance Senior Fellowship IA/S/17/2/503297
Department of Biotechnology BT/PR4983/MED/30/790/2012
Science and Engineering Research Board, Department of Science and Technology EMR/2015/000595

Title: A machine learning tool to identify bistable states from calcium imaging data

Authors: *A. VARMA[1], S. UDUPA[2], M. SENGUPTA[3], P. GHOSH[2], V. THIRUMALAI[4]; [1]Natl. Ctr. for Biol Sci., Bangalore, India; [2]Indian Inst. of Sci., Bangalore, India; [3]Washington University, Sch. of Med., Washington University, Med. Sch., Saint Louis, MO; [4]Natl. Ctr. For Biol. Sci., Bangalore, India

Abstract: The use of calcium imaging to map neuronal activation during behavioral tasks has significantly advanced our knowledge of nervous system function. However, current methods primarily focus on inferring spike probabilities and overlook the intrinsic dynamics of neurons, such as bistable membrane potential states. As a result, there is a lack of tools capable of converting calcium imaging signals into cellular state maps for bistable neurons.
Purkinje neurons (PNs) in the cerebellum are a prime example of neurons exhibiting membrane potential bistability. They can fire either tonically or in bursts and possess two distinct types of electrical activity, each contributing to intracellular calcium influx. The presence of bistability introduces complexity to the population code. Regrettably, no existing tool enables the conversion of these signals into maps of cellular state in bistable neurons.
To address this challenge, I developed CaMLsort, a novel tool specifically designed to classify the cellular state of PNs in larval zebrafish using only their calcium signals. CaMLsort employs a convolutional recurrent neural network (CNN-LSTM) trained on a simulated dataset with labeled states, and exhibits high classification accuracies when tested on real imaging data. Remarkably, despite being trained exclusively on zebrafish PNs, CaMLsort generalises well to other systems. The introduction of CaMLsort offers researchers the means to investigate the network and behavioral effects of the bistability population code. By leveraging this tool, we anticipate significant advancements in our understanding of the role played by bistability and its impact on neuronal function and behavior.

Disclosures: **A. Varma:** None. **S. Udupa:** None. **M. Sengupta:** None. **P. Ghosh:** None. **V. Thirumalai:** None.

Poster

PSTR378. Software Tools: Microscopy and Imaging

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR378.23/XX20

Topic: I.07. Data Analysis and Statistics

Title: Experience in Imaging Data Sharing with the ENIGMA Consortium: Insights and Collaborative Opportunities

Authors: *I. BRAMATI, P. MATTOS, T. MONTEIRO, M. MONTEIRO, F. MEIRELES, **F. TOVAR-MOLL**;
D'Or Inst. For Res. and Educ., Rio De Janeiro, Brazil

Abstract: Introduction: Data sharing is crucial for advancing research and collaboration in neuroimaging. The ENIGMA Consortium (Enhancing Neuro Imaging Genetics through Meta-Analysis) aims to unravel the intricate relationship between brain structure, function, and disease using advanced neuroimaging techniques. In this abstract, we share our experiences with data sharing in ENIGMA, highlighting valuable insights and collaborative opportunities. Methods: ENIGMA employs standardized data processing protocols and analytic workflows for cross-consortia initiatives. By pooling brain imaging data collected from multiple research centers, ENIGMA conducts collaborative large-scale meta- and mega analyses. Methodological harmonization, standardized scripts, and quality control ensure reliable results. We collaborated by sharing magnetic resonance imaging (MRI) data from D'Or Institute for Research and Education (IDOR), covering various brain-related disorders and control groups, including ADHD, OCD and ASPD. We shared only the results derived from standardized scripts, accessed using state-of-the-art image processing software on our IDOR infrastructure, not the imaging data files. We followed rigorous data anonymization and ethical guidelines from ENIGMA. Results: Collaborating with ENIGMA yielded valuable insights. Sharing our imaging data increased statistical power and generalizability. Larger sample sizes and diverse populations enabled robust results. Collaboration expanded research questions and integrated imaging data with other studies, enhancing understanding of brain structure, function, and disorders. The collaborative environment fostered scientific discussions, providing unique perspectives and interdisciplinary collaborations. Conclusion: Our data sharing experience in ENIGMA has been highly valuable. Collaborating and sharing imaging data improved our understanding of brain structure, function, and disorders. Sharing only results from standardized scripts and protocols ensures data privacy. ENIGMA provides a platform to share data, develop analysis protocols, and uncover new insights. By sharing our experiences, challenges, and solutions, we aim to advance discussions, foster collaboration, and contribute to ethical data governance frameworks for brain and health data.

Disclosures: **I. Bramati:** None. **P. Mattos:** None. **T. Monteiro:** None. **M. Monteiro:** None. **F. Meireles:** None. **F. Tovar-Moll:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.01/XX21

Topic: I.08. Methods to Modulate Neural Activity

Support: NIWC and DARPA Support N65236-19-C-8017

Title: An ex vivo model for non-invasive brain stimulation utilizing perpendicular planes of electrodes

Authors: *Y. LEE, V. JAIN, P. GROVER, M. FORSSELL;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Non-invasive electrical brain stimulation shows promising clinical applications, but is mostly limited to targeting superficial cortical regions. Generating electric fields far from electrodes is a challenge for planar electrode arrangements. However, human heads contain orthogonal surfaces on which electrodes can be placed, and some minimally invasive electrodes can be implanted to target the deep brain transnasally, opening up many possible configurations with uncommon electrode positions. Studying the resulting electric fields is usually done in simulation, but the effect of these fields on neural tissue has not been investigated.In this study, we developed an ex vivo setup to study the neural response of brain slices to electric fields from electrodes in perpendicular arrangements. The setup is schematically shown in Figure 1a. Electrode patterns to create electric fields on the slice were chosen, using electrode pairs on the same planar patch (either horizontal or vertical), or pairs using one electrode from each patch. The neural response was measured in cortical layer 5 with the distance between each patch and the stimulation target kept identical, and varied from 2 mm to 5 mm. Neural responses to stimulation were characterized by the amplitude of the fiber volley in the evoked local field potentials. The responses across pairs were also compared at the same injected current.The experiment reveals that at 2 mm distance from each patch, current thresholds for pairs of electrodes on the vertical patch were lower than for pairs on the horizontal patch. Moreover, the response amplitudes when injecting the same currents were 3.5 x larger for vertical pairs than horizontal pairs (Figure 1b). The electric fields for vertical pairs performed in simulation were 1.9 x and 2.5x greater than horizontal pairs, supporting the experimental results. At distances larger than 2 mm, only electrode combinations using both patches could stimulate the slice. The orthogonally placed electrode potentially can be utilized to stimulate the deep brain non-invasively.

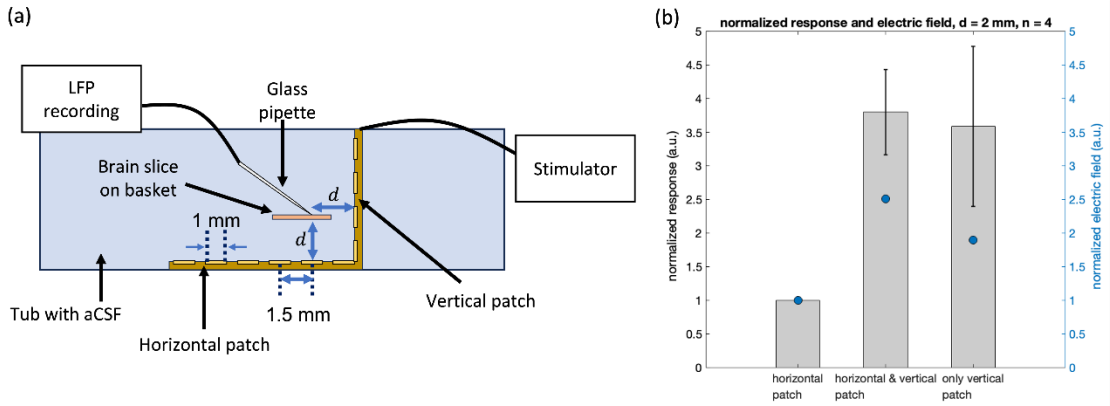


Figure 1. Ex vivo model setup and the main result. (a) Schematic illustration of the entire setup. Inside the tub filled with aCSF, the electrode patches, composed of 4x5 electrodes of 1 mm diameter in a grid at 1.5 mm pitch, were placed perpendicularly and connected to a current stimulator. The brain slice was suspended in a nylon-mesh basket allowing it to be moved with respect to the stimulation patches, modeling different locations in the brain. The distance (denoted as d) between each patch and the stimulation target in cortical layer 5 was kept identical, and varied from 2 mm to 5 mm. The glass pipette carefully touched the slice and measured local field potentials evoked by stimulation. Pairs consisting of electrodes on the same planar patch (either horizontal or vertical), or pairs using one electrode from each patch, were used to create electric fields on the slice. (b) Neural responses and simulated electric fields for d=2 mm. Both values are normalized to the value from the horizontal electrode pair (mean response V= 0.45 mV; field E=708 V/m). The bar graph shows the mean and standard deviation (N=4 slices from one mouse), and the normalized electric fields are marked by blue circles.

Disclosures: **Y. Lee:** None. **V. Jain:** None. **P. Grover:** None. **M. Forssell:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.02/XX22

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF Grant ECCS-1935841

Title: Enhanced In Vitro Stimulation of Neurons Using Updated Core-Shell Magnetoelectric Nanoparticles

Authors: ***M. SHOTBOLT**[1], E. ZHANG[2], A. SCOTT-VANDEUSEN[2], P. LIANG[3], S. KHIZROEV[2];
[1]Univ. of Miami, Miami, FL; [2]Univ. of Miami, Coral Gables, FL; [3]Cell. Nanomed, Irvine, CA

Abstract: Existing brain stimulation technologies provide an essential pathway for current and future treatments for Parkinson's and epilepsy, amongst other things. However, these technologies, such as deep brain stimulation and transcranial magnetic stimulation, have limitations in terms of invasiveness, spatial resolution, and stimulation depth. Magnetoelectric nanoparticles (MENPs) have emerged as a promising alternative for non-invasive and targeted brain stimulation. This study aims to present an improved nanoparticle formulation that enhances

neuron stimulation in vitro. We have iterated on previous fabrication techniques of MENPs with enhanced magnetoelectric properties and assessed their ability to stimulate neurons in vitro. By altering particle PEGylation from previous iterations, we were able to reduce the distance between the particles and the neuronal membrane while maintaining dispersion in solution. Additionally, by reducing the rate of crystal formation in the particle core, we enhanced the crystal structure, and thus the magnetoelectric effect of the particles, thereby generating stronger electric fields. The modified MENPs were compared to previous generations of MENPs in terms of their efficiency in evoking neuronal responses. Our updated MENPs formulation demonstrated a significant enhancement in neuron stimulation compared to earlier generation MENPs. The novel nanoparticles were able to evoke fast and reliable neuronal responses in vitro, indicating their potential for effective brain stimulation. The MENPs generated an estimated response rate of 97.16% of neurons as demonstrated by calcium fluorescence under magnetic stimulation. During concurrent particle treatment and magnetic stimulation, mean neuron firing intervals were reduced significantly compared to baseline firing ($p=0.00152$). The improved MENPs also addressed some of the limitations of existing brain stimulation technologies, such as invasiveness and spatial resolution. The updated nanoparticle formulation presented in this study offers a promising development for wireless in vitro stimulation of neurons using MENPs. Our findings suggest that the enhanced MENPs have the potential to overcome the limitations of current brain stimulation technologies and provide a more effective non-invasive method for stimulating neurons. Further research is needed to validate these results in vivo and explore the potential applications of this technology in the treatment of neurological disorders and the study of brain-machine interfaces.

Disclosures: **M. Shotbolt:** None. **E. Zhang:** None. **A. Scott-Vandeusen:** None. **P. Liang:** None. **S. Khizroev:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.03/XX23

Topic: I.08. Methods to Modulate Neural Activity

Title: Wireless deep brain stimulation with magnetoelectric nanoparticle-based neuromodulation approach

Authors: *C.-C. CHENG;
Inst. of Biomed. Engin., Natl. Yang Ming Chiao Tung Univ., Hsinchu City, Taiwan

Abstract: Manipulating neuronal activity in the central nervous system through electrical stimulation is crucial for clinical treatment of neurological disorders and for basic neuroscience research. Traditional electrode implantations might carry the risk of complications, infection, and damage caused by micromovements during daily activities. Recently, accumulating researches are focusing on developing neuromodulation technologies with less invasiveness. Multiple

magnetoelectrical nanoparticle-based neuromodulation approaches have been developed in last decade. However, the requirement of alternating magnetic field (AMF) at low intensity with high frequency or high intensity with low frequency limits the scale of magnetic apparatus, which cannot be further adapted into behavioral experiments, larger animals or future clinical treatments. Therefore, the magnetoelectrical nanoparticle-based neuromodulation approach with scalable magnetic apparatus is necessary. Here, we demonstrate a magnetoelectrical nanoparticle-based neuromodulation approach with low-intensity and low-frequency AMF to induce neuronal activation *in vitro* and in awake mice *in vivo*. In magnetoelectrical stimulation, magnetic field cannot be directly converted into electrical field. The mechanical force is initially generated by superparamagnetic nanomaterials with AMF application. Subsequently, the mechanical force is converted into electric field via piezoelectric nanoparticles. In this study, the magnetoelectrical nanoparticle-based neuromodulation approach can induce neuronal activity by directly activating voltage-gated sodium channel but not by activating intrinsic mechano-sensitive ion channel in neurons. The parameters of the magnetoelectrical stimulation were further optimized in hippocampal cultured neurons *in vitro*. Finally, we demonstrate that this magnetoelectrical stimulation can induce neuronal activity wirelessly in the deep brain region of awake mice *in vivo*. In conclusion, our wireless magnetoelectrical nanoparticle-based neuromodulation approach with low-intensity and low-frequency AMF can modulate neuronal activity *in vitro* and *in vivo*, which can be performed in a scalable magnetic platform. Therefore, this minimal-invasive untethered DBS approach has a great potential for applying in basic neuroscience researches and for future translational applications.

Disclosures: C. Cheng: None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.04/XX24

Topic: I.08. Methods to Modulate Neural Activity

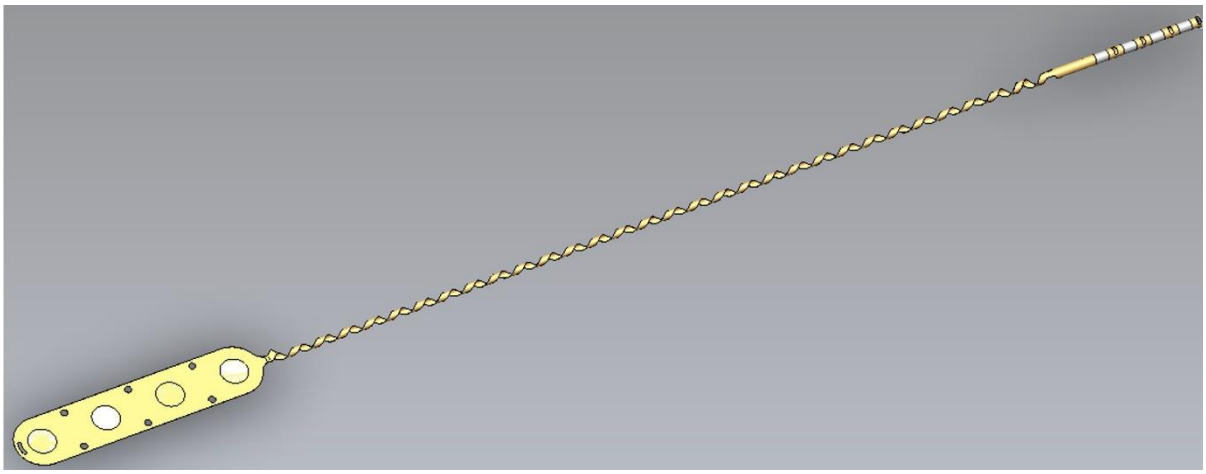
Support: NIH Grant R21MH120810

Title: Prototype Custom Flexible Leads Compatible with Chronic Neuromodulation IPGs

Authors: E. GRAF[1], M. TRIPLETT[1], H. E. DAWES[2], P. A. STARR[3], ***R.-U. HAQUE**[1]; [1]Lawrence Livermore Natl. Lab., Livermore, CA; [2]Neurolog. Surgery, UCSF, OAKLAND, CA; [3]Dept. of Neurol., Univ. of California, San Francisco, san francisco, CA

Abstract: Neurostimulation using invasive leads is a promising potential therapy for a variety of neural diseases, including psychiatric diseases. Short term invasive recordings have helped identify potential physiological biomarkers for specific psychiatric symptoms such as depression and anxiety. Further neural circuit analysis and biomarker discovery will be further accelerated with the fabrication of prototype custom-designed highly-flexible chronically-implanted neural

interfaces that can both deliver stimulation and recording capability. Here, we designed, fabricated, and demonstrated flexible arrays that can be directly connected to existing, FDA-cleared neurostimulation devices with wireless streaming capability. The lead connects to a Medtronic lead extender as a demonstration of how such custom leads can be made to work with chronically implanted neurostimulators. This approach could be used for other manufactures like Neuropace and is agnostic to the lead connecter interface and can be custom-made for virtually any approach. We believe these leads will address a significant need compared to those currently provided by manufacturers directly. First, researchers are typically limited to what manufacturers currently have available in production and do not typically make special, one-off leads. Additionally, there are also limitations in production availability once those parts go out of production, which can severely impact ongoing studies. In addition to these manufacturing limits, there are also technical limitations as well, including mechanical rigidity which could preclude access to certain parts of the brain. Here we address this by tuning the rigidity to the specific needs of the application. Spatial resolution (currently limited to 1cm spacing) and low channel count (4 contacts) are two other areas researchers can specify and modify with our manufacturing process to a maximum available in the neurostimulator (typically 8). and not limited by the available leads.
Prepared by LLNL under Contract DE-AC52-07NA27344.



Disclosures: E. Graf: None. **M. Triplett:** None. **H.E. Dawes:** None. **P.A. Starr:** None. **R. Haque:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.05/XX25

Topic: I.08. Methods to Modulate Neural Activity

Support: R01NS110823
GRANT12635723
GRANT12635707
Eugene McDermott Graduate Fellowship 202108

Title: Evaluation of Platinum/Iridium Microwire Arrays for Chronic Multi-Channel Stimulation of Rat Somatosensory Cortex

Authors: *T. SMITH[1], Y. WU[2], H. SRINIVASAN[3], F. FARUK[3], M. KAUL[4], A. A. KHAN[3], J. R. CAPADONA[6], S. F. COGAN[5], A. G. HERNANDEZ-REYNOSO[5], J. J. PANCRAZIO[5]; [1]Neuroscience/Bioengineering, [2]Materials Sci. and Engin., [3]Neurosci., [4]Biomed. Engin., [5]Bioengineering, Univ. of Texas at Dallas, Richardson, TX; [6]Biomed. Engin., Case Western Reserve Univ., Cleveland, OH

Abstract: Electrical stimulation of the somatosensory cortex via penetrating microelectrode arrays has emerged as a tool for restoring proprioceptive sensations in individuals with sensory loss. However, the current amplitudes required for producing sensory percepts chronically have been shown to change over time. This is partly due to multi-factorial failures at the neural interface observed during chronic in-vivo applications. Failures involving electrode de-insulation or gliosis alter impedance, while changes in charge storage capacity impact applied voltage magnitudes. In this study, we investigate the electrochemical changes at the neural interface for microwire arrays and highlight their stability for chronic multi-channel stimulation of rat somatosensory cortex. We implanted 3 rats with 12-channel Pt/Ir microwire arrays and monitored their weekly electrochemical properties alongside perception thresholds in a go/no-go behavioral paradigm. In the established paradigm, animals received current-controlled stimulus pulse trains delivered identically through 10 channels simultaneously. For electrochemistry, we used a 3-electrode configuration to measure the electrochemical impedance spectroscopy against Ag/AgCl and recorded 15 µA single-channel voltage transients against a transcranial stainless-steel screw to assess the maximum cathodal potential excursion (E_{mc}). Finally, we utilized non-linear regression to estimate perception thresholds, and linear regression to assess the stability of both thresholds and device electrochemistry up to 31 weeks post-implantation. The animals produced an average perception threshold of 1.9 nC/ph (9.5 µA) with a non-significant slope from zero ($p = 0.19$) of -0.05 nC/ph bi-weekly, indicating perceptual stability. Electrochemistry results displayed an increase in the slope of 0.5 mV/week ($R^2 = 0.7$) for average E_{mc}, while the 1 kHz impedance exhibited a decrease of -0.7 mΩ/week ($R^2 = 0.4$). Both slopes were statistically significant from zero ($p < 0.01$). Electrochemical analysis revealed a slight increase in E_{mc} and a decrease in impedance suggesting de-insulation. However, the demonstration of stable perception thresholds across the chronic period signifies that the arrays could produce consistent stimuli with negligible changes in material properties. Overall, preliminary data from this study demonstrates why Pt/Ir microwire arrays should be considered for chronic stimulation studies in rat somatosensory cortex. Future experiments will compare stimulation-evoked perception thresholds and performance against ultra-flexible silicon-based arrays.

Disclosures: **T. Smith:** None. **Y. Wu:** None. **H. Srinivasan:** None. **F. Faruk:** None. **M. Kaul:** None. **A.A. Khan:** None. **J.R. Capadona:** None. **S.F. Cogan:** F. Consulting Fees (e.g., advisory boards); Qualia Oto.. **A.G. Hernandez-Reynoso:** None. **J.J. Pancrazio:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.06/XX26

Topic: I.08. Methods to Modulate Neural Activity

Support: the MOST Grant 2022ZD0208604
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NSF of China grant 31771195
NSF of China grant 81790640
NSF of China grant 81773513
NSF of China grant 820712002
NSF of China grant 32100803

Title: Nanowire based artificial photoreceptors restore visual function in blind mice and monkeys

Authors: R. YANG[1], P. ZHAO[1], L. WANG[2], C. FENG[2], C. PENG[1], G. ZHENG[1], C. JIANG[3], Y. YUAN[2], ***B. YAN**[1], J. ZHANG[1];
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Abstract: Photoreceptor degeneration caused by retinitis pigmentosa (RP) or age-related macular degeneration (AMD) is a major cause of blindness. However, the remaining retinal cells and their brain projections are still functional by the disease. Retinal prosthesis using photovoltaic devices or materials has potential for restoring vision. Here, we developed gold (Au) nanoparticle-coated titania nanowire arrays and attached with ex vivo blind retina, achieving spatial and temporal resolution of 77.5 μm and 3.92 Hz respectively. The blind mice with nanowire arrays subretinally implanted enabled detection of drifting gratings and flashing objects at low light thresholds and exhibited visual acuity of 0.3 - 0.4 cpd in visually-evoked potentials and optomotor tests. Moreover, long-term in vivo calcium imaging indicated plasticity in visual cortical circuits after nanowire implant. The nanowire arrays showed good biocompatibility and stability for 54 weeks after subretinal implantation in monkeys, which could detect an LED of 0.5º in diameter at 10 μW·mm^-2 in visually-guided saccade experiments. Our results demonstrate the feasibility of nanomaterials as artificial photoreceptors to ameliorate visual impairments in patients with photoreceptor degeneration.

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Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.07/XX27

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF ECCS-2211082
DARPA

Title: Antibody-conjugated magnetoelectric nanoparticles for enhanced wireless brain stimulation

Authors: *V. ANDRE[1], M. ABDEL-MOTTALEB[2], Y. AKIN[3], S. CHEN[4], E. ZHANG[2], M. SHOTBOLT[5], X. JIN[7], P. D. GANZER[6], B. R. NOGA[8], P. LIANG[9], S. KHIZROEV[2]; [1]Biomed. Engin., Univ. of Miami, Miami, FL; [2]Univ. of Miami, Univ. of Miami, Coral Gables, FL; [3]Electrical and Computer Engin., Univ. of Miami, Miami, FL; [4]Chemical, Envrn. & Materials Engin., [5]Biomed. Engin., Univ. of Miami, Coral Gables, FL; [6]Univ. of Miami, Univ. of Miami, Miami, FL; [7]Indiana Univ. Sch. of Med., Purdue Univ., Indianapolis, IN; [8]Univ. of Miami Sch. of Med., Univ. of Miami Sch. of Med., Miami, FL; [9]Cell. Nanomed, Cell. Nanomed, Irvine, CA

Abstract: The use of magnetoelectric nanoparticles (MENPs) for neural stimulation shows great promise as a minimally invasive approach for modulating neural activity. Of these nanoparticles, $CoFe_2O_4$@$BaTiO_3$ core-shell nanostructures possess a remarkable magnetoelectric effect. This enables the generation of powerful electric fields in close proximity to the MENPs from the application of a remote magnetic field. However, the main obstacle in using these nanoparticles for neural stimulation is the limited efficiency of activation, largely due to the fact that the majority of MENPs are not in contact with the neuronal membrane at the time of stimulation. In our research, we tackle this challenge by developing magnetoelectric nanoparticles conjugated with anti-GluR2 antibody that can selectively bind to the AMPA GluR2 glutamate receptors in neuron dendrites. Our findings demonstrate considerably improved neuron stimulation efficiency, in comparison to MENPs without antibody conjugation. This advancement holds significant potential for the development of accurate and effective techniques in remotely modulating neural activity for clinical applications in DBS, Parkinson's disease, and brain-computer interfaces.

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Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

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Topic: I.08. Methods to Modulate Neural Activity

Support: Internal Department Funding

Title: Minimally-invasive electric stimulation techniques to target hippocampal theta in the urethane-anesthetized rat

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Abstract: Electrical brain stimulation shows significant potential for studying normal and treating abnormal brain function, owing to the electrical nature of the brain. Non-invasive techniques, while attractive due to their affordability, ease of use, and low-risk nature, have limited effectiveness and mechanisms that are not well understood. Moreover, their ability to target deep brain regions is restricted, often affecting only surface areas. A novel technique, Temporal Interference (TI), has emerged as a promising method for reaching deeper targets. In this study, we used a virtual rat head model to optimize the locations for skull-mounted electrodes to stimulate the hippocampus. The hippocampus is a deep brain region that spontaneously generates theta, which is the brain's largest amplitude rhythm, associated with learning, memory, and has possible antiepileptic effects. We employed a range of stimulation techniques and observed their effects on brain rhythms. Our results indicated that all stimulation methods, in a stimulus intensity-dependent manner, increased the likelihood of spontaneous theta, as well as the frequency and power of theta at the onset of the stimulus, which persisted after the stimulus. TI and amplitude-modulated (AM) stimulation generated phase-locked theta to the wave envelope of the stimulation. This study is significant in advancing our understanding of electrical stimulation techniques for deep brain modulation, potentially leading to safe, low-cost, non-invasive treatments or experimental tools that can target previously inaccessible brain structures.

Disclosures: B. Tessler: None. **S.E. Fox:** None.

Poster

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Program #/Poster #: PSTR379.09/XX29

Topic: I.08. Methods to Modulate Neural Activity

Title: Automated neural-behavioral data alignment for fully implantable DBS platforms

Authors: *M. E. ALARIE[1], N. R. PROVENZA[3], J. A. HERRON[4], W. F. ASAAD[2];
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Abstract: Sensing-enabled deep brain stimulation (DBS) devices have permitted unique insights into the correlation between neural activity and behavioral measures. Unfortunately, these sensing-enabled DBS platforms do not typically permit straightforward synchronization of neural data streams with external behavioral or environmental events. Unlike invasive, percutaneous neurophysiological research, precise synchronization of behavioral events with neural signals acquired by fully implanted devices requires novel approaches. Current idiosyncratic methods rely on manual injection of a typically single, initial, cross-channel artifact to provide a means of time-locking each data stream to the other. However, a more rigorous approach requires precisely timed, computer-driven signals injected directly into LFPs onboard bidirectional DBS devices for reliable and accurate data alignment. Here, we developed such a method for automated artifact injection via event triggered transcutaneous stimulation (TS). Specifically, we report on data collected onboard Medtronic's Percept PC™ in two patients with DBS of the subthalamic nucleus (STN) and globus pallidus internus (GPi) to treat Parkinson's Disease (PD). Task-triggered TS was transmitted through the EEG/EMG Headbox of the NeuroOmega recording system (Alpha Omega, Nazareth, Israel). We first quantified latencies within this computer-driven system by recording time differences between received task event markers and transmitted TS pulses measured by an oscilloscope (mean 2.60ms ± 2.88ms) and recorded directly from the NeuroOmega system (mean 5.12ms ± 2.79ms). Next, we provide analytic techniques for identifying TS artifacts across DBS conditions, comparing neural-behavioral alignment accuracy during DBS OFF (mean 25.6ms ± 21.8ms) and DBS ON (mean 5.6ms ± 8.5ms). Overall, we demonstrate a more rigorous approach for neural-behavioral alignment onboard fully implanted systems, reducing the need for multiple external recording streams while directly injecting behavioral events into device LFPs.

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Poster

PSTR379. New Approaches to Neural Stimulation

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH R01DC018621
NSF IOS-1942480

Title: Model predictive control of neural systems with data-driven forecasting

Authors: *C. FEHRMAN, C. MELIZA;
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Abstract: Neurons are highly nonlinear dynamical systems. While many methods exist to manipulate the activity of individual neurons and simple circuits, their biophysical complexities limit how precise this control can be. Improved control over neural activity would have numerous applications in experimental neuroscience, neuroprosthetics, and treatments for neurological disorders. One potential method for increased controllability of neural systems is the model predictive control (MPC) framework. MPC uses a mathematical model of the system to find optimal inputs to reach desired outputs. Using recent developments in data-driven forecasting, we demonstrate that relatively simple machine learning models can be used to predict and control neural responses to experimental input currents. Surprisingly, these models can be learned with limited information about the biophysics of neurons. Through simulation, we demonstrate that our framework allows for precise control of neuron spike timing in current clamp experiments.

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Poster

PSTR379. New Approaches to Neural Stimulation

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Program #/Poster #: PSTR379.11/XX31

Topic: I.08. Methods to Modulate Neural Activity

Support: NSTC 112-2321-B-A49-011-

Title: A rapid prototyping tool for closed-loop deep brain stimulatin system

Authors: *Y.-H. WU, Y.-H. OU-YANG, H.-C. LIN, C.-Y. LEE, M.-D. KER;
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Abstract: Deep Brain Stimulation (DBS) at different brain targets has been shown to be effective in the treatment of various neurological disorders such as Parkinson’s disease (PD), dystonia, essential tremor, chronic pain and epilepsy. However, studies have shown that conventional continuous DBS suppressed not only pathology but also physiological neuronal activity, which may worsen motor function. The demand of neuromodulation system is increasing in order to control neurological diseases. A closed-loop DBS system using National Instruments (NI) compatible platform is proposed to reduce power consumption and improve therapeutic efficacy for clinical research and development. The system can be connected to PD patient implanted with DBS lead for local field potential (LFP) recording. By analyzing the LFP data, observing the patient's response, and adjusting the stimulation parameters, the closed-loop control of the system is realized. The digital process and control functions in this system were designed in NI LabVIEW. It has flexible feature extracting functions and integrates physiological sensors to monitor patient symptoms. Dynamic threshold control based on latent

correlations of symptoms is proposed to improve system performance by analyzing information from deep brain signals and patient symptoms. In this work, a low power technique, personal model-based event-driven control, was implemented, which can reduce power consumption by at least 35% and achieve 99.53% accuracy. Additionally, a graphical user interface (GUI) is designed to display the physiological signals of patients and apply appropriate stimulation parameters. The GUI provides medical staff with a simple, safe, and intraoperative assessment system. The system is compliant with medical standards for electrical safety and electromagnetic compatibility. The system allows clinical researchers to find suitable stimulation parameters and adjustable procedures to establish closed-loop stimulation. The system can observe LFPs instantaneously, interprets beta-band power as it crosses a threshold, and provides feedback control of the stimulation. A more effective, time-saving and convenient rapid prototyping tool for closed-loop DBS system can be achieved. The system has good flexibility and extensibility. To this end, a smarter and more affordable DBS system could be developed to treat Parkinson's disease and other neurological disorders.

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Poster

PSTR379. New Approaches to Neural Stimulation

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant R01NS112176
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NRF Grant 2021R1A2B5B02002437

Title: Wincsware: software for real-time synchronization of voltammetry, electrophysiology and neuromodulation in vivo

Authors: *H. SHIN[1], Y. OH[1], A. GOYAL[1], J. ROJAS CABRERA, 55905[1], K. SCHEITLER[1], G. CAMERON[1], J. YUEN[1], W. DENNIS[1], D. EAKER[1], J. BOESCHE[1], I. MANDYBUR[1], B. SHARAF[1], D. JANG[2], C. D. BLAHA[1], K. E. BENNET[1], K. H. LEE[1];
[1]Mayo Clin., Rochester, MN; [2]Hanyang Univ., Seoul, Korea, Republic of

Abstract: Introduction: The advent of voltammetric techniques has enabled neuroscientists to probe changes in neurochemistry during pathologic disease states and experimental conditions. Recently, we developed the MAVEN (Multifunctional Apparatus for Voltammetry, Electrophysiology and Neuromodulation) for electrochemical and electrophysiological recording with electrical stimulation system that has enabled researchers to test hypotheses both *in vitro* and *in vivo*. Due to the increased complexity of user needs, and the desire for customizable control of experimental conditions, we have developed the WincsWare Software system.

Method: This software suite contains recording and stimulation modules with real-time analysis and visualization. The software enables data exchange wirelessly by Bluetooth or by wired connection using optical data transmission for increased transfer speeds. The optical connection can be synchronized with MRI scanners and other devices to mitigate signal interference. The software can display up to 10 channels of simultaneous recording from multiple hardware units. Result and discussion: The recording module is customizable to vary the voltammetric waveform including amperometry, fast scan cyclic voltammetry (FSCV), fast scan cyclic voltammetry (FCSWV), multiple cyclic square wave voltammetry as well as electrophysiology. The stimulation module facilitates development of complex stimulation waveforms that can be synced for artifact-free recording. Live data can be streamed and analyzed with third-party software using the relevant API, and the output can be fed back into the system to modulate stimulation parameters in a closed-loop manner.

Disclosures: **H. Shin:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic. **Y. Oh:** A. Employment/Salary (full or part-time):; Mayo Clinic, Navinetics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Navinetics. **A. Goyal:** None. **J. Rojas Cabrera:** None. **K. Scheitler:** None. **G. Cameron:** None. **J. Yuen:** None. **W. Dennis:** None. **D. Eaker:** None. **J. Boesche:** None. **I. Mandybur:** None. **B. Sharaf:** None. **D. Jang:** None. **C.D. Blaha:** None. **K.E. Bennet:** A. Employment/Salary (full or part-time):; Mayo Clinic, Navinetics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Navinetics. **K.H. Lee:** A. Employment/Salary (full or part-time):; Mayo Clinic, Navinetics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Mayo Clinic, Navinetics.

Poster

PSTR379. New Approaches to Neural Stimulation

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant 1R43MH135814-01

Title: A Hybrid Backscatter Radio System for Neural Recording and Stimulation

Authors: *R. J. GERTH[1], V. GO[2], C. L. HOWARD[1], N. ARMSTRONG[2], A. FERNANDO[2], D. LAZEGA[2], **J. C. MORIZIO**[2];
[1]Spike Neuro, Ann Arbor, MI; [2]Duke Univ., Durham, NC

Abstract: Wireless neural recording has greatly contributed to our understanding of the nervous system and neurological conditions. However, the development of wireless electrical stimulation

and neural recording has lagged, compelling researchers to rely on wired systems. This stems from wireless transmission of high-fidelity single-unit neural signals requiring high power demand and heavy batteries. Consequently, there is no lightweight, low-power, high channel-count wireless solution for active single-unit neural recording and electrical stimulation, hindering basic neuroscience and clinical translation.
Addressing this, Spike Neuro, LLC and the Wireless Electrophysiology laboratory at Duke University, Department of Electrical and Computer Engineering, have collaborated to create a unique low-power wireless headstage using a novel hybrid wireless radio system. Unlike traditional systems, our headstage leverages backscatter technology, utilizing an incident RF signal to transmit data, thereby reducing the need for a large and heavy batteries. This technique uses passive reflection and digital modulation of the incoming RF signal with hundreds of microwatts that can be digitally encoded for data communications.
Our early studies evaluated the feasibility of power consumption and data transmission with this innovative setup. The system's active components are contained in a base station with a passive chip antenna in the headstage, reducing the weight of the device. During testing, a Manchester encoded bit pattern was passed through the headstage located 5ft from the base station antennas. The headstage had a RF single pole, double throw (SPDT) switch with one pole connected to a 50 ohm load and the other an open load. The base station antenna creates a RF carrier signal such that when the SPDT is switched to the open load, the backscatter antenna is largely mismatched to 50 ohms, and maximally backscatters the incident RF transmit carrier signal. This modulated backscatter signal was collected by the base station, and demodulated to recover the original bit pattern signal. Importantly, in this setup, the headstage consumed less than 12mA at +/-5V, significantly below traditional systems, thus validating our goal to reduce power consumption. This wireless technology is an important step for supporting longer, novel experiments studying the neural basis of natural behaviors. In our upcoming work we will integrate electrical stimulation allowing experiments previously limited to tethered setups. Our innovation will benefit both fundamental neuroscience research and translational efforts focused on specific neurological conditions.

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Poster

PSTR379. New Approaches to Neural Stimulation

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Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Grant UH3NS100549-04

Title: Real-time detection of dual-device DBS artifacts for future adaptive algorithms

Authors: *R. BECHTOLD[1], N. PROVENZA[3], S. RAJESH[3], N. DIAB[3], S. A. SHETH[3], W. A. GOODMAN[3], J. A. HERRON[2];
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Abstract: Adaptive deep brain stimulation (aDBS) is an emerging strategy for optimizing DBS therapy that operates by titrating stimulation parameters responsively to neural activity. These algorithms can fall victim to large beat frequency artifacts (BFAs) inherent to patients implanted with two neurostimulators. These periodic artifacts occur due to minute differences between the internal clocks of the two devices, leading to slight deviations in their delivered stimulation frequency, even when programmed stimulation parameters are identical. The difference between the stimulation frequencies of each device is the frequency at which these BFAs occur. Detecting and ignoring these artifacts, thereby preventing unwanted changes to stimulation, is critical to the success of future aDBS technology. Here, we present a classification model to identify BFAs to prevent adaptive changes to stimulation, limiting unintended effects due to BFAs. We observe BFAs that have a mean duration of 8.98 (± 2.88) seconds in 2 patients with a primary diagnosis of obsessive-compulsive disorder (OCD) who have undergone DBS surgery and are dual-implanted with Medtronic Summit RC+S neurostimulators. We collected local field potentials while stimulation was delivered to capture the naturally occurring artifact. We used an offline simulation of the onboard classifier to identify an optimal threshold for delta band (1-4 Hz) power, which was most significantly distorted by the BFA, to classify artifacts. We tested our active BFA classifier onboard the patient devices during rest to characterize classifier performance in identifying BFAs and pausing stimulation changes. Across both patients and four total trials, lasting on average 13.3 (± 1.6) minutes, our classifier achieved an average accuracy of 86.2% (± 4.1%), a sensitivity of 93.6% (± 3.1%), and a specificity of 78.7% (± 5.1%). Our data suggest that a delta band power threshold is a useful metric in identifying dual-device artifacts in real-time allowing for holds on stimulation changes and preventing unintended adaptive changes to therapy. Our classifier demonstrates the ability to effectively program dual-implanted devices with aDBS algorithms while circumventing cross-device interference.

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Poster

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Topic: E.05. Brain-Machine Interface

Support: NIH BRAIN UG3NS107688
Blackrock Neurotech Contract to University of Minnesota
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Title: Pre-clinical testing of a penetrating auditory nerve interface

Authors: *L. RIETH[1], I. SONDH[2], K.-H. DYBALLA[5], M. LEBER[6], J. CREW[6], K. HÜBNER[7], A. P. HEILLER[2], W. NOGUEIRA VAZQUEZ[5], L. JOHNSON[3], G. GHOSE[3], W. M. THOMAS[9], R. GURGEL[9], D. J. WARREN[10], S. ZUNIGA[3], D. CHIEFFE[3], A. LOVELAND[3], L. LARSON[3], R. FRANKLIN[6], A. SAMII[5], A. J. OXENHAM[4], S. STRAHL[8], C. BATSOULIS[8], D. SIEBER[12], F. SOLZBACHER[11], M. ADAMS[3], T. LENARZ[5], H. H. LIM[2];
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Abstract: Cochlear implants (CIs) are the most successful sensory neural prosthetic to date with >1 million implanted, which have provided or restored meaningful hearing for patients. Considerable resources have been invested to improve CI performance in the last 25 years, but resulted in modest improvement for real-world tasks such as understanding speech in noise. Spreading of the stimulation current due to the low conductivity of bone between the electrode and nerve, and the conductivity of perilymph around the cochlear electrode are putative factors limiting the number independent channels. We have developed a penetrating electrode, the auditory nerve Utah slanted electrode array (AN-USEA) fabricated by Blackrock Neurotech. The array is surgically placed in the auditory nerve, and driven by a MED-EL SYNCHRONY 2 cochlear stimulator. Our study will evaluate if more selective stimulation can be achieved through an intraneural interface and safely generate more natural hearing in human subjects. We report the device architecture used for Auditory Nerve Implant (ANI) devices, and bench tests such as impedance measurements and stimulation characterization from fully functional devices. Pilot studies with wired ANIs to develop a chronic cat model were also performed, and an optimized surgical procedure was developed to maintain the health of the animals and stimulation performance of the ANIs. Animal health was maintained for longer than the 6-month endpoints to be used in preclinical studies. The electrode impedances increased with implantation but remained < 70 kΩ in at least one animal, which suggests current can be delivered effectively. Nerve engagement was evaluated by evoked auditory brainstem recordings (eABRs) collected longitudinally. At least one animal maintained very convincing eABRs for longer than the 6-month pre-clinical endpoint supporting the viability of the cat model, surgical approach, and electrode technology. These studies have also evaluated the activation thresholds, forward masking paradigms to help confirm auditory nerve fibers are recruited, and channel independence through masking with separate electrodes and achieved highly promising results. The surgical approach for NHP studies has continued to be developed, focusing on implantation, insertion, and stabilization methods, which are more technically demanding than previous human cadaver studies. The impedances of implanted electrodes and eABR measurements were used to

evaluate the electrode-tissue interface and nerve engagement, respectively. Data from large animal models will be used to gain approvals for first-in-human implants of the ANI devices.

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Poster

PSTR379. New Approaches to Neural Stimulation

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Topic: D.01. Somatosensation

Support: Japan Society for the Promotion of Science (JSPS) Fellows to N.K. [#19J21897]
Meiji Yasuda Life Foundation of Health and Welfare to N. K.
JSPS KAKENHI to K.N [#JP18H04082 and #JP18HKK0272]
JST-Mirai Program to K.N [#JP20349063]
JST-MOONSHOT program to K.N [#JPMJMS2012–2-2–2]
NSERC Discovery Program to K.M [RGPIN-2017-06790]

Title: Ia-sensory inputs from peripheral nerve stimulation are greater than those from motor point stimulation in the soleus muscle

Authors: *N. KANEKO[1], A. SASAKI[2], K. L. FOK[3,4], H. YOKOYAMA[5], K. NAKAZAWA[1], K. MASANI[3,4];
[1]Univ. of Tokyo, Tokyo, Japan; [2]Univ. of Miami Miller Sch. of Med., Miami, FL; [3]KITE Res. Inst. – Univ. Hlth. Network, Toronto, ON, Canada; [4]Univ. of Toronto, Toronto, ON, Canada; [5]Tokyo Univ. of Agr. and Technol., Tokyo, Japan

Abstract: Neuromuscular electrical stimulation (NMES) is a non-invasive technique that induces muscle contraction to restore, support, or replace motor function in individuals with neurological disorders. NMES can be classified into two types: peripheral nerve stimulation (PNS) and motor point stimulation (MPS). Sensory inputs induced by PNS and MPS are an essential factor for neuroplasticity to improve motor function. Our recent studies have found that MPS does not elicit the Hoffman reflex in the soleus muscle as PNS does, indicating that PNS could induce greater Ia-sensory inputs than MPS. Here, we used a conditioning paradigm that combines transcutaneous spinal cord stimulation (tSCS) with PNS or MPS to characterize their Ia-sensory inputs. Thirteen individuals participated in this study. We applied MPS and PNS on the soleus muscle as conditioning stimuli and tSCS to the lumbosacral spinal cord as test stimuli. We recorded PNS- and MPS-conditioned spinal reflexes induced by tSCS at five inter-stimulus intervals (ISIs) and four intensities (20 conditions for each conditioning stimulus). Our results showed that all PNS conditioning significantly decreased the amplitudes of spinal reflexes, indicating post-activation depression. The PNS-conditioned spinal reflexes had greater depression for shorter ISIs and higher conditioning stimulus intensities. Meanwhile, MPS showed depression in 12 of 20 conditions and intensity dependence but did not show a clear ISI dependence as PNS did. Additionally, our results indicated that PNS elicited greater depression than MPS in 18 of 20 conditions. Since post-activation depression is associated with a transient decrease in the amount of neurotransmitter released from Ia afferent nerve terminals, the extent of the depression reflects the degree of Ia-sensory inputs. Therefore, we conclude that PNS induces greater Ia-sensory inputs than MPS.

Disclosures: **N. Kaneko:** None. **A. Sasaki:** None. **K.L. Fok:** None. **H. Yokoyama:** None. **K. Nakazawa:** None. **K. Masani:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.17/XX37

Topic: I.08. Methods to Modulate Neural Activity

Support: NSF CAREER 1653080

Title: Electrical stimulation-induced action potential interactions lead to variations in peripheral neural output: an in vivo study

Authors: *L. MADDEN, R. LIU, S. IVANESCU, T. M. BRUNS;
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Abstract: Electrical neuromodulation of the peripheral nervous system can provide treatment for a number of neurological conditions, such as bladder disorders and chronic pain. These therapies often deliver stimulation to neural structures transmitting ongoing neurophysiological activity. Both computational and clinical studies have suggested that interactions between physiologically-induced and electrical stimulation-induced action potentials can occur, modulating the signals that reach the central nervous system. Differences in the relative frequencies of the action potentials from each source and the size of the targeted axons can lead to differences in endpoint neural output, which may affect the consistency of therapeutic results. Here we characterized changes in primary afferent neural output in response to different electrical stimulation frequencies imposed onto ongoing activity in the pudendal nerve of an in vivo feline model. Anesthetized felines were implanted with a cuff electrode around a pudendal nerve and microelectrode arrays in the ipsilateral S1 and S2 dorsal root ganglia (DRG). Physiological action potentials were induced via cutaneous brushing of the perineal region, administered by a computer-controlled actuator. Electrical stimulation was administered during brushing via the cuff electrode at 10 frequencies spaced logarithmically from 0.5 Hz to 30 Hz at the motor threshold amplitude and at twice the motor threshold amplitude. DRG recordings were also performed during brushing without electrical stimulation. Spike sorting analysis was performed on the raw microelectrode data, and further analyses were performed on the sorted results to extract interspike interval (ISI) distributions and spike counts per unit. Statistical analyses were performed to examine how the ISI distributions change in response to stimulation frequency. Preliminary results demonstrate a non-monotonic trend between mean neural output ISI and electrical stimulation frequency. We also found variations in output response to frequency across different units, with ISI distributions shifting or adding a second peak. The outcomes of this study will inform the development of continuous and closed-looped electrical neuromodulation systems and may shed light on how stimulation frequency relative to

endogenous neural activity can influence therapeutic outcomes. These results may also have implications for peripheral motor and central nervous systems.

Disclosures: **L. Madden:** None. **R. Liu:** None. **S. Ivanescu:** None. **T.M. Bruns:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.18/XX38

Topic: E.05. Brain-Machine Interface

Support: NIH/NRSA Training Grant NS105595
NIH/NINDS Grant U01NS123127
T&C Chen BMI Center
Boswell Foundation

Title: Qualitative and temporal experiences of ICMS-elicited tactile sensations are affected by visual context

Authors: *I. A. ROSENTHAL[1], L. BASHFORD[1], D. A. BJA°NES[1], K. PEJSA[1], B. LEE[2], C. LIU[2], R. A. ANDERSEN[1];
[1]Caltech, Pasadena, CA; [2]USC, Los Angeles, CA

Abstract: Artificial tactile sensations can be elicited through intra-cortical microstimulation (ICMS) of primary somatosensory cortex (S1). For a successful closed-loop brain-machine interface (BMI), ICMS must be processed as part of the real-world environment, but there has been little work on how ICMS is integrated with other sensory inputs. Here, a tetraplegic patient implanted with 2 microelectrode arrays (Blackrock Neurotech) in S1 received ICMS (300Hz, 0.5s) at varying current amplitudes while observing visual stimuli. Visual and ICMS stimuli were delivered at varying temporal offsets from one another (0, 150, or 300ms). Visual stimuli were presented using a VR headset and were either abstract (a dot moving to the end of a line) or realistic (a robotic arm tapping a first-person-perspective human arm). The participant reported the respective order of visual contact and ICMS-evoked sensations, or if they were perceived simultaneously. Task performance was not affected by learning over time, visual condition, or current amplitude. ICMS was perceived as temporally lagging behind visual input, with the point of subjective simultaneity (PSS) occurring when ICMS slightly preceded visual stimuli. The temporal binding window between ICMS and vision was extended when visual stimuli were realistic, meaning that the participant reported that the stimuli felt simultaneous over larger temporal offsets. This finding suggests that the biological relevance of visual stimuli causes the brain to better synchronize ICMS with vision. Additionally, the participant's verbal descriptions of the qualitative nature of perceived sensations were influenced by the realistic visual condition, becoming more consistent with the visual depiction of touch. Electrophysiological data was also recorded from S1 during catch trials in which no ICMS was delivered. A significant percentage

of channels were tuned to visual stimuli during catch trials, with a large degree of overlap in channels between the abstract and realistic conditions. Representational similarity analysis (RSA) revealed that neural patterns across the S1 arrays were not segregated by visual condition, indicating that S1 represents ICMS-relevant information from vision similarly across the abstract and visual conditions. The perceptual differences between abstract and realistic visual conditions are therefore likely to arise not in S1, but elsewhere within the sensory processing stream. Uncovering how S1 encodes touch-related stimuli and how ICMS is interpreted in a multisensory context is necessary to understand the uses and limitations of ICMS for BMIs implemented in real world environments.

Disclosures: I.A. Rosenthal: None. **L. Bashford:** None. **D.A. Bja°nes:** None. **K. Pejsa:** None. **B. Lee:** None. **C. Liu:** None. **R.A. Andersen:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.19/XX39

Topic: I.08. Methods to Modulate Neural Activity

Title: In Vivo Concurrent Sciatic Nerve Electrical Stimulation and Evoked Muscle Recording with A Custom Designed Neurostimulator System

Authors: *A. ERSOZ[1], S. GERSHANOK[2], T. COHEN-KARNI[3], D. J. WEBER[4]; [1]Mechanical Engin., [2]Materials Sci. and Engin., [3]Biomed. Engineering; Materials Sci. and Engin., [4]Mechanical Engineering; Biomed. Engineering; Neurosci. Inst., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Neurostimulator systems with electrical stimulation and recording capabilities are essential to modulate neurons and measure their physiological responses. For electrical stimulation applications, continuous monitoring of the voltage potential is important to ensure the voltage is restricted within safety limits. Biopotential measurements, such as electromyograms, are also important for monitoring and controlling the physiological responses to neurostimulation. We have developed an integrated platform for closed-loop control of neurostimulation. The system is portable and supports wireless communication of data and control parameters. Our system generates simultaneous constant-current stimulation pulses with maximum 1.5 mA on up to 4 channels based on user-specified settings. The system also provides 4 bipolar EMG channels that can be used to record spontaneous activity and evoked responses. An integrated Bluetooth radio enables wireless control and data capture. This system is unique in that it has built-in voltage transient measurement circuits that shows ohmic voltage drop and polarization voltage of the electrode to ensure stimulation signal in electrochemical safe limits and power-management circuitry to regulate battery voltage in certain voltage limits (3.3 V, 5V, -5V) and recharge the battery via USB. *In vivo* tests were performed to verify the system. Biphasic symmetric constant-current stimulation signals were applied to a rat sciatic nerve

through needle electrodes. Voltage transients in response to the constant-current stimulation were monitored to observe polarization of the electrode. EMG electrodes were placed intramuscularly in the Tibialis Anterior and Lateral Gastrocnemius muscles to measure evoked responses. The stimulation signal intensity to produce extraneural activation was biphasic symmetric 500 µA with 10 Hz and 200 µs pulse widths. Polarization voltages of the needle electrodes were within safe limits such as 100 mV in response to the stimulation pulses. The EMG response recorded ~0.7 V peak voltage with 5 ms duration following stimulation artifacts. We verified that the system could perform constant-current stimulation, bidirectional wireless data transmission, voltage transients monitoring in response to stimulation signals, and evoked muscle recordings. This custom designed neurostimulator system will be used to support studies of feedback-controlled neuromodulation applications for neurorehabilitation.

Disclosures: A. Ersoz: None. **S. Gershanok:** None. **T. Cohen-Karni:** None. **D.J. Weber:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Bionic Power Inc, Iota Biosciences Inc, Neuronoff Inc, Neuroone Inc, Reach Neuro Inc.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.20/XX40

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH grant R01NS062092
NIH grant K24-NS088568 (SSC)

Title: Synchronous two-site electrical stimulation leads to superposition and nonlinear responses

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[1]Neurol., Massachusetts Gen. Hosp., Boston, MA; [2]Hlth. Sci. and Technol., MIT, Cambridge, MA

Abstract: Improving the therapeutic utility of electrical stimulation for treating neurological disorders requires a better understanding of the brain's response to novel stimulation patterns. Here, we probed the effect of synchronous two-site single-pulse direct electric stimulation (SPES) in humans. We hypothesized that response to two-site SPES would be a linear superposition of the two single-site stimulation responses.
Simultaneous SPES at 4 and 7 mA of two distinct sites was performed while recording intracranial EEG in patients implanted with depth electrodes for localization of their seizure focus. Analysis was restricted to channels responsive to at least one of the two single-site SPES or to joint stimulation. The normalized waveforms of the responses to simultaneous SPES for individual channels were compared to linear combinations of the single-site responses. Channels

whose response to the simultaneous stimulation was larger (smaller) in magnitude to linear combinations for at least 100ms were termed as superlinear (sublinear). Area under the curve (AUC) of the normalized waveforms was also computed.
In a total of 545 channels across 3 patients, 9 pairs of sites, and 2 stimulation amplitudes, 508 (93.2%) channels showed a linear response, consistent with our hypothesis. Interestingly, 17 (3.1%) showed a sublinear and 20 (3.7%) showed a superlinear response. At 4mA, linear: 90.9%, sublinear: 5.7%, and superlinear: 3.3%, while at 7mA, linear: 94.6%, sublinear: 1.5%, and superlinear: 3.9%. Responses at individual channels were consistent across the two stimulation amplitudes. The AUC for simultaneous stimulation is closely related to the sum of the AUCs for the single-site stimulation responses (Fig. 1).
The essentially linear responses seen in most locations implies that dual site low amplitude stimulation can be useful for activation of neuronal populations without saturation as a means of avoiding side effects. The less frequent but still present superlinear and sublinear responses may be useful for exciting specific regions of the brain in previously unexpected ways.

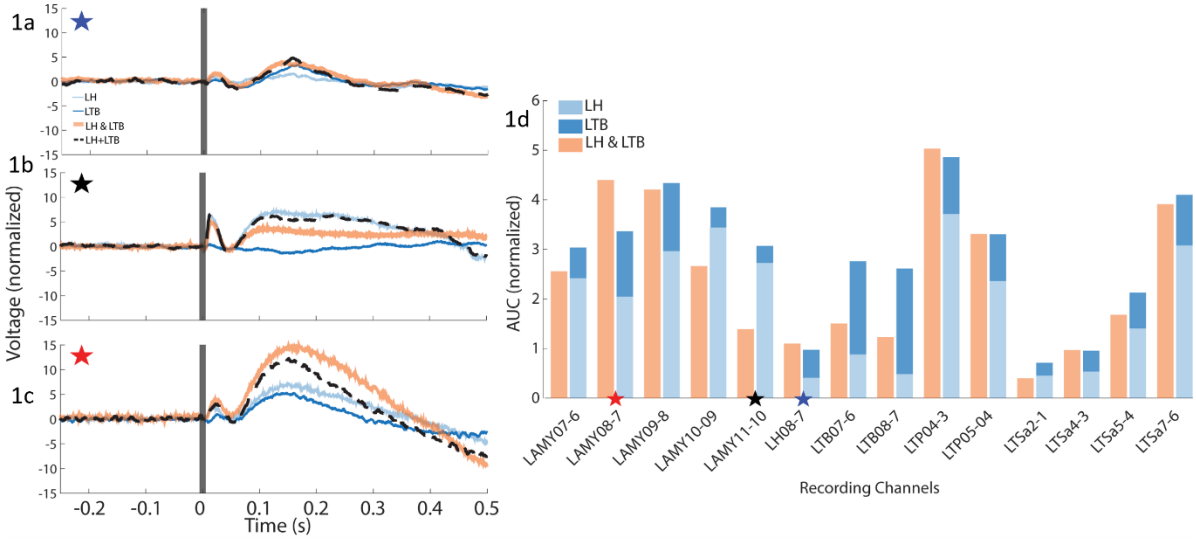

Figure 1: Response at various recording channels to simultaneous two-site stimulation in left hemisphere at middle temporal (LH) and inferior temporal (LTB) lobe for a patient, SPES at 7mA. In all plots, the light blue line shows the response observed for stimulation at LH alone, dark blue shows the response to stimulation of LTB alone, orange shows response for simultaneous stimulation of LH and LTB, and the dashed black line shows the sum of the two single site stimulation waveforms. The vertical thick grey line in 1a-1c denotes the time at which stimulation is delivered. **1a:** Normalized waveforms detected at channel LH08-7, demonstrating linear response. **1b:** Normalized waveforms detected at channel LAMY11-10, demonstrating sublinear response. **1c:** Normalized waveforms detected at channel LAMY08-7, demonstrating superlinear response. **1d:** Area under the curve (AUC) for z-normalized waveforms is plotted for the 14 recording channels responsive to stimulation at both LH and LTB. The red star corresponds to the superlinear response seen in 1c, black star to sublinear response in 1b, and blue star to linear response in 1a. The AUC curve for LH&LTB closely follows LH+LTB, but differs at superlinear and sublinear response, which is verified by the waveform plots.

Disclosures: D. Sehgal: None. **A.C. Paulk:** None. **J.D. Peralta:** None. **P. Hadar:** None. **S.S. Cash:** F. Consulting Fees (e.g., advisory boards); Beacon Biosignals. **R. Zelmann:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR379.21/XX41

Topic: I.08. Methods to Modulate Neural Activity

Support: JSPS Grant-in-Aid for JSPS Fellows 19J22132
JSPS Grant-in-Aid for Early-Career Scientists JP20K14274
JSPS Grant-in-Aid for Scientific Research on Innovative Areas 18H05523
JSPS Grant-in-Aid for Scientific Research (A) 21H04909

Title: Effects of transcranial alternating current stimulation (tACS) on multiple alpha components: An MEG study

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Abstract: Posterior alpha oscillations at approximately 10 Hz play a critical role in perception and cognition. Previous studies have shown that alpha-tACS targeting the posterior region can enhance alpha-band power after stimulation, suggesting potential benefits in perceptual and cognitive functions. However, there is an argument that tACS effects are highly variable and not replicable, and the overall understanding of the underlying mechanisms of tACS aftereffects remains limited. Furthermore, while alpha-band components have been observed in brain regions beyond the posterior, such as temporal tau and parietal mu, it remains unclear whether and how non-focal tACS influences these distributed alpha components. Therefore, we performed MEG measurements before and after tACS to elucidate the effects of tACS on individual alpha-band components. Eighteen participants received 20 minutes of tACS with Cz and Oz montage or sham stimulation on separate days. MEG data were recorded for 10 minutes before and after stimulation. Alpha oscillations were decomposed into five components originating from the visual, parieto-occipital, auditory, left sensorimotor, and right sensorimotor areas (Takahashi & Kitazawa, 2017). We selected the sensors that best reflected each component based on the forward modeling. The peak frequency and power of each component were calculated from each selected sensor. We evaluated the frequency mismatch between component frequency and stimulation frequency, and its effect on alpha power changes. We found that the alpha components slower than the stimulation frequency are suppressed, while those faster than the stimulation frequency are enhanced; these relationships were observed for the parieto-occipital and auditory components. The observed stimulation frequency-dependent changes are consistent with the spike timing-dependent plasticity model (Vossen et al., 2015). The other three components, including the visual component, did not show these systematic changes by tACS. These results suggest that posterior alpha-tACS, typically using Cz and Oz montage, predominantly affects the parieto-occipital component rather than the visual component. The present findings provide additional support for the mechanisms underlying tACS aftereffects and, for the first time, demonstrate the potential for differential effects between components with slightly different frequencies within the alpha band.

Disclosures: **S. Shibusawa:** None. **T. Kawashima:** None. **K. Amano:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.22/XX42

Topic: I.08. Methods to Modulate Neural Activity

Title: Cortical calcium dynamics evoked by Temporal Interference (TI) electrical stimulation in vivo

Authors: *P. DZIALECKA, N. ZABOURI, S. BARNES, N. GROSSMAN;
Imperial Col. London, LONDON, United Kingdom

Abstract: Temporal interference (TI) is a novel electrical brain stimulation technique that can target deep brain structures noninvasively. It is based on the simultaneous application of two kHz electric fields, which generate a field with an amplitude-modulation (AM) oscillating at the difference frequency where the applied fields overlap. Direct neuronal responses to TI stimulation have been tested using single-cell patch recordings in vivo (Grossman et al., 2017) and local field potential recordings *ex vivo* (Esmaeilpour et al., 2021). While electrophysiological recordings have a high temporal resolution, the spatial resolution is limited. In addition, concurrent TI electrical stimulation is challenging due to nonlinear hardware artefacts. Herein, we examine for the first time the direct response of large-scale neural networks to TI stimulation *in vivo*. We used widefield calcium imaging to track the activity of large cortical populations of Thy1-GCaMP6s mice undergoing TI stimulation and control (i.e., traditional AC) stimulation. We found that TI stimulation reliably evoked neuronal oscillations at the difference frequency of the applied fields and in the second harmonic. The threshold of evoking calcium oscillations is approximately a few folds higher than traditional AC, depending on the carrier frequency. The locus of the evoked oscillations is located between the stimulating electrodes, as predicted by a finite element method (FEM) modelling of the TI fields, with a delayed response in the contralateral brain region. This work provides direct evidence of the cortical response to TI electrical stimulation *in vivo* and the underlying spatiotemporal complexity.

Disclosures: **P. Dzialecka:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. **N. Zabouri:** None. **S. Barnes:** None. **N. Grossman:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent. Other; Company co-founder (TI Solutions AG).

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR379.23/XX43

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH BRAIN initiative RFANS20006 (NINDS; project number 1RF1NS126143-01A1)

Title: Characterizing the multiomic impact of direct electrical stimulation of the human cortex in vivo

Authors: *H. MOORE[1], G. KONOPKA[2], B. C. LEGA[3], A. KULKARNI[4];
[1]Neuroscience, Neurolog. Surgery, Univ. of Texas, Southwestern Med. Ctr., Dallas, TX; [2]Neurosci., UT Southwestern Med. Ctr., Dallas, TX; [3]Neurosurg., [4]Neurosci., UT Southwestern Med. Ctr., Dallas, TX

Abstract: The underlying mechanisms of therapeutics using brain stimulation in humans are not understood. In particular, it is unknown how brain stimulation impacts gene expression in acute and chronic temporal periods. Although therapeutic approaches like deep brain stimulation are becoming more widely used, it is impossible to study stimulation-induced genetic changes without tissue samples which at best could be collected post-mortem. To overcome these extremely limiting circumstances, we have implemented an experimental design in which tissue is collected from neurosurgical patients immediately after direct cortical stimulation in vivo. By collecting and flash-freezing tissue within 30 minutes of stimulation in human neurosurgical patients in vivo, we have had the opportunity to gain insight into the near-immediate impact of direct brain stimulation on cell-type specific gene expression and chromatin structure. Here we present our preliminary findings from integrated single nucleus RNA and ATAC sequencing experiments of directly stimulated versus adjacent unstimulated pre-motor cortical tissue from five patients. Our analysis has revealed cell-type specific, novel patterns of differential gene expression and chromatin accessibility that occur when the human cortex is stimulated in vivo. Our results indicate that excitatory neurons are particularly influenced by the stimulation program. We also detail our first efforts toward untangling stimulation-mediated gene expression changes in the human cortex ex vivo using microelectrode array stimulation programs in human organotypic slice culture. Our unique integrated approach of applying stimulation paradigms in human brain tissue both in vivo and ex vivo highlights the strengths of our interdisciplinary team with members possessing a wide range of specializations from neurogenomics to neurosurgery. These results represent a key first step in our quest to understand exactly how brain stimulation supports neural function at the cellular level.

Disclosures: H. Moore: None. **G. Konopka:** None. **B.C. Lega:** None. **A. Kulkarni:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

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Program #/Poster #: PSTR379.24/XX44

Topic: I.08. Methods to Modulate Neural Activity

Support: National Science Foundation (NSF) 1707865
Kuwait Foundation for the Advancement of Sciences (KFAS) CB20-62SL-01

Title: Effects of focused ultrasound on synaptic transmission at the Drosophila Larval Neuromuscular Junction

Authors: *S. ALSHUAIB[1], W. S. MÜLLER[2], G. EHNHOLM[4], Y. OKADA[3], J.-W. LIN[1]; [1]Boston Univ., Boston, MA; [2]Div. of Newborn Med., [3]Div. of Newborn Medicine, Dept. of Med., Boston Children's Hosp. & Harvard Med. Sch., Boston, MA; [4]Dept. of Neurosci. and Biomed. Engin., Aalto Univ., Aalto, Finland

Abstract: Ultrasound neuromodulation has made significant strides recently in the field of neuroscience, yet the underlying cellular mechanisms remain to be elucidated. While most research has focused on the impact of ultrasound on neuronal excitability, effects on synaptic transmission have not been addressed. We employed intracellular recordings in Drosophila larval neuromuscular junction to investigate the influence of Focused Ultrasound (FUS) on synaptic transmission.

Suction electrode stimulation was used to induce excitatory postsynaptic potentials (EPSPs), 10 stimuli at 10 Hz. The FUS protocol included twenty tone bursts (2.1 MHz , 0.3-0.9 MPa) delivered at 1 kHz with a duty cycle of 10%. A 20 ms US stimulus was synchronized with the onset of electric stimulation.

Synchronized delivery of FUS and electric stimulation generated two datasets that were analyzed separately. In preparations with EPSP delay longer than 20 ms, FUS influenced action potential initiation and propagation. In preparations with synaptic delay shorter than 20 ms, the transmitter release process occurred during US tone burst.

The main parameter affected by FUS was synaptic delay. In preparations where electric stimulations triggered single EPSPs, synaptic delay was reduced in 5 out of 7 preparations. The delay reduction ranged from 0.2 to 5.3 ms and was independent of FUS tone burst duration. Overall, FUS accelerated action potential during initiation or propagation. Mechanistically, we propose that synaptic acceleration could be due to FUS induced membrane depolarization in axons. We observed small depolarizing transients in muscle fibers and we believe that this depolarization may initiate AP earlier or increase AP conduction velocity.

In three preparations, strong electric stimulation induced multiple EPSPs, presumably due to repetitive firing of action potentials at ~20-30 Hz. In 2 out of 3 preparations the amplitudes of the second EPSP, occurring after US tone burst, was reduced. We hypothesize that this reduction could be a result of US affecting calcium dynamics, AMPA receptors or the shape of the second action potential. Determining FUS effects on synaptic transmission will deepen our understanding of its cellular mechanism and brings this technology closer to therapeutic applications.

Disclosures: S. Alshuaib: None. **W.S. Müller:** None. **G. Ehnholm:** None. **Y. Okada:** None. **J. Lin:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

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Program #/Poster #: PSTR379.25/XX45

Topic: I.08. Methods to Modulate Neural Activity

Support: NIH Brain Initiative Grant U01NS128612

Title: An Ecosystem of Technology and Protocols for Adaptive Neuromodulation Research in Humans: Year 1 experience with the BIC-BCI2000 construct

Authors: *K. MILLER[1], M. A. VAN DEN BOOM[1], F. MIVALT[1], I. KIM[1], V. KREMEN[1], G. SCHALK[2], D. HERMES[1], P. BRUNNER[3], G. WORRELL[1];
[1]Mayo Clin., Rochester, MN; [2]Tianqiao and Chrissy Chen Inst., Shanghai, China; [3]Washington Univ. in St. Louis, Saint Louis, MO

Abstract: This describes initial work toward an ecosystem for adaptive neuromodulation in humans by illustrating implantation of CorTec's BrainInterchange (BIC) device in a beagle canine and connecting it with the BCI2000 software environment. We initially performed a proof-of-principle implant with bilateral multiscale grids, demonstrating feasibility of the technology. We subsequently performed a right-sided craniotomy and implanted arrays in the epidural space over the putative sensorimotor, visual, and auditory brain regions in a beagle canine. The amplifier and power induction coil were placed on the right flank immediately behind the forelimb. Post-operatively, we successfully interfaced the BCI2000 software with the implanted BIC. Stable signals have been recorded over 2 months and recordings are ongoing. Robust task-associated voltage changes have been observed as 1) decreased power in low frequency oscillations (below 20Hz) and 2) broadband power increases (from 1-200 Hz). These are topologically (and separately) specific for sensorimotor and visual changes in their expected cortical regions. With further development and validation, the BIC/BCI2000 ecosystem will become an important tool for research into new adaptive neuromodulation protocols in humans.

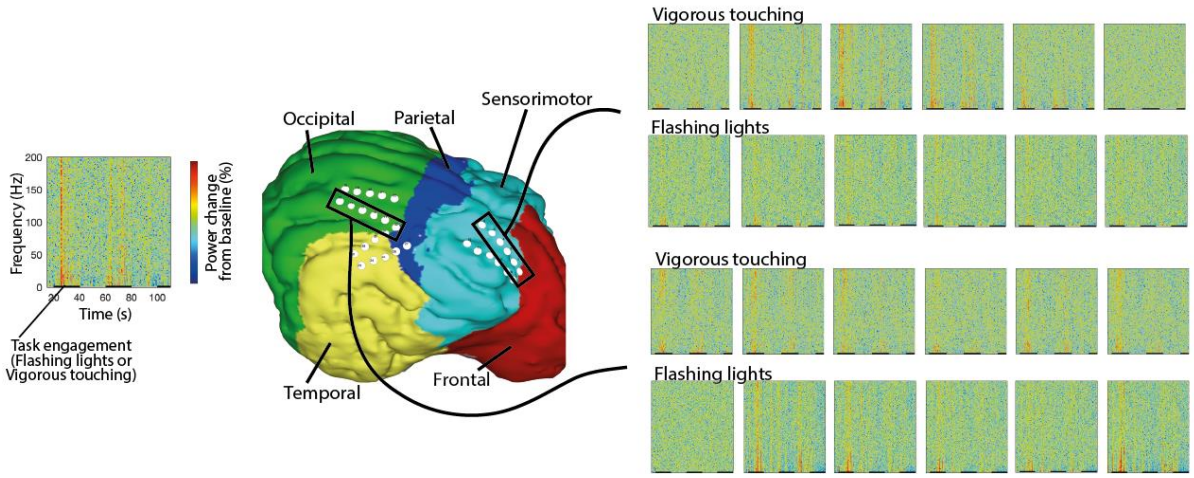


Figure: Functional activity. Spectrograms from a sensorimotor electrode bank and an occipital bank illustrating robust task-associated voltage changes have been observed as decreased power in low frequency oscillations (below 20Hz) and broadband power increases (from 1-200 Hz).

Disclosures: K. Miller: None. **M.A. van den Boom:** None. **F. Mivalt:** None. **I. Kim:** None. **V. Kremen:** None. **G. Schalk:** None. **D. Hermes:** None. **P. Brunner:** None. **G. Worrell:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.26/XX46

Topic: I.08. Methods to Modulate Neural Activity

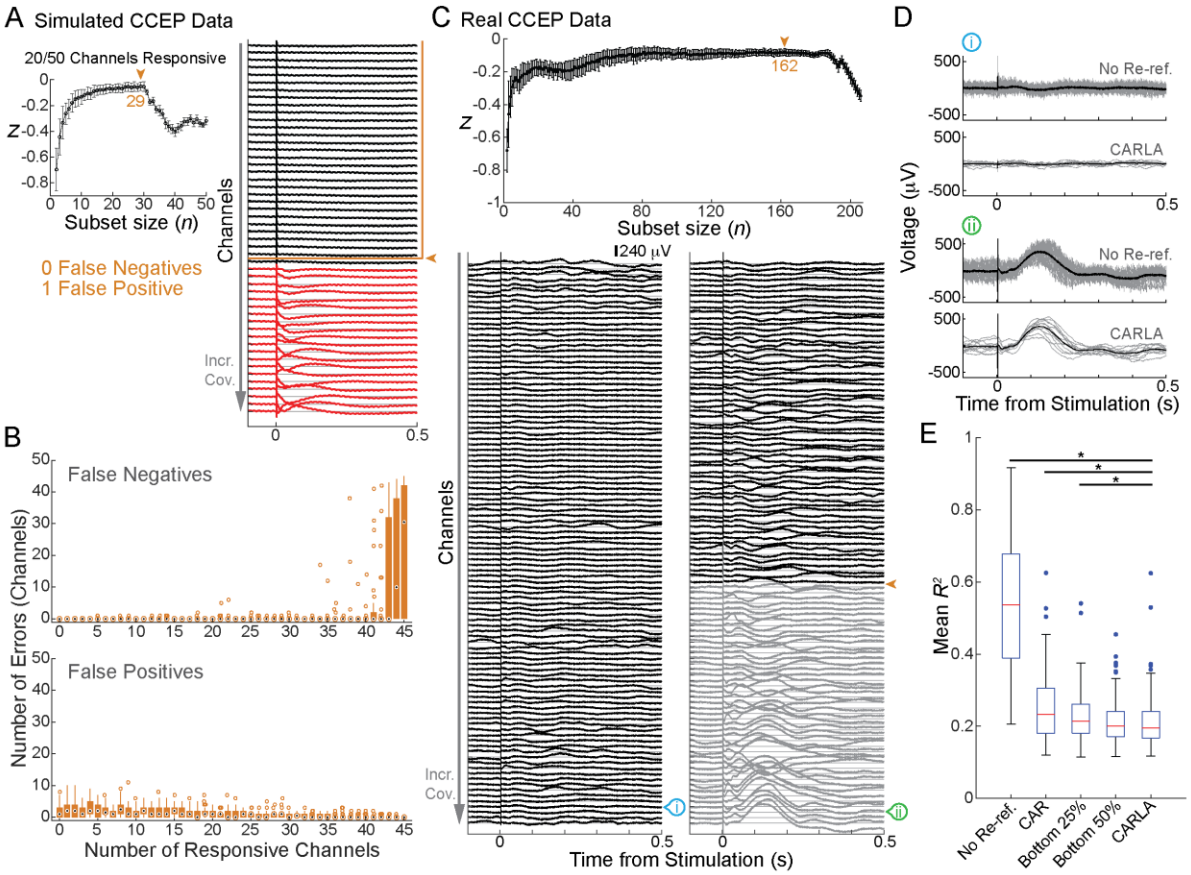
Support: National Institute of Mental Health of the National Institutes of Health, Award Number R01MH122258
National Institute of General Medical Sciences, Award Number T32GM065841
American Epilepsy Society, Award Number 937450

Title: Carla: adjusted common average referencing for cortico-cortical evoked potential data

Authors: *H. HUANG[1], G. OJEDA VALENCIA[2], N. M. GREGG[3], G. M. OSMAN[3], M. N. MONTOYA[2], G. A. WORRELL[3], K. J. MILLER[4], D. HERMES[2];
[1]Med. Scientist Training Program, [2]Physiol. and Biomed. Engin., [3]Neurol., [4]Neurologic Surgery, Mayo Clin., Rochester, MN

Abstract: Connectivity in the human brain can be mapped by electrical stimulation during intracranial EEG measurements. The raw cortico-cortical evoked potential (CCEP) data is often contaminated by noise, which is reducible by re-referencing. Common average referencing

(CAR) removes common noise and preserves response shapes, but it can introduce bias from responsive channels. We address this issue with an adjusted, adaptive CAR algorithm termed "CAR by Least Anticorrelation (CARLA)". CARLA was tested on simulated CCEP data and real CCEP data collected in 4 human participants who had stereo EEG electrodes implanted for epilepsy surgery evaluation. The algorithm is briefly as follows. First, channels are ordered by increasing mean cross-trial covariance post-stimulation. Iteratively, the n channels with the lowest covariance form a subset, and z quantifies the mean correlation between the most anticorrelated channel and all other re-referenced channels. The optimal common average is constructed from the n-subset whose z is least negative. We simulated CCEP data with 50 channels, true responses in 0 to 45 channels, 30 times per number of responsive channels. CARLA's error was quantified: false negatives (FN) indicate responsive channels erroneously included in the optimal common average and false positives (FP) indicate non-responsive channels erroneously excluded. Median FN was 0 for <44 responsive channels, and median FP was <3 throughout. On real CCEP data, signal quality was quantified with the mean R^2 between all pairs of channels, which represents inter-channel dependency and is low for well-referenced data. CARLA re-referencing produced significantly lower mean R^2 than CAR, CAR with fixed bottom quartile of channels by covariance, and no re-referencing (Wilcoxon signed-rank tests, $p < 0.01$, Bonferroni-corrected). CARLA minimizes bias in re-referenced CCEP data by adaptively selecting the optimal subset of non-responsive channels. It showed high specificity and sensitivity on simulated CCEP data and lowered inter-channel dependency compared to CAR on real CCEP data.



Performance of CARLA on simulated and real CCEP data. A, *z* vs. *n* (left), and mean time series across 12 trials sorted by increasing cross-trial covariance (right), for one iteration of simulated CCEP data where 20 of 50 channels are responsive. Channels in black are non-responsive, channels in red contain a true evoked potential. The optimal common average size determined by CARLA is labeled by the arrowhead. **B,** Boxplots summarizing false negatives and false positives for 30 simulated iterations at each number of responsive channels out of 50. **C,** *z* vs. *n* (top), and mean time series across 12 trials sorted by increasing cross-trial covariance (bottom), for real CCEP data from a stimulation site in participant 1. The optimal common average size determined by CARLA, at 162 of 206 channels, is labeled by the arrowhead. Channels in black are included in the optimal common average, channels in gray are excluded. **D,** The channels labeled "i" and "ii" in **C** before and after CARLA re-referencing. Individual trials are in gray and the mean across trials is in black. **E,** Boxplots of average R^2 for different re-referencing methods, across 82 stimulation sites in all participants. Asterisis (*) indicate statistical significance by pairwise Wilcoxon signed-rank tests (Bonferroni-corrected $p < 0.01$).

Disclosures: H. Huang: None. **G. Ojeda Valencia:** None. **N.M. gregg:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Investigator for the Medtronic Deep Brain Stimulation Therapy for Epilepsy Post-Approval Study. **G.M. Osman:** None. **M.N. Montoya:** None. **G.A. Worrell:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Investigator for the Medtronic Deep Brain Stimulation Therapy for Epilepsy Post-Approval Study. **K.J. Miller:** None. **D. Hermes:** None.

Poster

PSTR379. New Approaches to Neural Stimulation

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR379.27/XX47

Topic: I.08. Methods to Modulate Neural Activity

Support: Air Force Office of Scientific Research Grant FA9550-20-1-0061

Title: Amperometric analysis of exocytosis in chromaffin cells stimulated with a 5 nanosecond electric pulse

Authors: *A. BALAJI[1], G. L. CRAVISO[2], N. LEBLANC[3], J. YOON[1];
[1]Dept. of Electrical and Biomed. Engin., Univ. of Nevada, Reno, Reno, NV; [2]Dept. of Pharmacol., Univ. of Nevada Sch. of Med., Reno, NV; [3]Dept. of Pharmacol., Univ. of Nevada, Reno Sch. of Med., Reno, NV

Abstract: We have been investigating the potential for nanosecond electric pulses (NEPs) to serve as a novel electric stimulation method to modulate neurosecretion, using isolated bovine adrenal chromaffin cells as a cell model. Fluorescence imaging of intracellular calcium concentration ($[Ca^{2+}]_i$) and monitoring exocytosis by total internal reflection fluorescence microscopy have shown that a train of ten, 5 ns, 5 MV/m pulses, delivered at 1 Hz, causes longer-lived increases in $[Ca^{2+}]_i$ as well as a longer duration of exocytotic events, respectively, than evoked by ten applications of the nicotinic receptor agonist 1,1-dimethyl-4-piperazinium (DMPP) at 1 Hz. Also, there was a delay of several seconds in the onset of exocytosis in cells stimulated with the NEP train. The goal of this study was to work toward identifying the nature of the differences between nicotinic-receptor mediated and NEP-mediated exocytosis using carbon fiber amperometry. Chromaffin cells were stimulated with a single 5 ns pulse using tungsten rod electrodes or with a single application of DMPP (100 μM) delivered by a pressure ejection system. Amperometric spikes were recorded using a carbon fiber electrode (CFE) held at +800 mV and positioned close to the surface of the cell. To protect the amplifier circuitry during NEP exposure, the amplifier was briefly disconnected from the CFE using a series of reed relays, resulting in a 4 ms gap in data recording after pulse delivery. Like DMPP, a NEP evoked a rapid burst of spikes in all cells exposed to the stimulus. However, there were differences in the responses that included a shorter duration of the evoked spikes (90 s or less) for DMPP-stimulated cells versus a more sustained duration of evoked spikes (sometimes exceeding 3 minutes) in NEP-stimulated cells. In the case of the latter, the highest concentration of spikes (i.e., highest spike rate) occurred during the first minute following the pulse. Finally, whereas the onset of spikes in cells stimulated with DMPP was less than 500 ms, the onset of spikes in cells stimulated with a 5 ns pulse was delayed in the majority of cells (n=7/8), ranging from 1 to 7.5 s post-stimulus with an average delay of 3.8 ± 1.1 s (SEM). Only one cell had an onset less than 500 ms. The results presented here provide the first evidence of exocytotic events triggered by a single 5 ns pulse in chromaffin cells quantified by amperometry. Further, they demonstrate the distinguishing features of exocytosis evoked by NEP when compared to physiological stimulus, which will continue to be investigated with respect to spike characteristics. These findings are crucial for understanding the efficacy of NEP as a new tool for stimulating neurosecretion.

Disclosures: A. Balaji: None. **G.L. Craviso:** None. **N. Leblanc:** None. **J. Yoon:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.01/XX48

Topic: E.05. Brain-Machine Interface

Support: Stanford Center for Mind, Brain, Computation and Technology
Stanford Institute for Human-Centered Artificial Intelligence / Wu Tsai Neurosciences Institute

Title: A non-invasive silent speech interface with flexible and high-density surface electromyography arrays

Authors: *T. BENSTER[1], G. WILSON[2], Y. LEE[3], R. ELISHA[3], J. THELWELL[3], J. M. HENDERSON[4], Z. BAO[3], S. DRUCKMANN[3];
[1]Stanford Univ. Neurosciences Phd Program, Stanford, CA; [2]Stanford Univ., San Francisco, CA; [4]Dept Neurosurgy, [3]Stanford Univ., Stanford, CA

Abstract: Silent speech interfaces (SSIs) facilitate soundless verbal communication, offering benefits for individuals with communication disorders and providing a discreet option for automatic speech recognition. The most advanced SSIs to date harness surface electromyography (sEMG) to capture voltage signals from speech articulators. However, these typically depend on individually-wired electrodes and machine learning pipelines with manually designed features, limiting their applicability outside controlled settings. In this work, we utilize a flexible, high-density (HD) sEMG array made of sticky conductive polymers on a stretchable substrate for superior silent speech signal acquisition. Using Structured State Spaces for Sequence Modeling (S4), we decoded offline a 50-word corpus with 87% accuracy, maintaining consistent performance across more than four hours of continuous recording, and not yet saturating performance with dataset size. We demonstrate a significant average decrease in WER of over 5% by pooling data from 15 sessions across three participants. Lastly, we demonstrate a new state-of-the-art for silent speech decoding (26% WER) using large language models on a preexisting dataset. Our results indicate that flexible HD-sEMG arrays are well-suited for silent speech interfaces and may enable general-purpose use across an open vocabulary.

Disclosures: **T. Benster:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reflex Technologies, Inc. **G. Wilson:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reflex Technologies, Inc. **Y. Lee:** None. **R. Elisha:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Reflex Technologies, Inc. **J. Thelwell:** None. **J.M. Henderson:** None. **Z. Bao:** None. **S. Druckmann:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.02/XX49

Topic: E.05. Brain-Machine Interface

Support: This research was supported by X-mind Corps program of National Research Foundation of Korea(NRF) funded by the Ministry of Science, ICT (No.2022H1D8A303866311)
This study was supported by the National Police Agency and the Ministry of Science, ICT & Future Planning (Grant no: 1711174175)
This work was supported by a Research Grant of Pukyong National University(2022)
This work was supported by the Pukyong National University Research Fund in 2022(202212510001)
This study was supported by Startup Growth Technology Development Project(Strategic) (No.1425174568)

Title: Decoding electroencephalographic signals for inner speech words in brain-computer interface using echo state networks

Authors: D.-H. LIM[1], H. PARK[2], M.-J. JO[1], S.-H. KO[3], Y. CHO[4], J. KWON[6], ***H.-H. KIM**[5]; [1]Dept. of Computer Engin., [2]Dept. of Materials Sci. and Engin., [3]Dept. of IT Convergence and Application Engin., [4]Dept. of Biomed. Engin., [5]Pukyong Natl. Univ., Busan, Korea, Republic of; [6]Res. Inst., 4N Inc., Daejeon, Korea, Republic of

Abstract: Various circumstances, such as neurological disorders like aphasia, the biological aging process, or privacy considerations, may lead to situations where one's capacity for verbalizing thoughts aloud is inhibited or deemed inappropriate. Additionally, when the aim is to control a variety of Internet of Things (IoT) devices utilizing a Brain-Machine Interface (BMI), the use of Inner Speech proves to be suitable. This study goal was developed a decoder for Electroencephalographic Brain-Machine Interface (EEG-BMI) for individuals experiencing these situations. We conducted an experimental protocol to measure and decode neural activities associated with the mental representation of specific inner speech words. Techniques for EEG signal analysis and classification were applied to identify distinct patterns related to inner speech word recall. The experimental group comprised a carefully selected sample of 40 healthy individuals aged between 20 and 30, none of whom exhibited indications of psychiatric disorders. This ensured a homogeneous and representative participant population for the study. Participants wore a 16-channel EEG device and performed tasks within a shielded room to minimize environmental noise interference. Subjects engaged in an inner speech task, focusing on each of the ten assigned words for a duration of five seconds each. In the initial phase of EEG signal pre-processing, an implementation of real-time Independent Component Analysis (ICA) was employed to reduce signal artifacts. The Power Spectral Density (PSD) was then derived for each individual frequency band of the EEG signal to facilitate feature extraction. Feature selection was executed using the Fisher ratio, a widely utilized statistical method known for its effectiveness in high-dimensional data. An EEG decoder, based on the Echo State Network

(ESN) paradigm, was designed and implemented to decode the EEG data using ten readouts with a winner-takes-all policy. In the offline evaluations, a 10-fold cross-validation approach was employed, which yielded an average validation accuracy of 0.93864. Moreover, in the online evaluations, the decoding accuracy for each of the two target words exceeded 90%. These findings provide evidence that the decoders can successfully decode the EEG signals associated with inner speech words. These results lay a critical foundation for the advancement of innovative technologies and devices aimed at facilitating the brain-machine interface, and underscore the practical potential of their implementation within the field of neuroscience.

Disclosures: **D. Lim:** None. **H. Park:** None. **M. Jo:** None. **S. Ko:** None. **Y. Cho:** None. **J. Kwon:** None. **H. Kim:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.03/XX50

Topic: E.05. Brain-Machine Interface

Support: NSF IUCRC BRAIN Award 2137255
University of Houston CLASS Early Career Research Progress Grant

Title: Decoding acoustic characteristics produced during overt speech with deep learning from scalp electroencephalography (EEG)

Authors: *A. R. CRAIK[1], H. R. DIAL[2], J. L. CONTRERAS-VIDAL[3];
[2]Communication Sci. and Disorders, [3]Electrical and Computer Engin., [1]Univ. of Houston, Houston, TX

Abstract: In 2019, over 7 million people in the U.S. reported living with neurological disorders that hamper their ability to produce speech, significantly reducing their quality of life. Typical speech interfaces for this patient population, including eye-tracking devices and P300 spellers, involve the energy-intensive selection of individual letters or words, which is a slow and unnatural process. Neural-based speech interfaces that decode the acoustic characteristics of speech directly have been proposed as a more natural mechanism for these patients ([1] Nature, 568 (7753): 493). Here, Mel-Frequency Cepstral Coefficients (MFCCs) of the audio signal generated through continuous overt speech are decoded with deep learning from EEG. Nine neurologically intact participants were recruited (NSF IUCRC BRAIN Award 2137255) and fitted with a 64-channel Brain Products ActiChamp Plus EEG system, with four additional sensors placed around the eyes for use in the removal of eye artifacts. Each participant sat in a shielded room to limit external noise and were presented with sentences on a display to read aloud. A Brain Products Stimtrak was employed to synchronize the spoken output with EEG data. To capture the full speech spectrum, sentences were selected from passages designed to have a similar phonetic representation as compared to the English language (Caterpillar,

Rainbow, Grandfather passages). All data were pre-processed through a pipeline that includes filtering, line noise removal, adaptive noise cancellation for the removal of EOG contamination, and ICA for the removal of EMG contamination. The clean EEG data were then passed to the deep learning models for MFCC prediction (Convolutional Neural Network with three convolutional blocks). EEG data were segmented into windows of 0.3 seconds at 200 Hz and centered around the corresponding MFCCs as derived from the audio signal (200 Hz). Model performance was evaluated with Mel-Cepstral Distortion (MCD), a common metric for assessing the effectiveness of speech processing systems (lower MCD implies higher generation quality). The resulting average MCD value from each participant, as predicted from the final 10% of the data following four training sessions, range from 22.5 to 29.0 dB (overall average of 25.7 dB). As a comparison, in [1], where decoding was based on ECoG, median MCD values ranged from 5.1 to 6.6 dB. The effective decoding of acoustic characteristics from continuously produced overt speech is a first step towards a non-invasive speech BCI. Such a system has the potential to act as a universal BCI control signal, which opens up significant opportunities for the commercial translation of speech BCI.

Disclosures: A.R. Craik: None. **H.R. Dial:** None. **J.L. Contreras-Vidal:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.04/XX51

Topic: E.05. Brain-Machine Interface

Title: Piccom: electrooculogram tool for communication by pictograms in cerebral palsy patients.

Authors: *G. D. C. LOPEZ-ARMAS[1], Y. NAVA-RAZO[2], A. ARENAS-PEREZ[3], J. MARGAIN-MORENO[4], L. I. GUERRERO-LINARES[5];
[1]Mecatrónica, Ctr. De Enseñanza Técnico Industrial, Guadalajara, Mexico; [2]Mecatrónica, Ctr. de Enseñanza Tecnica Industrial, guadalajara, Mexico; [3]NXP Semiconductors, Guadalajara, Mexico; [4]Univ. de Guadalajara, Guadalajara, Mexico; [5]Tyndall Natl. Inst., Univ. Col. Cork, Cork, Ireland

Abstract: Introduction: Patients with spastic quadriplegia cerebral palsy (SQCP) are marked by difficulties in establishing an effective communication process; in this sense, augmentative and alternative communication (AAC) has been developed for the nonverbal communication system. This system is divided into two categories; the first one is unaided techniques like gestures, body language, facial expression, and sign language, and the second one is aided systems such as pen and paper, pictograms, and devices to interpret the patient. The electrooculogram (EOG) is an affordable tool that can help record the electric difference potential between the cornea and retina produced by blinking. Our goal was to create a communication system for patients with cerebral palsy, which includes an electronic device that works with EOG sensors to detect double-eye

blinking. The patient matches a highlighted pictogram with their needs or wishes through a smartphone app that displays multiple pictograms simultaneously, continually changing the highlight until a match is found. Once a match is made, communication is established, and the system can recognize the patient's needs and provide a short sentence through a sound translation of the pictogram. **Methodology:** We enrolled five healthy adults and three patients with SQCP for this study. All patients gave written informed consent; also, Helsinki guides were respected. Previously all participants were familiarized with pictograms for AAC before using the system; firstly, we standardized blinking by EOG tool for the normal subject and the signal taken from the user's eye in a range of 1 to 30Hz; this stage is powered by a 5v voltage that comes from the USB connector of the mobile device; an ADC of a microcontroller samples the signal obtained from the previous stage; this microcontroller is programmed so that each electrical pulse corresponding to a double bilking eye sends a command via serial communication to the mobile application. The application was created using the MIT app inventor, which contains a bank of pictograms of AAC used and standardized internationally. **Results:** This project focuses on the user's experience with the interface by allowing them to navigate through the categories using the command received via serial communication; this system adapts to the user's cognitive abilities and motor skills but is limited by the number of words and phrases the user knows, as well as their mobility since only voluntary blinking is required for use. People diagnosed with SQCP can communicate easily, improving their social interactions and overall quality of life and facilitating communication with their caregivers

Disclosures: G.D.C. Lopez-Armas: None. **Y. Nava-Razo:** None. **A. Arenas-Perez:** None. **J. Margain-Moreno:** None. **L.I. Guerrero-Linares:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.05/XX52

Topic: E.05. Brain-Machine Interface

Support: NSERC RGPIN - 2020-06757

Title: A flexible machine learning approach to EMG biofeedback for accessible exercise interventions

Authors: *S. L. TOEPP[1], A. J. NELSON[2];
[1]Kinesiology, McMaster Univ., Hamilton, ON, Canada; [2]McMaster Univ., McMaster Univ., Stoney Creek, ON, Canada

Abstract: Neurological injuries caused by stroke, spinal trauma, or degenerative disease can severely impact voluntary control and reduce participation in everyday activities. Disuse of the effected sensory and motor pathways can exacerbate functional decline, making exercise-based rehabilitation interventions extremely important. Electromyography (EMG) biofeedback training

can support exercise in severely motor impaired individuals where meeting minimum strength or dexterity requirements of traditional resistance and aerobic exercise is difficult. Machine learning techniques can leverage surface EMG to recognize and reinforce muscle activity patterns that reflect the performance of prescribed training exercises. We have developed a machine learning procedure that maps 6 different muscle activation patterns to the controls of a block stacking game. Our procedure accommodates any number of sensors, and with appropriate sensor placement, movement cues, and signal quality, it can train virtually any movement pattern. With this flexibility, training can be tailored to the specific needs of each individual user, which is a key requirement for diverse neurological populations. We also store data from each training session to create a "global" databank that our algorithm uses to minimize the impact of between-session inconsistencies in electrode placement and other factors. The collected data may also be used by clinicians to study the rehabilitation process. In healthy volunteers, we show that our approach supports stable control of the biofeedback game during training of one upper or lower limb, inter-limb coordination, and neck and shoulder movements. We also apply our approach in two individuals with advanced multiple sclerosis, providing proof of concept for its use as an accessible mode of exercise. The result of our development is a useful rehabilitation tool with significant potential for application in motor rehabilitation. Future work will explore applications of this technology in other common scenarios, such as stroke, cerebral palsy, or spinal cord injury, where severe motor impairment limits access to exercise. There may also be also post-surgery applications for our method, such as following knee or hip arthroplasty where EMG feedback could be used to safely reinforce joint movement in the face of extreme pain and poor stability.

Disclosures: **S.L. Toepp:** None. **A.J. Nelson:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.06/XX53

Topic: E.05. Brain-Machine Interface

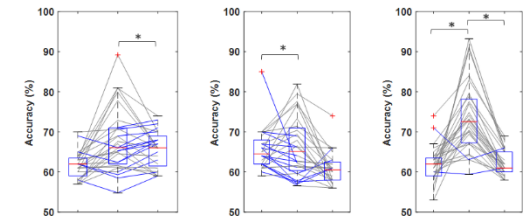
Support: the National Research Foundation of Korea (NRF) grant (No. 2021R1I1A3060828)
Sabbatical Leave Grant at Handong Global University(HGU-2023)

Title: Task-to-task transfer learning for user-centered motor imagery brain-computer interface

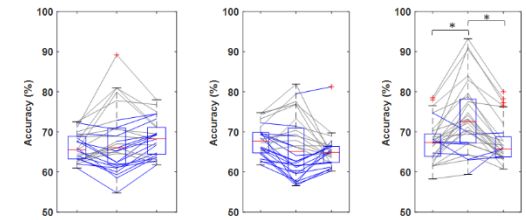
Authors: *D. GWON[1], M. SONG[2], M. AHN[2];
[1]Handong Global Univ., Pohang-Si, Gyeongsangbuk-Do, Republic of, Korea, Republic of;
[2]Handong Global Univ., Pohang, Gyeongsangbuk-Do, Korea, Republic of

Abstract: Motor imagery (MI) plays a crucial role in brain-computer interface (BCI) applications, involving the mental simulation of movements without muscle contractions.

However, the conventional MI paradigm poses challenges, including lengthy calibration times, repetitive sessions leading to fatigue, and a higher prevalence of low-performers. To address these limitations, we propose a novel approach utilizing task-to-task transfer learning with motor execution (ME) and motor observation (MO), which are considered more user-friendly paradigms. The ME involves active physical movement and feedback, fostering higher concentration levels and the MO is a passive paradigm based on observing movements, resulting in reduced fatigue. By utilizing both tasks, we aim to perform MI classification using few/zero-shot learning, based on an Riemannian classifier, employing Riemannian geometry. We conducted MI, ME, and MO experiments involving 28 subjects performing left- and right-hand movements (50 repetitions each). The signal, filtered from 5 to 40Hz, was analyzed for accuracy by shifting in 0.2-second increments from -2 to 3 seconds, with a window size of 3 seconds. The accuracy evaluation included a 10x10 cross-validation for within and 4 cross-validations, including 20% for few-shot learning. The accuracy represents the performance achieved within the optimal time window for each subject. Figure 1 shows that the ME training model is 64.46%, statistically no different from the performance within MI (66.49 %) ($p > 0.05$). Conversely, the MO training model exhibited lower accuracy compared to within-MI classification. Regarding few-shot learning, no significant differences were observed in the ME (67.78 %) and MO (65.13 %) training models when compared to within-MI performance. These findings suggest that transfer learning enables the classification of MI within subjects using ME and MO that are relatively easy tasks. Thus, we believe that this novel approach can improve the usability of the existing MI-BCI systems, particularly for patient groups with movement disability.



Zero-shot training									
Train	MO	ME	MI	ME	MI	MO	ME	MO	MI
Test	ME	(Within)	ME	MI	(Within)	MI	MO	(Within)	MO
Average	61.71	67.85	65.67	65.46	66.21	60.82	61.85	73.62	62.07



Few-shot training									
Train	MO	ME	MI	ME	MI	MO	ME	MO	MI
Test	ME	(Within)	ME	MI	(Within)	MI	MO	(Within)	MO
Average	66.22	67.85	68.47	67.78	66.21	65.13	67.52	73.62	67.40

* Wilcoxon signed the rank test $p < 0.05$

Figure 1. Zero/few-shot accuracies of ME, ME, and MO

Disclosures: **D. Gwon:** None. **M. Song:** None. **M. Ahn:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.07/XX54

Topic: E.05. Brain-Machine Interface

Support: MSIT, IITP Grant 2017-0-00432

Title: Improving classification performance of MI-based BCI in inefficient subjects using hybrid imagery trainning combined with motor and somatosensory stimuli: An approch focused on deep learning method in four-classes motor imagery

Authors: *J. WOO[1], S. PARK[3], J. HA[1], L. KIM[2];
[1]Bionics Res. Ctr., [2]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Hanyang Univ., Seoul, Korea, Republic of

Abstract: The impediments to the advancement and utilization of brain-computer interface (BCI) technology can be attributed, in part, to the inefficiencies observed in information transfer rates and reliability. Efforts to improve performance on inefficient subjects of imagery-based BCI involve technological advancements in algorithms and enhancements in human factors such as training method. The principal aim of this study was to improve the classification effectiveness of a four-class motor imagery-based BCI (four-class: left hand, right hand, right foot, and tongue) of inefficient subjects, combining motor and somatosensory activity, with the intent of offering a superior operational and imaginary standard. Fifteen healthy individuals was enrolled in this experimental protocol, comprising two experimental paradigms: (1) control-condition, exclusively employing motor imagery, and (2) hybrid-condition, a combination of motor imagery and somatosensory stimuli, specifically using a rough textured ball. Applying the deep learning algorithm based on EEGNet across both paradigms, approximately 67% was employed as training data, while around 33% served as testing data. The outcomes were evaluated through a five-fold cross-validation process. Test data of control-condition and hybrid-condition for all participants resulted in average accuracy of 60.83±24.86% and 70.68±19.07%, respectively. The aforementioned results demonstrate an enhancement of approximately 7% when compared to the mean accuracies (control-condition: 53.48±21.17%, hybrid-condition: 63.31±17.11%) achieved with the Minimum Distance to Mean algorithm utilizing geodesic filtering. Within the sub-group identified as inefficient subjects, the hybrid-condition showed a significant enhancement in performance, achieving an accuracy level of 58.02%, reflecting an improvement of 17.26% in comparison to the accuracy rate under the control-condition (40.77%). Conversely, within the sub-group identified as efficient subjects, the difference in accuracy between control-condition (83.75%) and hybrid-condition (85.15%) was only 1.4%. Deep learning algorithm based on EEGNet have helped improve overall performance for the four-class motor imagery classification. The hybrid-condition paradigm delivered enhanced classification for inefficient subjects in the imagery-based BCI, while generating superior event-related desynchronization patterns in motor and somatosensory regions relative to the control-condition. Hybrid-imagery strategy may offer the potential to increase the efficacy of motor imagery-based BCIs, especially for inefficient subjects.

Disclosures: **J. Woo:** None. **S. Park:** None. **J. Ha:** None. **L. Kim:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.08/XX55

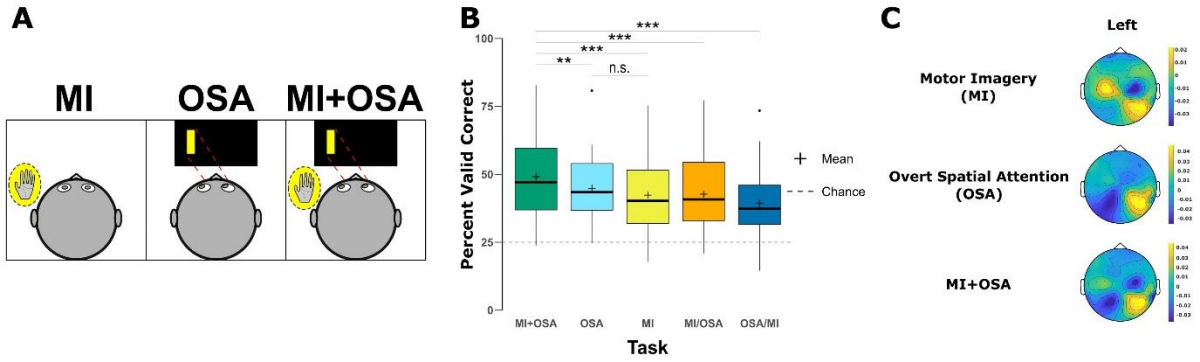
Topic: E.05. Brain-Machine Interface

Support: NIH AT009263

Title: Integrating simultaneous motor imagery and spatial attention for EEG-BCI control

Authors: *D. FORENZO, Y. LIU, J. KIM, Y. DING, T. YOON, B. HE;
Carnegie Mellon Univ., Pittsburgh, PA

Abstract: EEG-based brain-computer interfaces (BCI) are non-invasive approaches for replacing or restoring motor functions in impaired patients and enabling direct brain-to-device communication in the general population. Motor imagery (MI) is one of the most used BCI paradigms, but its performance varies across individuals and certain users require substantial training to develop control. In this study, we propose to integrate MI simultaneously with OSA, a recently proposed paradigm, to develop a new combined method for BCI control (Fig. A). We studied a cohort of 25 human subjects (mean age: 25.5, 24 right-handed, 15 male) and evaluated their ability to control a virtual cursor in one- and two-dimensions over 5 BCI sessions. The subjects used 5 different BCI paradigms: MI, OSA, MI and OSA simultaneously towards the same target (MI+OSA), and MI for one axis while OSA controls the other (MI/OSA and OSA/MI). Our results show that MI+OSA reached the highest average online performance in 2D tasks at 49% Percent Valid Correct (PVC), and statistically outperforms using both MI alone (42%) and OSA alone (45%) (Fig. B). MI+OSA had a similar performance to each subject's best individual method between MI alone and OSA alone (50%) indicating that the new method may be able to use the strongest available features for decoding. Nine subjects reached their highest average BCI performance using MI+OSA suggesting that in some cases, combining MI and OSA can produce better decoding than either individual feature set. Electrophysiological analysis reveals that the combined MI+OSA method produces both MI and OSA features simultaneously (Fig. C), and that there is no significant difference in the strength of the features between MI+OSA and each individual method. Overall, the results show that integrating MI and OSA together leads to improved performance over both individual methods at the group level and is the best BCI paradigm option for some subjects. This work proposes a new BCI control paradigm that integrates two existing paradigms and demonstrates its value by showing that it can improve users' BCI performance.



Disclosures: **D. Forenzo:** None. **Y. Liu:** None. **J. Kim:** None. **Y. Ding:** None. **T. Yoon:** None. **B. He:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.09/XX56

Topic: E.05. Brain-Machine Interface

Support: Samuel F. Hulbert Chair Endowment

Title: Superimposition of flashing indicators on a physical device in a mixed reality environment for EEG prosthetic arm control

Authors: *A. W. L. CHIU[1], Z. HE[2], B. FISHER[1], Y. DAI[2], E. PADGETT[2], R. SU[2], J. SUSLOWICZ[1], J. SONG[2];
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Abstract: Motion artifacts can negatively impact the performance of EEG-based motor rehabilitation applications. Steady-state visual evoked potential (SSVEP) prosthetic control uses flashing targets (such as LEDs) placed along the strategic locations in the physical environment. Depending on the placement of these physical stimuli, they may be obstructed due to the prosthetic device's or the user's movement, which could be problematic. Using only a low-cost NREAL Headset (~$1,000 USD, 540p, 30FPS), we develop a system where real-time interactions between real-world scenes and digitally created content can be captured by superimposing the virtual flashing indicators on the headset. The system consists of three physical components: a streaming webcam that captures live video of a prosthetic arm, a laptop that processes the live stream and generates simulated flashing light effects at corresponding positions on the prosthetic with adjustable frequencies, and an augmented reality headset that displays the real-time video to the user with an immersive mixed-reality view. We test our system using an open-source 3D-printed robotic arm from InMoov. Four simple shapes or markers (triangle, square, pentagon, hexagon) are positioned along the robotic arm. Here, each shape would be tuned to a particular flashing frequency, adjustable between 1-10 Hz, and corresponds to a particular arm motion. Instead of a traditional SSVEP study where a particular flashing frequency would only show up in one target, multiple copies of each shape are placed in such a way that at any robotic arm orientation and position, at least one copy of each shape is visible. Using the Canny Edge Detection technique, structural information from vision objects can be extracted such that the superimposed image of each simple shape would flash at a particular frequency in the augmented environment. We test the arm moving speed to measure the consistency of shape detection when the prosthetic arm moves at different speeds (Still 0 cm/sec; Slow 3 cm/sec; and Swift 10 cm/sec). We find that at low flashing frequency (<4Hz), the shape detection accuracy remains high (>98%). The shape detection accuracy drops from 94% (for still) to 86% (for slow) and 77% (for swift) at high flashing frequency (>9Hz). Furthermore, we found that the accuracy of stationary SSVEP detection using canonical coherence analysis (CCA) when flashing stimuli are applied in the mixed reality environment (77% accuracy, 25.40 Bit/Min) is only slightly lowered when compared to the real-world physical environment (80% accuracy, 28.83 Bit/Min) if screen refresh rate and the flashing frequencies are carefully selected.

Disclosures: **A.W.L. Chiu:** None. **Z. He:** None. **B. Fisher:** None. **Y. Dai:** None. **E. Padgett:** None. **R. Su:** None. **J. Suslowicz:** None. **J. Song:** None.

Poster

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Topic: E.05. Brain-Machine Interface

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Reality Labs at Meta

Title: High-frequency modulation of motoneuron activity during movement preparation and cancellation

Authors: *B. ZICHER[1], S. AVRILLON[1], J. IBAÑEZ[2,1], D. FARINA[1];
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Abstract: Oscillatory synchronization has been widely reported as a mechanism of modulating neural population activity. In the motor cortex, beta (13-30Hz) and gamma (30-60Hz) oscillations have been studied in the context of movement preparation, execution, and cancellation. We developed an experimental paradigm that explores these oscillations in the firings of populations of spinal motoneurons during various motor states. Brain and muscle activity were recorded with electroencephalography (EEG) and high-density electromyography (HD-EMG) during a 'GO'/'NO-GO' task. In each trial, subjects (N=9, all males, ages 21-38 years) were asked to hold an isometric contraction of ankle dorsiflexion and to prepare for a ballistic movement when presented with a warning stimulus. When the imperative cue arrived 1s later, they either had to go ahead with the fast contraction ('GO' trial) or to cancel the preparation without releasing the stable contraction ('NO-GO' trial). The two types of trials were presented randomly in equal amounts. The firings of an average of 32 motor units (MU) per subject were decomposed from the HD-EMG signals using blind source separation methods. The spiking activities were summed to get the cumulative spike train (CST), and the spectral content of the CST was analysed during the changing brain states. From the 'NO-GO' trials, where subjects successfully cancelled the preparation and kept the force stable (94.68 ± 3.80% SD), three main time intervals were analysed: -2s to 1s, -1s to 0s and 0s to 1s relative to the imperative cue that occurred at 0s. These represented the baseline, preparatory and preparation cancellation

periods. We observed a significant change in beta power in the MU activity during the trials. Beta levels were increased in the last interval (0s to 1s) compared to the first ($p < 0.01$) and second intervals ($p < 0.001$). The same changes could be observed at the brain level in the EEG recordings ($p < 0.05$ and $p < 0.001$). Furthermore, significant corticomuscular coherence in beta was found in seven out of nine subjects in the preparation cancellation period. Similar changes were also seen in lower (8-12Hz) and higher (30-45Hz) frequencies at the MU level, with only the power in low gamma also increasing at the brain level. These results suggest that the activity of spinal motoneurons is modulated by high frequency cortical oscillations during states when force is held constant.

Disclosures: B. Zicher: None. **S. Avrillon:** None. **J. Ibañez:** None. **D. Farina:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.11/XX58

Topic: E.05. Brain-Machine Interface

Support: JSPS KAKENHI Grant Number 21J20955
JSPS KAKENHI Grant Number 20H05923

Title: Repeated voluntary up-and down-regulation of human sensorimotor rhythm magnitude stabilizes excitatory cortical state to suppress momentary relaxation: a double-blind, randomized, sham-controlled study

Authors: *S. IWAMA[1], A. MATSUOKA[3], J. USHIBA[2];
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Abstract: The adaptive contraction of muscles controlled by sensorimotor neural network composes of dexterous human behavior. However, it is unclear how the network subserving motor control enables both rapid and stable motor output in the primary motor cortex (M1) as well as its release. Here, to investigate the human ability of voluntary excitability control of M1, we developed an experimental paradigm aided by non-invasive brain-computer interfaces (BCI) based on the established neural representation of sensorimotor excitability, namely, sensorimotor rhythm (SMR; Takemi et al., 2018).In the present study, participants underwent a two-day training in the bidirectional volitional control of SMR magnitude in response to visual cues indicating the desired cortical state, that is the excitatory and idling state indexed by event-related spectral perturbation. During the online BCI control based on SMR in EEG signals, we asked participants to repetitively perform motor attempt and voluntary relaxation of right fingers. For a 20-second task period in a trial, participants tried bidirectional modulation of the corticomotor excitability as indexed by SMR-ERD magnitude derived from the left hemisphere. Along with the instruction, participants received online feedback of SMR-ERD magnitude as the

bar height, and the half received yoked-sham neurofeedback of SMR-ERD, that is the feedback of other participant EEG data.First, we analyzed the scores acquired during the training of SMR control; participants acquired one point when the magnitude of SMR-ERD reached the instructed state. The total scores acquired in each session were subjected to a two-way repeated measures ANOVA considering BCI type (Verum or Placebo) and time (number of sessions). A significant interaction was found ($F = 2.16$, $p < 0.05$) and post-hoc Holm test revealed a significantly higher scores in the last session of BCI training than those in the middle session of day 1. Moreover, the time course of SMR-ERD magnitude revealed that the verum group exhibited a shorter time period to reach excitatory state (i.e., SMR-ERD induction with motor attempt) than that to reach relaxation. The effect was not found in the sham group, and conversely, the time period to release induced SMR-ERD was rather shorter in the sham group than that of verum-group. Collectively, voluntary control of SMR-ERD magnitude with the aid of closed-loop neurofeedback stabilizes excitatory state of M1, suggesting the release of aberrantly contracted muscles are rather refractory and requires involvement of additional inhibitory activities.

Disclosures: **S. Iwama:** None. **A. Matsuoka:** None. **J. Ushiba:** A. Employment/Salary (full or part-time):; LIFESCAPES Inc.. F. Consulting Fees (e.g., advisory boards); LIFESCAPES Inc..

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.12/XX59

Topic: E.05. Brain-Machine Interface

Support: NIH R01 NS124564
NIH R01 AT009263
NIH T32 EB029365

Title: Transcranial focused ultrasound improves brain-computer interface by amplifying the targeted activity in visual cortex

Authors: *J. KOSNOFF[1], K. YU[1], C. LIU[3], B. HE[1,2];
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Abstract: An estimated 1 in 50 Americans suffer from paralysis. This condition is cureless, but brain-computer interfaces (BCI) can offer symptom relief through controlling an external device with brain signals. Current BCI outcomes leave room for improvement, which may be achieved through use of neuromodulation technologies. In this study, the effects of transcranial focused ultrasound (tFUS) were examined on a motion-onset visual evoked potential (mVEP) BCI speller. Subjects stared at an intended letter on a virtual keyboard while lines flashed subsequently across the keyboard's rows and columns. When a line moved across the key the subject was looking at, an mVEP was generated. The timing of these mVEPs were decoded into

a keyboard row and column index. Three experimental conditions were tested in a random order: non-modulated (NM; no active tFUS), sham tFUS (decoupled active ultrasound), and pulsed tFUS (2 MPa estimated peak-peak pressure, 3 kHz pulse repetition frequency, 167 μs pulse duration, 500 ms sonication duration) applied to the left hemisphere (LH) V5, a region associated with processing visual motion. Healthy subjects were recruited to the study and were excluded if their NM BCI classifier was at a random chance. The resultant data from 14 subjects (7 M / 7 F) were used for analysis. Behavioral performance was measured by the Euclidean distance error between the BCI decoder's predicted letter and the subject's intent, normalized with respect to the trial's ultrasound dosage and the number of BCI scans. Electrophysiological LH V5 data were extracted using MNE Python's minimum norm source imaging analysis with FreeSurfer segmented regions (Brodmann Label *MT_exvivo-lh*) and filtered between 3.5 and 7 Hz. Neural data were normalized against their trial specific baseline and examined in terms of the N200 amplitude, calculated by taking the peak-peak amplitude between the most negative peak in the 120 - 350 ms post stimulus window and the positive peak directly preceding it. Statistical analysis was performed with a two-tailed Wilcoxon Rank Sum test and Bonferroni α correction. Behavioral results show a significant ($\alpha = 0.05/3 = 0.0167$) decrease in Euclidean error for tFUS (9.00% ± 11.6) compared to both sham (14.2% ± 14.5) and NM (14.1% ± 12.6) conditions. LH V5 analysis yields a significant ($\alpha = 0.05/3 = 0.0167$) increase of the N200 mVEP theta band amplitude in tFUS condition (3.95 ± 2.32 pA*m) over both sham (3.22 ± 1.72 pA*m) and NM (3.64 ± 2.05 pA*m) conditions. Here we show, for the first time, that tFUS modulation at V5 can improve BCI outcomes. Neural analysis suggests that the mechanism of action of this improvement may be related to amplification of targeted brain signals.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.13/XX60

Topic: E.05. Brain-Machine Interface

Support: NIH Grant P41 EB018783
Stratton VA Medical Center
NVIDIA Corp

Title: Enhancing BCI-based color vision assessment

Authors: M. DIRAIMO[1], J. CARP[2,1,3], ***J. NORTON**[3,1,2];
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Abstract: Recent research in our laboratory has demonstrated a non-invasive brain-computer interface (BCI)-based method for color vision (CV) assessment. Unlike most existing CV

assessments, our method is fully automatic, does not require the user's active participation, and may provide a more precise definition of color vision deficiencies (CVDs). Early experiments have concentrated on two-dimensional linear grid searches of a color space to identify a dichromatic test source (i.e., 525 (green (*g*)) and 625 (red (*r*)) nm) that is the same color (i.e., a *metamer*) as a monochromatic reference source (i.e., 590 nm (amber)). People with vs. without CVDs identify different light sources as metamers. Grid searches are slow, requiring the collection of a substantial amount of data. To enhance the speed of BCI-based CV assessment for the identification of congenital CVDs, we developed a new protocol that reduces the dimensionality of the search to one by using light sources with equal illuminances (137 lux, Urceli MT-912). The reference source produced this illuminance at 150 D/A units. Sixteen different settings of the test source ($T_{1 \cdots 16}$) (i.e., 107 g, 0 r; 105 g, 10 r; 99 g, 20 r; etc. in D/A units) that emitted light at this illuminance were also identified. Three participants (S_1-S_3) completed a behavioral task (repeated 4 times) where they identified settings of the test source that were most similar in color to the reference source ($T_{metamer}$). Then, they completed an EEG experiment (8 channels over occipital cortex, 1200 Hz) where they looked at a visual stimulus that alternated between the reference source and the test sources (ordered from T_1 to T_{16}, 3 s each, 10 runs/participant, 8 mins of data total) at 10 Hz. We predicted that $T_{metamer}$ would elicit the smallest steady-state visual evoked potential (SSVEP) (measured using canonical correlation analysis). Preliminary results support our prediction. The settings of the test source that the participants behaviorally identified ($T_{9.3}$, T_{10}, and $T_{9.5}$) as being $T_{metamer}$ were similar to those that elicited the smallest SSVEPs (T_{11}, T_6, T_{10}), although there was an unexpected peak in SSVEP size for S_2 at T_9. Results of these pilot experiments suggest our new protocol may enhance the speed of BCI-based CV assessment. With refinement and confirmation—which we will achieve through the recruitment of additional participants, including people with CVDs—this protocol may enable the fast and automatic identification of congenital CVDs in children, people with cognitive impairments, or those with severe motor deficits who are poorly served by existing assessments of CV.

Disclosures: **M. DiRaimo:** None. **J. Carp:** None. **J. Norton:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); NVIDIA Corp.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.14/XX61

Topic: E.05. Brain-Machine Interface

Support: JST Moonshot R&D (JPMJMS2012)

Title: Thirty-minute motor imagery exercise aided by EEG sensorimotor rhythm neurofeedback increases corticospinal excitability: a sham-controlled study

Authors: *T. FUJIMAKI[1], S. IWAMA[2], M. TAKEMI[1], J. USHIBA[2];
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Abstract: Neurofeedback training with electroencephalogram (EEG)-based brain-computer interfaces (BCIs) and motor imagery exercise can volitionally modulate the excitability of the sensorimotor cortex without overt movement. However, it remains unclear how much neurofeedback enhances corticospinal excitability and how long the induced excitability lasts. Here, we investigated changes in corticospinal excitability and the duration of treatment effect after a 30-minute BCI-based neurofeedback training, which aimed to regulate sensorimotor rhythm (SMR) in scalp EEG. Ten right-handed healthy adults were recruited in this study and performed kinesthetic motor imagery of right index finger movement with online feedback of SMR. The participants received either real-time feedback of event-related desynchronization (ERD) of SMR magnitude from the contralateral sensorimotor cortex (SM1) or yoked-sham placebo feedback. To evaluate the corticospinal excitability change, robotic transcranial magnetic stimulation (TMS) was used before and after about 30 minutes of neurofeedback training. Amplitudes of motor-evoked potentials from the right first dorsal interosseous were recorded and fitted to a stimulus-response curve (SRC) to estimate variables reflecting the net corticospinal output. EEG analysis revealed that SMR-ERD occurred in both groups' alpha and beta frequency bands during motor imagery exercise. However, corticospinal excitability was transiently enhanced only in the group receiving veritable neurofeedback. The plateau of the SRC increased and remained elevated at 15, 30, and 45 minutes after the intervention compared to the pre-exercise level. In contrast, the placebo group did not exhibit a comparable increase in the SRC plateau. These findings suggest that providing individuals with real-time feedback from their brain activity during motor imagery enhances corticospinal excitability and this modulation effect lasts for at least 45 minutes. Future studies should use power analysis from the present results to determine sample size and demonstrate these effects in a double-blind randomized controlled trial.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.15/XX62

Topic: E.05. Brain-Machine Interface

Support: JST Moonshot R&D (JPMJMS2012)

Title: Bci-based neurofeedback with muscle relaxation enhances inhibitory activity in human sensorimotor cortex

Authors: *H. KURASHIKI[1], S. IWAMA[1], J. USHIBA[2];
[1]Dept. of Biosci. and Informatics, Fac. of Sci. and Technol., [2]Grad. Sch. of Sci. and Technol., Keio Univ., Kanagawa, Japan

Abstract: Neurofeedback training using electroencephalogram (EEG)-based brain-computer interfaces (BCIs) has recently gained attention as a new therapeutic approach to restore motor function in patients with neurological diseases. However, it remains unclear whether rehabilitative BCI is applicable as a treatment for hyperkinetic neurological diseases such as dystonia which hampers voluntary muscle relaxation due to thalamo-cortical dysrhythmia. This proof-of-concept study experimentally tested whether BCI-based neurofeedback, which trains subjects to self-modulate sensorimotor rhythms (SMR) in EEG, a biomarker of the thalamo-cortical loop, allows users to lead the motor circuit to an inhibitory state during muscle relaxation in healthy subjects. We asked right-handed healthy subjects (n=20, 19-31 years, 8 female) to increase SMR event-related synchronization (SMR-ERS) during muscle relaxation after a right-hand grasping movement, aided by visual feedback. The study employed a single-blind, sham-controlled design; subjects were assigned to the real feedback group (Real), where the feedback was computed from their own contralateral primary sensorimotor cortex, or the sham feedback group, where the feedback was computed from others. To control the contraction level of right finger muscles before muscle relaxation exercise, we instructed subjects to maintain force within the range of 5-25% of maximal voluntary contraction, displayed as visual feedback before muscle relaxation. We assessed changes in SMR-ERS amplitudes, other ERS from different electrodes and frequency bands, and force in pre- and post-neurofeedback training using qualitative visualization, repeated-measures ANOVA, and post hoc t-tests (Bonferroni correction). Time-frequency and topographic map visualization showed time-, frequency-, and region-specific increases in SMR-ERS only in Real. We also found a significant interaction (group x session, $F(1,18) = 4.492$, $p = 0.048$) and a simple main effect ($F(1,9) = 20.098$, $p = 0.002$) in Real. Post hoc t-test revealed a significant increase in SMR-ERS in Real ($t(9) = 4.483$, $p = 0.009$, Cohen's $d = 1.418$), but not in other electrodes or frequency bands. Furthermore, force visualization revealed the onset and subsequent reduction in force during the experiment. There was only significant interaction between session and time ($F(5.361,54) = 2.548$, $p = 0.027$), but no simple main effect was found. These results suggest that BCI-based neurofeedback could specifically modulate cortical activity, which reflects the thalamo-cortical loop. This approach is expected to lead to a new training system for treating hyperkinesia in focal dystonia.

Disclosures: H. Kurashiki: None. **S. Iwama:** None. **J. Ushiba:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); He is the founder and representative director of LIFESCAPES Inc. He receives a salary from this company. The company does not have any relationships with the device or setup used in the current study.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

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Program #/Poster #: PSTR380.16/XX63

Topic: E.05. Brain-Machine Interface

Support: KAKENHI Grant Number 20H05923

Title: Improving the motor imagery skill through functional electrical and mechanical stimulation

Authors: *K. KUSANO[1], M. HAYASHI[2], J. USHIBA[2];
[1]Grad. Sch. of Sci. and Technol., Keio Univ., Yokohama-shi, Japan; [2]Keio Univ., Keio Univ., Kanagawa, Japan

Abstract: Brain-Computer Interfaces (BCI), which utilize event-related desynchronization (ERD) observed over contralateral sensorimotor cortices (SMC), have emerged as a promising treatment for stroke hemiplegia. However, the efficiency and scope of BMI rehabilitation remain challenging due to the weakness of ERD during motor imagery (MI) in patients with the compromised sensorimotor function of their paralyzed limbs. Increasing SMC excitability prior to BCI training could potentially improve treatment outcomes. Here, we investigated whether instructing somatosensory MI sensations as a pre-conditioning strategy can enhance MI ability and ERD intensity. Five healthy right-handed individuals participated in this study. Each participant underwent five stimulating sessions, with each session containing 20 trials. In addition, three evaluation sessions, comprised of pre-, mid-, and post-stimulation phases, were performed. Whole-head high-density scalp EEG was recorded throughout the experiments. During the stimulating sessions, functional electrical stimulation (FES) was administered to the extensor digitorum communis muscle of their non-dominant hand. Simultaneously, mechanical stimulation involving hand extension induced by an exoskeleton robot was employed. Participants were asked to focus on sensory sensations rather than engaging in MI. During the evaluation sessions, participants were instructed to perform MI of non-dominant hand extension. Only visual cues indicating “Rest” and “Imagery” were presented without feedback on their MI performance. We examined the immediate effects by assessing ERD intensity in the contralateral (right) SMC during the stimulating sessions. Subsequently, we investigated whether somatosensory instruction improved MI performance by analyzing changes in ERD intensity across the evaluation sessions. As a result, during the stimulating sessions, the contralateral ERD was observed during the stimulation period in the alpha (8~13 Hz) and beta (15~30 Hz) bands in all participants. Also, higher ERD was confirmed in the alpha and beta bands in the post-stimulation evaluation session in 4 out of 5 participants. These findings suggest that incorporating FES and mechanical stimulation as pre-conditioning can improve MI performance. Further investigations involving healthy individuals and studies with stroke patients hold the potential to enhance the efficacy of BCI-based rehabilitation strategies.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.17/XX64

Topic: E.05. Brain-Machine Interface

Title: Investigating cortical neural signatures in Parkinson's disease

Authors: K. ZENG[1,3], A. BHATTACHARYA[1], ***R. ANNIROOD**[2], N. DESAI[1], X. LI[3], R. CHEN[1,4];
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Abstract: Cortical neural signatures in Parkinson's disease
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Background: Changes in resting-state brain activities have been demonstrated in Parkinson's disease patients. However, the nature of abnormal cortical neural activities in Parkinson's disease remains uncertain. The aim of the present study was to comprehensively explore the cortical neural signature of PD.
Method: We recorded resting-state magnetoencephalographic (MEG) signals in patients with Parkinson's disease (N = 30) on and off medications, and in healthy age- and sex-matched participants (N = 32). A series of neural indexes including absolute and relative power, aperiodic components, beta burst and cross-frequency coupling, were extracted from the MEG data.
Results: 1) Absolute power analysis showed enhanced alpha, beta and gamma power in PD; 2) Relative power analysis showed a widespread increase in theta and low alpha power, as well as a reduction of beta and gamma power in PD; 3) Aperiodic components: greater aperiodic exponents and offset was found in PD; 4) Beta burst: a higher percentage of longer beta bursts along with the increase in their incidence rate was observed in PD.; 5) cross-frequency coupling: phase amplitude coupling between beta oscillation and broadband gamma activity (50-150 Hz) was elevated in PD.
Conclusion: We found several neural signatures of PD in resting state MEG. Combining these features in future studies may lead to better understanding of the pathophysiology of PD and the mechanisms underlying specific PD symptoms, and could potentially lead to use of MEG in the clinical care of PD.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.18/XX65

Topic: E.05. Brain-Machine Interface

Title: Assessing motor function of chronic stroke survivors through the combination of non-invasive EMG biomarker features

Authors: *N. TACCA[1], I. BAUMGART[1], B. SCHLINK[1], A. KAMATH[1], M. DARROW[1], L. WENGERD[2,1], D. FRIEDENBERG[1], E. MEYERS[1];
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Abstract: Deficits in upper limb function are profoundly disabling and significantly affect one's ability to complete activities of daily living. The current standard of care in rehabilitation is limited due in part to the significant variability among stroke survivors. This increases the difficulty in developing personalized therapy protocols that target a patient's specific weaknesses. As novel methods emerge to enhance the current state of rehabilitative care, an objective measure to track and predict functional ability has the potential to help inform and personalize therapy protocols. Non-invasive, high-density electromyography (HD-EMG) has emerged as a simple-to-use yet powerful technique to collect a range of distinct neurophysiological motor information in humans. HD-EMG data can be decomposed into various features to investigate different properties of neuromuscular control, with recent studies demonstrating changes in these features that occur after stroke and throughout therapy. Co-contraction index (CCI), muscle synergy composition, and motor unit coherence each capture unique aspects of function and have been shown to be altered after stroke. Importantly, as function returns, these features trend toward that of healthy motor function, highlighting the potential of these methods to serve as useful biomarkers. However, previous studies have largely explored these EMG features as candidate biomarkers in isolation, limiting their potential clinical utility. Here, we demonstrate the ability to predict hand and wrist function based on a combination of EMG biomarker features decomposed from a 150 electrode HD-EMG forearm sleeve. Able-bodied subjects (N=14) and chronic stroke subjects undergoing intensive therapy (N=7) performed cued sets of 12 functional hand and wrist movements while EMG was recorded. Three biomarker feature sets were decomposed from the EMG data, namely CCI by movement, muscle synergy composition, and motor unit coherence by movement. These biomarker feature sets were combined into a single correlated latent feature set using canonical correlation analysis. We demonstrate that the correlated latent features were able to dissociate between able-bodied, mild, and severe stroke subject groups based on their upper-extremity Fugl Meyer hand sub-score. A 3-fold cross-validation with logistic regression classifier achieved an average accuracy of 90.48 +/- 4.76% in predicting subject groups across all folds. These results demonstrate the feasibility of predicting functional motor ability through the combination of EMG biomarker features, which can help monitor and guide therapy.

Disclosures: **N. Tacca:** None. **I. Baumgart:** None. **B. Schlink:** None. **A. Kamath:** None. **M. Darrow:** None. **L. Wengerd:** None. **D. Friedenberg:** None. **E. Meyers:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.19/XX66

Topic: E.05. Brain-Machine Interface

Support: Ohio Third Frontier GR121423

Title: Non-invasive neurotechnology innovation for individuals with spinal cord injury (sci)

Authors: A. KAMATH[1], *S. COLACHIS[1], L. WENGERD[3], I. BAUMGART[5], J. KECKLER[1], T. H. LUCAS, JR[4], D. FRIEDENBERG[2];
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Abstract: Spinal Cord Injury (SCI) survivors with resultant tetraplegia have consistently ranked restoration of hand function as a top priority to maximize their independence and quality of life. While promising advancements have been made with surgically implanted, high-performance brain-computer interface controlled functional electrical stimulation (BCI-FES), these systems are currently limited to lab use, are not ready for immediate translation, and are less accessible to many due to the surgical requirements. In response to the SCI community feedback, we have been developing a non-surgical neuro-orthotic that provides upper limb reanimation via a Functional Electrical Stimulation (FES) sleeve that is controlled non-invasively by the user with their preferred control methods (button, tablet, sip-and-puff, electromyography (EMG), etc.). In this study, we have been gathering feedback on preferred FES control systems from a wide range of individuals with tetraplegia due to SCI, developing these systems based on gathered feedback, and evaluating system functionality using standardized clinical assessments. We have enrolled two participants (C5 ASIA A; C6 ASIA C) to date and begun evaluating their functional abilities with and without their preferred FES control modality using the Toronto Rehabilitation Institute Hand Function Test (TRI-HFT) and the Grasp and Release Test (GRT). Both study participants expressed the most interest in an EMG control modality, among all non-invasive options provided. To address participant feedback, we modified our FES sleeve technology to enable recording of motor intention signals from the same sleeve electrodes using proprietary EMG-FES hardware. With this EMG controlled FES system both participants have already been able to perform activities of daily living that they were previously unable to perform without caregiver assistance (ex. drinking from and pouring from a mug, manipulating pillows, etc.). These improvements have been measured thus far using the TRI-HFT. For many of these functional activities, our sleeve technology was able to decode motor intention from EMG signal despite the lack of palpable movement. Additionally, we have been able to calibrate the following functional movements, among others, for both participants using the FES sleeve: Key

Pinch Grip, Hand Extension/Flexion, Palmar Grip, Tip-to-Tip Pinch. Our results preliminarily indicate the effectiveness of an EMG controlled FES orthotic for individuals with SCI, despite lacking palpable movement in many cases. With this non-invasive technology, individuals with SCI may one day soon be able to use their own hands again and regain independence.

Disclosures: **A. Kamath:** A. Employment/Salary (full or part-time):; Battelle Employee. **S. Colachis:** A. Employment/Salary (full or part-time):; Battelle Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **L. Wengerd:** A. Employment/Salary (full or part-time):; Battelle and OSU Employee. **I. Baumgart:** A. Employment/Salary (full or part-time):; Battelle Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder. **J. Keckler:** A. Employment/Salary (full or part-time):; Battelle Employee. **T.H. Lucas:** A. Employment/Salary (full or part-time):; OSU Employee. 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.; Grant PI. **D. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Employee. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patent Holder.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.20/XX67

Topic: E.05. Brain-Machine Interface

Title: Functional motor recovery after intention-driven functional electrical stimulation therapy using a wearable sleeve: a case study in two chronic stroke survivors with hemiparesis

Authors: *I. BAUMGART, M. DARROW, N. TACCA, L. WENGERD, D. A. FRIEDENBERG, E. C. MEYERS;
Battelle Mem. Inst., Columbus, OH

Abstract: Background: Functional electrical stimulation (FES) has been recognized for decades as a method to retrain the motor system after stroke. Administering FES with the subject's volitional movement attempt has been proposed to drive recovery by pairing ascending sensory feedback from FES-enabled movement with descending motor commands, however clinical evidence is mixed. Key therapy elements are now emerging that suggest FES rehabilitation can be enhanced by synchronizing FES with the user's volitional motor intention, functionalizing the limb for task-oriented rehabilitation, and providing a range of FES enabled movements. Methods: Our group has recently developed the NeuroLife ® Sleeve, a wearable forearm sleeve that contains a high-density grid of embedded electrodes capable of simultaneous EMG recording and FES delivery. During task-oriented practice, intention-driven FES was delivered

via operator control twice weekly and EMG control once weekly for 8 weeks. Results: Using the wearable sleeve interface, we were able to set up FES configurations and EMG decoding algorithms before 57 ± 3 minutes of task practice at each 120-minute session. 6 functional movements were created for participant A and 5 for participant B. Participants received an average of 148 ± 9 individual stimulation events per therapy session with an average duration of 2.50 ± 0.03 seconds per event. At the end of the therapy period, participants A and B improved their scores beyond the minimal clinically important difference (MCID) for Box and Blocks Test (BBT; A: +6, B: +7), the Action Arm Research Test (ARAT; A: +7, B: +12), the Fugl Meyer Upper Extremity section (UEFM; A: +11, B: +9), and the 9-Hole Peg Test (9HPT; A: 158 sec, B: 54 sec, both previously unable). These improvements persisted during the 10-week follow-up period (BBT A: +10, B: +7; ARAT A: +9, B +12; UEFM: A: +9, B: +8, 9HPT A: 97 sec, B: 91 sec). Both participants increased the Amount of Use and Quality of Movement sub-scores of the Motor Activity Log, and both reported personally important improvement (A: ability to tie shoes, B: spontaneous use of impaired arm while cooking). Conclusions: The results from this case series are in agreement with previous studies that demonstrate improvement after intention-driven FES therapy. The addition of task-oriented practice across a variety of movements with intention-driven FES may have produced synergistic effects to the benefit of these participants. The persistence of the improvements in these stroke survivors may indicate neuroplastic effects of this approach.

Disclosures: I. Baumgart: A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **M. Darrow:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **N. Tacca:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **L. Wengerd:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **D.A. Friedenberg:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute. **E.C. Meyers:** A. Employment/Salary (full or part-time):; Battelle Memorial Institute.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.21/XX68

Topic: G.04. Emotion

Title: Estimation of eustress state during factory work using EEG

Authors: *C. FURUTA[1], Y. MURATA[2], H. TANAKA[3], N. NOGUCHI[3], A. ITO[3], K. UEDA[2], N. YAMADA[2], Y. HAMA[3], K. NAGATO[2];
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Abstract: In recent years, mechanization has been promoted to remove the physical burden and hazardous work of factory workers, and productivity has been improved through the development of numerical modeling and digital twin technology.On the other hand, no progress

has been made in addressing the psychological burden of factory workers, which has become a factor hindering productivity. If a model can be created for the workers' psychological state of commitment to labor, and if data acquisition and feedback can be performed in real time, it can be expected to lead to improved productivity. Stress is a well-known concept that is closely related to workers' psychological work efforts.Stress is classified into "good stress (eustress)" and "bad stress (distress).Although there have been studies on the relationship of distress to labor, a method to discriminate between eustress and distress in a simple way that does not interfere with factory operations in real time has not yet been established. It is considered important to increase eustress and decrease distress in order to improve workers' psychological state of commitment to work. In order to apply feedback that increases eustress and reduces distress in real time, it is first necessary to estimate the stress state of factory workers. In this study, we conducted an evaluation using EEG measurement to estimate the stress state in factory work. As a factory task, we designed a task in which the participants were required to follow a model and make a screw. Each of the four participants performed the task with a time limit of 2 minutes. Four trials of each of three difficulty levels with different numbers of screws were performed, for a total of 12 trials. Electroencephalograms (EEG) were measured while the task was being performed. EEG was measured from EEG electrodes placed near Fz, Fp1, and Fp2 on the forehead so as not to interfere with the work. Immediately after the completion of each difficulty level task, a questionnaire for subjective evaluation of the stress state was administered. The EEG results showed that the three subjects had greater Fmθ power at the medium difficulty level. This suggests that an EEG sensor applied to the forehead does not interfere with factory work and may be used as an indicator to measure the degree of work concentration.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.22/XX69

Topic: E.05. Brain-Machine Interface

Support: Georgia CTSA, NIH-NCATS UL1TR002378

Title: Address Practical Constraints of Adaptive Stimulus Selection Algorithm by Thompson Sampling in ERP-based Brain-Computer Interfaces

Authors: *Z. OU[1], J. E. HUGGINS[3], T. MA[2];
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Abstract: Motivation: A P300 Event-Related Potential (ERP)-based Brain-Computer Interface (BCI) speller is a non-invasive tool that assists people with disabilities "type" words without using a physical keyboard. The system displays a series of events on the virtual screen and searches for the elicited target P300 ERP responses among a group of non-target ones. The row-column paradigm (RCP) and checkerboard paradigm (CBP) are two commonly used stimulus presentation methods.
The conventional sequence size for testing is usually large and independent of previous EEG data. Although recent studies have proposed data-driven stimulus selection methods and achieved high spelling efficiency, the results have been limited to relying on classifier scores as inputs due to the inability to conduct offline real data analysis. Therefore, it is important to examine the performance under practical circumstances by integrating real-participant EEG signals.
Method: Building on the previous work[1], we simulate data from offline de-identified EEG datasets and incorporate a training process for a binary classifier to emulate the real data collection . We incorporate a statistical language model into the algorithm by updating the conditional P0. The algorithm is modified to accommodate response delay and refractory effects. To address response delay, we employ the rule of cross-iteration update and quantify the time delay associated with physiological constraint and classifier training. For refractory effects, we partition the extracted EEG signals into multiple training pools by the interval between non-target stimuli in two consecutive target stimuli within a sequence. We adopt a similar stopping rule and establish the evaluation criteria: character-level prediction accuracy, spelling time, information transfer rate (ITR), and utility measure.
Result and Conclusion: The statistical language model prior greatly improves the ITR and utility. Our results underscore the robustness of the algorithm under physiological and practical constraints. This will provide useful guidance for the parameter setup of real-time implementation before moving on to the phase of real-time data collection on participants.
Reference:[1] Ma, et al. Adaptive sequence-based stimulus selection in an ERP-based brain-computer interface by Thompson sampling in a multi-armed bandit problem In *2021 IEEE International Conference on Bioinformatics and Biomedicine (BIBM)*, pp. 3648-3655. IEEE, 2021.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.23/XX70

Topic: E.05. Brain-Machine Interface

Support: The study was funded by the dtec.bw – Digitalization and Technology Research Center of the Bundeswehr (MEXT project). The dtec.bw is funded by the European Union – NextGenerationEU

Title: Semi-automated motor hotspot search (SAMHS): a framework towards an optimised approach for motor hotspot identification

Authors: *D. AGBOADA[1], M. OSNABRUGGE[1,4], R. RETHWILM[2], C. KANIG[1,4], F. SCHWITZGEBEL[3], W. MACK[1], M. SCHECKLMANN[4], W. SEIBERL[2], S. SCHOISSWOHL[1,4];
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Abstract: Background: Motor hotspot identification represents the first of two steps in the determination of the motor threshold and is the basis for the specification of stimulation intensity used for various Transcranial Magnetic Stimulation (TMS) applications. The level of experimenters' experience and the methodology of motor hotspot identification differ between laboratories. The need for an optimised and time-efficient technique for motor hotspot identification is therefore substantial.
Objective: With the current work, we present a framework for an optimised and time-efficient semi-automated motor hotspot search (SAMHS) technique utilising a neuronavigated robot-assisted TMS system (TMS-cobot). Furthermore, we aim to test its practicality and accuracy by a comparison with a manual motor hotspot identification method.
Method: A total of 32 participants took part in this dual-centre study. At both study centres, participants underwent manual hotspot search (MHS) with an experienced TMS researcher, and the novel semi-automated hotspot search procedure with a TMS-cobot (cobot hotspot search, CHS) in a randomised order. Resting motor threshold (RMT), and stimulus intensity to produce 1 mV (SI1mV) peak-to-peak of motor-evoked potential (MEP), as well as MEPs with 120% RMT and SI1mV were recorded as outcome measures for comparison.
Results: Compared to the MHS method, the CHS produced lower RMT, lower SI1mV and a trendwise higher peak-to-peak MEP amplitude in stimulations with SI1mV. The duration of the CHS procedure was longer than that of the MHS. However, accuracy of the hotspot was higher for the CHS compared to the MHS.
Conclusions: The SAMHS procedure introduces an optimised motor hotspot determination system that is easy to use, and strikes a good balance between accuracy and speed. This new procedure can thus be deplored by experienced as well as beginner-level TMS researchers.

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Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.24/XX71

Topic: E.05. Brain-Machine Interface

Support: y the Challengeable Future Defense Technology Research and Development Program through the Agency For Defense Development(ADD) funded by the Defense Acquisition Program Administration(DAPA)

Title: Zero-calibration for the daily use of P300-based brain-computer interfaces

Authors: *D. HEO, S. P. KIM;
Biomed. Engin., Ulsan Natl. Inst. of Sci. and Technol., Ulsan, Korea, Republic of

Abstract: To advance non-invasive brain-computer interfaces (BCIs) toward practical usage, it is critical to address the issue of the recalibration of decoders. Few studies, however, have examined the effect of decoder calibration over days. This study aims to address this issue by comparing calibration-based versus calibration-free decoders over multiple days and to propose a solution to avoid cumbersome daily calibration. A calibration-based decoder was rebuilt every day using the training data obtained on the same day while a calibration-free decoder was built only using the training data on the first day and fixed for the use on successive days. We investigated differences in the performance of P300-based BCIs using either the calibration-based or calibration-free decoders. Then, to avoid daily calibration, we developed a new method to extract daily invariant event-related potentials (ERPs) from observed EEG on each day using the sparse dictionary learning (SDL). Seventeen healthy participants used a P300-based BCI system to operate a household appliance for five days. We evaluated the accuracy of P300-based BCIs online using the calibration-based decoder. Then, we simulated the performance of the same P300-based BCIs using the calibration-free decoder offline and observed lower accuracy than using the calibration-based decoder. In contrast, when we simulated using the proposed method to extract ERPs together with the calibration-free decoder, accuracy increased to the level of using the calibration-based decoder: accuracy improvement was 2.35%, 3.33%, 2.55%, 1.76%, and 2.55% on each day. In light of this result, the proposed method may offer a solution to the issue of daily calibration for P300-based BCIs, which would be essential for bringing non-invasive BCIs into daily life.

Disclosures: D. Heo: None. **S.P. Kim:** None.

Poster

PSTR380. Non-Invasive Neurophysiology and Biofeedback: Therapeutic Applications

Location: WCC Halls A-C

Time: Tuesday, November 14, 2023, 8:00 AM - 12:00 PM

Program #/Poster #: PSTR380.25/XX72

Topic: G.04. Emotion

Title: Developing Affective Brain-Computer Interfaces on Low Dimensional Riemannian Manifolds

Authors: *H. FANG[1], M. WANG[1,2], Z. ZHANG[1], Y. YANG[1,2,3,4];
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Abstract: Affective brain-computer interface (aBCI) uses neural signals to decode the brain's affective state, which has wide applications such as augmenting human-computer interaction in daily lives and quantifying mood symptoms of neuropsychiatric disorders such as depression in clinical settings. Accurate emotion decoding from neural signals is key in developing aBCI. Current emotion decoders mainly use machine learning methods such as support vector machine (SVM) or convolution neural network (CNN) to classify valance and arousal states from electroencephalogram (EEG) signals. These methods extract channel-wise temporal-spectral features and use spatial filters to aggregate them to decode valance and arousal states in high-dimensional Euclidean space. On the other hand, emotion is reflected by the activity of multi-site brain networks; thus, emotion could be better decoded from brain network functional connectivity. However, decoding emotions from functional connectivity is challenging because of two main reasons. First, functional connectivity is quantified by functional connectivity matrices, which have special geometric properties such as symmetric positive definiteness and are best represented in Riemannian manifolds rather than Euclidean space. Second, due to the large channel number of EEG, functional connectivity matrices are usually of high dimension. Here, we propose an emotion decoding algorithm on low-dimensional Riemannian manifolds. We first extract three typical types of functional connectivity matrix from EEG, i.e., covariance, coherence, and cross-power spectrum density. Next, we generalize the classical Euclidean linear discriminant analysis (E-LDA) approach to Riemannian discriminant analysis (RDA) and apply it to extract low-dimensional embedded manifolds from the raw high-dimensional functional connectivity matrices. Last, we use k-nearest neighbors (K-NN) algorithms based on Riemannian distance metrics to achieve emotion decoding. We comprehensively evaluated our proposed algorithm on several public human EEG emotion datasets and showed the proposed algorithm outperforms traditional SVM and CNN methods. Our results hold promises to achieve accurate emotion decoding for designing future aBCIs.

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