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Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.01

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

Title: Astrocytic ephrin-B1 regulates PV inhibitory synapse development in the CA1 hippocampus

Authors: *S. SUTLEY[1], A. Q. NGUYEN[1,2], T. SHOFF[2], L. NGUYEN[1], V. SANTHAKUMAR[2,3], I. M. ETHELL[1,2];
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Abstract: Impaired inhibition, inhibitory synapse dysfunction, and parvalbumin (PV) interneuron hypofunction are thought to underlie the development of hyperactive neuronal networks in neurodevelopmental disorders. Although astrocytes are critical regulators of synapse development, few astrocytic proteins have been established as contributing to inhibitory synapse development. While previous work has implicated trans-synaptic ephrin/EphB signaling in excitatory synapse development, the role of ephrin/EphB signaling in inhibitory synapse development has not been described. We recently made a new discovery linking astrocytic ephrin-B1 to the development of connections between inhibitory PV neurons and CA1 pyramidal cells (PCs) in the hippocampus. Conditional deletion of ephrin-B1 from astrocytes during the postnatal (P14-P28) developmental period impaired PV->PC connectivity. We found reduced numbers of PV/VGAT positive presynaptic sites near PC somata, reduced IPSC and mIPSC amplitude in recordings from CA1 PCs, increased sensitivity to PTZ induced seizures, reduced sociability, and increased repetitive behaviors in mice with postnatal astrocytic ephrin-B1 deletion. Overexpression of astrocytic ephrin-B1 from P14-P28 using AAV viral vector transfection increased PV->PC connectivity, with overexpression mice showing an increase in both PV/VGAT positive presynaptic sites and IPSC amplitude recorded from CA1 PCs. In mice expressing the excitatory opsin in PV neurons, overexpression of astrocytic ephrin-B1 increased the amplitude of optically evoked PV mediated IPSCs in PCs. We propose that astrocytic ephrin-B1 positively regulates PV-PC connectivity through removal of EphB receptor from PV boutons. We find that deletion of astrocytic ephrin-B1 increases expression of EphB in PV boutons and increases the number of EphB puncta near astrocytes, suggesting reduced removal of EphB by ephrin-B1 deficient astrocytes. Conversely, overexpression of astrocytic ephrin-B1 reduces expression of EphB in PV boutons, suggesting increased removal of EphB by astrocytes. EphB signaling in PV cells may negatively regulate inhibitory synapse formation by influencing ErbB4 signaling, as we find reduced ErbB4 activation in KO animals. Altogether, our findings suggest that direct interactions between astrocytic ephrin-B1 and EphB receptor in PV boutons regulate the establishment of PV perisomatic inhibitory synapses. Our work describes a novel mechanism by which astrocytes regulate PV-PC connectivity and represent a point of intervention which can be used to correct impaired inhibitory circuits in neurodevelopmental disorders.

Disclosures: **S. Sutley:** None. **A.Q. Nguyen:** None. **T. Shoff:** None. **L. Nguyen:** None. **V. Santhakumar:** None. **I.M. Ethell:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.02

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

Support: NIDA RO1

Title: Ephb-ephrinb cis interaction in filopodial movement

Authors: *V. BACCINI[1], Y.-T. MAO[2], M. B. DALVA[2];
[1]Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA; [2]Thomas Jefferson Univ., Philadelphia, PA

Abstract: Proper brain function relies on the formation of specific synaptic connections between neurons. During normal brain development, neurons extend filopodia from dendrites that can initiate synapse formation between partner neurons. One mechanism mediating the initiation of synaptogenesis is the postsynaptic receptor tyrosine kinase EphB2. EphB2 acts as a decision-maker in the tips of dendritic filopodia to determine which filopodia initiate synaptogenesis. Differences in the rate of EphB kinase activation enable filopodia to decide whether to make contact or seek other targets: Fast EphB signals (<1min rise time) cause filopodial retraction, while filopodial stabilization and synapse initiation are mediated by a slow EphB signal activation (>4min). However, it is unknown how EphB kinase activity is modulated to drive these distinctive cellular behaviors.
We propose that *cis* binding of ephrinB3 (ligand) to EphB2 (receptor) may be a mechanism to regulate the rate of EphB signaling. Here using biochemistry, immunocytochemistry, and proximity ligation assay, we show that EphB2 *cis*-interacts with ephrinB3 via a fibronectin type III (FN3) domain of EphB2. Mutation of three amino acids in the second FN3 domain decreases the EphB2-ephrinB3 *cis* interaction. In contrast, EphB2 mutants that lacks the canonical ephrin-B binding domain expressed in the same cell as ephrin-B3, show normal PLA and co-IP, suggesting that FN3s are necessary and sufficient for the EphB-ephrinB *cis* interaction. Using genetically encoded indicator GPhosEphB and live-cell imaging, we find the EphB2-ephrinB3 *cis* interaction slows EphB kinase activation. To directly test whether the EphB-ephrinB3 *cis* interaction can drive filopodial behavior, we tagged EphB2 and ephrinB3 with photodimerization partners (CRY2 and CIBN) and then optogenetically induced the EphB2-ephrinB3 *cis* interaction. Focal induction of the EphB2-ephrinB3 *cis* interaction alters the fate of filopodial movement to drive filopodial stabilization. In contrast, focal activation of EphB2 alone drives fast EphB activation and filopodial retraction. Consistent with this model, EphB2 and ephrinB3 localize in the tips of dendritic filopodia. These data suggest that FN3-mediated EphB2-ephrinB3 *cis*-interaction can modulate EphB kinase signaling to generate different functional outcomes in

cellular behavior. Given the role of ephrinB3 in controlling synapse number, and EphBs essential function in regulating the excitatory synapse formation, these data suggest an attractive model for the coordinated action of these proteins pre- and post-synaptically during synaptic development.

Disclosures: V. Baccini: None. **Y. Mao:** None. **M.B. Dalva:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.03

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

Title: Engineered Adhesion Molecules Drive Synapse Organization

Authors: *W. D. HALE[1,2], T. C. SUDHOF[2], R. L. HUGANIR[1];
[1]Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD;
[2]Mol. and Cell. Physiol., Stanford Univ. Sch. of Med., Stanford, CA

Abstract: In the central nervous system (CNS), cell adhesion molecules localize to synapses to control synapse formation and shape synaptic properties. These synaptic adhesion molecules (SAMs) come from diverse gene families that share little homology at the primary sequence level. Despite a lack of homology, many SAMs drive the formation of synaptic contacts when overexpressed in cultured neurons. A major question in synapse biology is how the diverse plethora of SAMs share a functionally similar relationship to synapse formation, despite lacking common functional domains. We hypothesized that high-affinity adhesion might be a common property of SAMs that facilitated their effects on synapse formation. We tested this hypothesis by generating a novel synaptic adhesion pair with no vertebrate homologues, dubbed ‘Barnoligin’ and ‘Starexin.’ Barnoligin and Starexin form a specific adhesion complex with one another, but not with other synaptic proteins. The reconstitution of the Barnoligin-Starexin adhesion complex in cultured neurons drives the formation of synapses, and the effect on synapse formation is directional, with Barnoligin driving only postsynaptic assembly and Starexin driving only presynaptic assembly. A GPI-anchored version of Starexin forms a functional adhesion complex but does not drive synapse assembly, demonstrating that in addition to adhesion, intracellular signaling is also required for SAM-driven synapse formation. We conclude that the property of high-affinity adhesion is essential for the synaptic function of SAMs and propose that the diversity and specificity of SAM complexes improves target specificity of synapsing neurons. Furthermore, we present Barnoligin and Starexin as tools for probing adhesion complexes broadly and for specifically perturbing patterns of synaptic connectivity.

Disclosures: W.D. Hale: None. **T.C. Sudhof:** None. **R.L. Huganir:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.04

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

Support: R01 GM134035-01
R01 GM134035-03S2

Title: Alternative splicing of Teneurin mediates transsynaptic interactions and synapse formation

Authors: *J. ALVARADO[1], J. LI[1], Y. XIE[1], X. JIANG[3], R. SANDO[4], S. P. KORDON[1], K. LEON[1], T. C. SUDHOF[4], M. ZHAO[1], D. ARAC-OZKAN[2];
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Abstract: The brain processes information by transmitting signals between neurons at the synapse, creating a diverse network of transsynaptic connections. Development of this brain circuitry depends on interactions between receptors, ligands, and cell adhesion molecules. Teneurins (TENs), a family of highly conserved type-II transmembrane cell adhesion proteins, play an important role in brain development across species, regulating synapse formation and guiding axons to correct targets (Li et al., 2018; Vysokov et al. 2018). We report the 2.9-Å cryo-electron microscopy structure of the human TEN2 extracellular region (ECR) in complex with its binding partner Latrophilin-3 (LPHN3), an adhesion G-protein coupled receptor (GPCR). We show that TEN2 is comprised of five domains (EGF; Ig-like domain; β-propeller; β-barrel; and toxin-like domain), and the N-terminal lectin domain of LPHN3 interacts with the β-barrel. Furthermore, we use cell-aggregation assay, flow cytometry, and size exclusion chromatography to show that an alternative splice site on the β-propeller of TEN2 mediates the interaction with LPHN3 via conformational geometry rather than altering the TEN2-LPHN3 binding site at the β-barrel. This alternative splicing has profound effects on TEN2 function. We used artificial synapse formation assay with PSD-95 and GABA(A)α2 postsynaptic markers to show which TEN2 isoforms induced excitatory or inhibitory synapses. The TEN2 isoform without the alternatively spliced sequence in the β-propeller (-SS) forms excitatory synapses in conjunction with LPHN3 and a third LPHN-binding partner, fibronectin leucine-rich transmembrane protein (FLRT). On the other hand, the other TEN2 splice variant (+SS), which cannot interact with LPHN3 due to the lack of rotational accessibility to the LPHN-binding site, induces inhibitory synapse formation. Taken together, these results show that alternative splicing acts as a molecular switch that determines the binding partner for TEN across the synaptic cleft, thereby inducing synaptic specificity. Moreover, this highlights the significance of molecular structure and its impact on function, providing the foundation for further studies on the peculiar barrel structure of TEN and its role in neurodevelopment.

Disclosures: J. Alvarado: None. **J. Li:** None. **Y. Xie:** None. **X. Jiang:** None. **R. Sando:** None. **S.P. Kordon:** None. **K. Leon:** None. **T.C. Sudhof:** None. **M. Zhao:** None. **D. Arac-Ozkan:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.05

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

Support: HHMI

Title: Minimal molecular code for synaptic specificity in the fly visual system

Authors: J. YOO[1], P. MIRSHAHIDI[1], A. NERN[2], S. A. LOCASCIO[1], S. L. ZIPURSKY[1], Y. Z. KURMANGALIYEV[1];
[1]UCLA, Los Angeles, CA; [2]Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Big data provides a broadly applicable approach to uncovering the molecular basis of synaptic specificity. First, EM level connectomes provide cell type specific connectivity. Second, single cell sequencing provides a detailed description of cell-type specific transcriptomes during development. Third, maps of cell surface protein interactions provide maps of potential molecular interactions between axons and dendrites of identified neuron types during development. And finally, genetic approaches provide ways for assessing function of these interactions regulating circuit formation. Here we show how these multiple types of big data can be utilized to elucidate the mechanisms of synaptic specificity in the Drosophila visual system. This provides a general strategy for uncovering synaptic specificity across species. T4/T5 neurons are direction-selective neurons. There are eight subtypes with discrete differences in synaptic specificity between them. Based on single cell sequencing we proposed that connectivity of each subtype is specified by gene modules, one dendritic and two axonal programs. These modules comprise a small number of differentially expressed genes encoding cell surface proteins as possible candidates for differences in synaptic specificity. Here we focused on cell surface proteins regulating the synaptic specificity of a discrete subclass of these neurons. To narrow down candidates for proteins uncovering synaptic specificity, we determined the developmental transcriptome of closely related neuron types post-synaptic to different T4/T5 neurons. Here we report the differential expression of different Beat and Side protein combinations expressed in different pre and postsynaptic cells and demonstrate through genetic analysis that these proteins regulate synaptic specificity. Together these studies and others from our lab and other groups established that three large families of immunoglobulin-containing cell recognition molecules(Dscams, DIPs/Dprs and Beats/Sides) regulate synaptic specificity in Drosophila.
References:Schmucker et al. (2000) PMID: 10892653;
Ozkan et al.(2013) PMID: 23827685*; Tan et al. (2015)* PMID: 26687360*; Carillo et al. (2015)* PMID: 23827685*; Xu et al. (2018)* PMID: 30467079*; Li et al.(2017)* PMID: 28829740; *Sanes and Zipursky(2020)* PMID: 32359437

Disclosures: **J. Yoo:** None. **P. Mirshahidi:** None. **A. Nern:** None. **S.A. LoCascio:** None. **S.L. Zipursky:** None. **Y.Z. Kurmangaliyev:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 028.06

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

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Title: The cell surface receptor LRP4 promotes synaptic growth, active zone organization, and synaptic maturation through a downstream SR-protein kinase mechanism.

Authors: *A. DEPEW[1], J. BRUCKNER[3], K. M. O'CONNOR-GILES[4], T. J. MOSCA[2];
[1]Alison DePew, [2]Thomas Jefferson Univ., Thomas Jefferson Univ., Philadelphia, PA; [4]Brown Univ., [3]Brown Univ., Providence, RI

Abstract: During synapse development, signaling must transpire between pre- and postsynaptic cells to ensure precise coordination of cellular processes. Cell surface receptors are essential for receiving signals at the synapse and transducing them into downstream changes. As such, these receptors often serve as master regulators which direct multiple cellular events to promote development. The receptor LRP4 likely functions as a master regulator at the mammalian neuromuscular junction (NMJ), where it is required for distinct aspects of pre- and postsynaptic development, though our understanding of what pathways it engages to organize synapses remains incomplete. Recent work demonstrated that LRP4 is required for synapse development in both fly and mammalian brains, further indicating conserved mechanisms for this receptor and underscoring its importance in neuronal function. Despite this essential nature and conservation across species, how LRP4 regulates the cellular mechanisms that lead to these developmental defects in neurons remains largely unknown. Specifically, the downstream mechanisms of LRP4 are largely undetermined. Understanding the mechanisms by which LRP4 functions during development will provide unique insight into developmental and neurodegenerative disorders like myasthenia gravis or ALS, that can arise when LRP4 function is impaired. To better understand how LRP4 functions to organize synaptic connections, we studied its role at the fly NMJ, a highly accessible, well-characterized synapse which allows for in-depth study of the cellular mechanisms underlying development. We find that LRP4 is expressed in presynaptic motoneurons and localizes adjacent to active zones. *lrp4* mutants show defects in growth, cytoskeletal and active zone organization, maturation, and neurotransmitter release. These widespread developmental defects indicate that LRP4 may serve as a master regulator of multiple downstream pathways. To investigate a downstream mechanism through which LRP4

may function, we assessed the kinase SRPK79D, which functions in active zone trafficking at the NMJ, and with LRP4 in the CNS, but whose role in synaptic growth, maturation, cytoskeletal organization, and LRP4-dependent processes are unknown at peripheral synapses. Interestingly, loss of SRPK79D results in similar growth, cytoskeletal, and maturation phenotypes, and SRPK79D genetically interacts with LRP4 to influence many of these organizational aspects. We propose that LRP4 functions presynaptically via SRPK79D in motoneurons to regulate multiple aspects of synaptic development, furthering our understanding of mechanisms of synapse development.

Disclosures: **A. DePew:** None. **J. Bruckner:** None. **K.M. O'Connor-Giles:** None. **T.J. Mosca:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 028.07

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

Support: NIH Grant R01-NS110907
NIH Grant R00-DC013059
Sloan Foundation
Whitehall Foundation

Title: Synaptic development in diverse olfactory neuron classes uses distinct temporal and activity-related programs

Authors: M. AIMINO[1], A. DEPEW[2], L. RESTREPO[1], *T. J. MOSCA[2];
[1]Neurosci., [2]Thomas Jefferson Univ., Thomas Jefferson Univ., Philadelphia, PA

Abstract: Developing neurons must follow specific molecular, cellular, and temporal programs to establish synapses capable of comprising a functional circuit. However, the vast diversity in class, morphology, and function of brain neurons raises important questions. Do all classes of neurons use the same, or different, organizational mechanisms to form synapses and achieve functional and morphological maturation? Do neurons within the same sensory circuit that share a common goal of detecting natural stimuli, develop on similar timescales and use identical molecular approaches to form the correct number of synapses? To investigate these questions, we used the Drosophila antennal lobe, a model olfactory circuit with remarkable genetic access and synapse-level resolution. Using tissue-specific genetic labeling of active zones, we performed a quantitative analysis of synapse formation in multiple classes of olfactory neurons throughout development and adulthood. We found that olfactory receptor neurons (ORNs), projection neurons (PNs), and local interneurons (LNs) each have unique time-courses of synaptic development, addition, and refinement, demonstrating that each class follows a distinct developmental program. Furthermore, food-sensing and pheromone-sensing subtypes of ORNs

exhibited variations in their time-courses, revealing that even neurons of the same class do not develop in exactly the same way. This raised the possibility that these classes may also have distinct cellular requirements for synapse formation. To examine this, we genetically altered neuronal activity in each class of neuron and observed differing effects on synapse number based on the neuronal class examined. Silencing neuronal activity in ORNs, PNs, and LNs impaired synaptic development but only in ORNs did enhancing neuronal activity influence synapse formation. Intriguingly, ORNs and LNs demonstrated similarly impaired synaptic development with overexpression of a constitutively active version of the master, activity-dependent kinase, GSK-3β, suggesting that neuronal activity and GSK-3β function in a common pathway. However, ORNs also demonstrated impaired synaptic development with GSK-3β loss-of-function, suggesting additional activity-independent roles. Ultimately, our results suggest that the requirements for synaptic development are not uniform across all neuronal classes with considerable diversity existing in both their developmental timeframes and molecular requirements. These findings provide novel insights into the mechanisms of synaptic development and lay the foundation for future work to determine their underlying etiologies.

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Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.08

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

Support: HFSP

Title: Layer 5 pyramidal neurons form transient, active, and recurrent circuits at the inception of neocortex in the living embryo

Authors: *M. MUNZ[1], A. BHARIOKE[1], B. ROSKA[2];
[1]Inst. of Mol. and Clin. Ophthalmology Basel, Basel, Switzerland; [2]Friedrich Miescher Inst. For Biomed. Resear, Basel, Switzerland

Abstract: Pyramidal to pyramidal neuron connections comprise a majority of the connections in cortical circuits, yet the assembly of these circuits remains poorly understood. Layer 5 pyramidal neurons are amongst the earliest born cortical neurons and show highly recurrent connectivity in the adult. Thus, they may form early pyramidal to pyramidal neuron circuits. Here, we show that embryonic layer 5 pyramidal neurons, identified through single cell transcriptomics, are active and show two phases of circuit assembly. The first phase consists of a novel recurrent circuit motif, forming at E14.5, at the inception of neocortex. Using two photon calcium imaging, visually guided patch clamp recordings, and pharmacology, all in vivo, as well as electron microscopy, we found that neurons in both layers of the first phase display active somas and neurites, voltage-gated sodium conductances, responses to glutamatergic agonists, and functional

synapses. Additionally, neurons show high correlations both within and between layers of the recurrent circuit motif. Through single cell RNA sequencing, we found that embryonic layer 5 pyramidal neurons already display three transcriptomic types, which correspond to the three adult layer 5 cell types. The first phase of circuit assembly, at E14.5, consists of only neurons of the embryonic near-projecting type. By E15.5, neurons in the upper layer undergo programmed cell death and, simultaneously, the activity of all embryonic layer 5 pyramidal neurons decreases. Therefore, this novel motif of cortical development is transient.
The second phase of circuit assembly follows the traditional inside-out pattern of cortical development, with neurons migrating from a layer within the subplate to the layer within neocortex that will form layer 5. Neurons corresponding to all three adult layer 5 subtypes are now present within cortex. We again show that neurons display active somas and neurites, voltage-gated sodium conductances, and functional synapses. Interestingly, during this phase, the activity within the dendrites is greater than that within the somas, possibly reflecting the presence of synaptic inputs. As at E14.5, pairs of embryonic layer 5 pyramidal neurons show high correlations throughout this phase.
In summary, within the embryonic cortex, layer 5 pyramidal neurons form primordial active pyramidal neuron circuits, and demonstrate a novel active, transient pyramidal-to-pyramidal neuron recurrent circuit motif, right at the inception of the neocortex.

Disclosures: **M. Munz:** None. **A. Bharioke:** None. **B. Roska:** None.

Poster

028. Synapse Formation

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

Support: Human Frontier Science Program (RGP0019/2016)
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Agence Nationale de la Recherche (Projects DynHippo)
Agence Nationale de la Recherche (NanoPlanSyn)
Agence Nationale de la Recherche (soLIVE)
Agence Nationale de la Recherche (DopamineHub)

Title: Transient dopamine-NMDA receptor interaction governs hippocampal synaptic and network maturation

Authors: *N. BENAC[1], G. SARACENO[2], C. BUTLER[2], N. KUGA[3], Y. NISHIMURA[4], P. SU[5], T. SASAKI[6], M. PETIT PEDROL[2], R. GALLAND[2], F. LIU[7], Y. IKEGAYA[8], J.-B. SIBARITA[9], L. GROC[10];
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Sendai, Japan; [7]Ctr. Addiction & Mental Hlth., Ctr. Addiction & Mental Hlth., Toronto, ON, Canada; [8]Grad Sch. Pharma Sci, Univ. Tokyo, (d) TOKYO YAKKA UNIV LIB, Tokyo, Japan; [9]CNRS / Univ. Bordeaux, CNRS / Univ. Bordeaux, Bordeaux, France; [10]CNRS - Univ. of Bordeaux, Ctr. Natl. de la Recherche Scientifique, Bordeaux, France

Abstract: The dopamine system controls the maturation of excitatory synapses through protein kinases involved in other modulatory processes and glutamatergic NMDA receptor (NMDAR). A physical interaction between NMDAR and dopaminergic D1 (D1R) has been speculated to play a central role in the dopamine-glutamate interplay, providing in particular molecular specificity. However, tools to directly capture the live receptor interaction at the single molecule level have been lacking, questioning its functional role. Here, we developed a multidimensional spectral single molecule localization microscopy approach (MS-SMLM) to track over development the surface interplay between surface D1R and NMDAR at the nanoscale and with millisecond temporal resolution. D1R-NMDAR interaction is transient and upregulated onto immature neurons in a Casein Kinase (CK)- and mGluR-dependent manner. Functionally, the interplay is required for synaptogenesis and tunes early *in vivo* hippocampal network activity. Thus, MS-SMLM shed the first lights on the developmentally-regulated transient interaction between dopamine and glutamate receptors and its role during synaptogenesis.

Disclosures: **N. Benac:** None. **G. Saraceno:** None. **C. Butler:** None. **N. Kuga:** None. **Y. Nishimura:** None. **P. Su:** None. **T. Sasaki:** None. **M. Petit Pedrol:** None. **R. galland:** None. **F. Liu:** None. **Y. Ikegaya:** None. **J. Sibarita:** None. **L. Groc:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

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Program #/Poster #: 028.10

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

Support: Drexel University Dean's Fellowship

Title: Investigating the role of microtubule-associated motor protein KIFC1 at the synapse.

Authors: *S. GUHA, H. MURALIDHARAN, P. W. BAAS;
Neurobio. and Anat., Drexel Univ. Col. of Med., Philadelphia, PA

Abstract: In addition to serving their necessary roles of architecture and transport in both axons and dendrites, microtubules are crucial for the maintenance of synaptic connections. On the presynaptic side, microtubules fortify the distal axon and ensure that it does not pull away from the postsynaptic side. On the postsynaptic side, microtubules fortify dendritic spines by transiently invading them. Synapse loss is downstream to many different neurodegenerative pathways. Surprisingly little has been done, however, to explore the microtubule-based mechanisms that ensure the fidelity of the synapse during health and how those mechanisms fail during disease. Here we have investigated a potential role for KIFC1, a kinesin-related motor

protein best known for its role in mitosis, but recently shown to also play critical roles in various aspects of the life of the neuron. In non-neuronal cells and *in vitro* assays, KIFC1 crosslinks microtubules to prevent them from sliding, binds to membrane proteins, and interacts with the plus end of the microtubule via EB1 and EB3 - all of which our present results demonstrate pertain to synapses. In studies on cultured rat hippocampal neurons, our results show dramatic synapse loss when KIFC1 is pharmacologically inhibited, with both sides of the synapse affected in different ways. Conversely, when we express a rigor mutant of KIFC1 or simply overexpress wild-type KIFC1, synapses are fortified against loss when challenged by disease mechanisms. The functional readout of this loss is reduced neuronal activity, recorded from hippocampal neurons cultured long-term on multi-electrode arrays. We posit that KIFC1 malfunction may underlie synapse loss during diseases. Moreover, new generations of drugs that strengthen KIFC1's attachment to microtubules may be a powerful tool for treating neurodegenerative diseases.

Disclosures: S. Guha: None. **H. Muralidharan:** None. **P.W. Baas:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.11

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

Title: Role of C. elegans RAPGEFin Synapse Development at the Neuromuscular Junction

Authors: *R. LAMB, S. J. CHERRA, III;
Neurosci., Univ. of Kentucky, Lexington, KY

Abstract: Synapses are a crucial component of neural circuits, and improper synapse formation can result in the development of neurological disorders. The RAPGEF subfamily of GEFs are associated with multiple neurological disorders such as schizophrenia, amyotrophic lateral sclerosis, and myoclonic epilepsy. Guanine Exchange Factors (GEFs) are a family of proteins that regulate GTPase signaling cascades through activation. In murine models, disruption of RAPGEF6 function resulted in reduced anxiety behaviors, increased long term potentiation, but no changes in gross brain morphology. In *C. elegans,* the RAPGEF protein, PXF-1, has a canonical role in epithelial development through activation of RAP-1. Additionally, PXF-1 has been identified as a potential modulator of synaptic transmission, but its role in neurons is still unknown. To determine how PXF-1 regulates synapses, we used the *C. elegans* neuromuscular junction (NMJ) as our model. We hypothesized that PXF-1 contributes to neural circuit function by promoting synapse development at the NMJ. To measure changes in NMJ function, we used aldicarb, an acetylcholinesterase inhibitor. We found that two independent mutant alleles of *pxf-1* caused the animals to become resistant to aldicarb in comparison to wild type animals. To further investigate how PXF-1 affects NMJ activity, we used a genetically-encoded calcium indicator, GCaMP. Mutations in *pxf-1* caused a decrease in frequency of GCaMP signal but no changes in

amplitude. Together, these data indicate that PXF-1 modulates presynaptic function. We then used fluorescently tagged synaptic proteins to investigate whether *pxf-1* mutants displayed alterations in motor neuron synapse number or morphology. There were no changes in the density of motor neurons synapses; however, we observed a decrease in the intensity of vesicle proteins in *pxf-1* mutant animals. There were no differences in the intensity of active zone proteins in *pxf-1* mutants. Active zone protein complexes and the actin cytoskeleton coordinate the abundance and localization of synaptic vesicles at presynaptic terminals. Since *pxf-1* mutants displayed no changes in active zone proteins, we examined whether *pxf-1* mutants altered the actin cytoskeleton in cholinergic terminals using a GFP labeled filamentous actin binding protein. We found that actin filaments were decreased in *pxf-1* mutants. Overall, our findings indicate that *pxf-1* modulates the actin cytoskeleton to promote synapse development. Overall, our work provides new insight into how RAPGEFs regulate nervous system function and how disruption of RAPGEF function may contribute to the development of certain neurological disorders.

Disclosures: R. Lamb: None. **S.J. Cherra:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.12

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

Support: John S. Rogers Science Program

Title: The role of thrombospondin in larval Drosophila melanogaster neuromuscular junction structure and locomotor function

Authors: *N. A. VELAZQUEZ ULLOA[1], G. WOODS[1], E. LOWENSTEIN[3], K. COREY[1], A. WOOTEN[2], A. OSGOOD[1], D. MENDOZA ORTIZ[4], I. MAXWELL[1], S. LEE[1];
[1]Biol., [2]Biochem. and Mol. Biol., Lewis & Clark Col., Portland, OR; [3]Oregon Hlth. and Sci. Univ., Portland, OR; [4]UNAM, Mexico City, Mexico

Abstract: Thrombospondin (TSP) is an extracellular matrix protein that is involved in synaptogenesis of central nervous system mammalian glutamatergic synapses. The role of TSP at the neuromuscular junction (NMJ) in *Drosophila melanogaster* has not been described. *D. melanogaster* has a single *D-tsp* gene, while mammals have 5. This allows for targeted investigation of this single conserved *tsp* gene to determine its contribution in synapse formation at the 3rd instar larval NMJ. We hypothesized that *D-tsp* would be necessary for normal NMJ formation and locomotor behavior. Moreover, the NMJ in *D. melanogaster* is a glutamatergic synapse, and as such can provide information that can be compared and contrasted between *D. melanogaster* and mammals. To determine the role of *D-tsp* at the NMJ of *D. melanogaster*, we knocked it down specifically in neurons, muscles or in neurons and muscles at the same time

using the Gal4-UAS system. Here we focus mainly on the results we have with neuronal knockdown achieved by crossing *w;elav-GAL4* flies with two different *UAS-tsp_RNAi* lines, *w29399* or *w34661*, which have different predicted knockdown efficiency. The female 3rd instar larvae progeny of either parental (no knockdown) or experimental (knockdown) crosses was dissected, stained and imaged with a confocal microscope for anatomical analyses of their NMJs at muscle 4 in segments A3 and A4. For locomotor function experiments, 3rd instar larvae from parental to experimental crosses were collected and their locomotor activity recorded in a gridded arena and subsequently analyzed. In addition, qPCR was performed to validate the knockdown of tsp, and our preliminary results show the decrease we were expecting in the experimental crosses. All neuronal knockdown results presented come from at least two independent experiments, and we found no differences in survival between parental (control) and experimental conditions. Analysis of the NMJ images showed significant differences in perimeter and area for one of the experimental crosses after the data was normalized by muscle area. We also observed significant differences in distance traveled and zones distribution, a measure of how far away from the starting position a larva gets. Our preliminary results for *D-tsp* knockdown also show differences in NMJ structure that we want to follow up on. Our results thus far suggest that *D-tsp* plays a role at the NMJ of 3rd instar *D. melanogaster* larvae and contributes to normal NMJ development and larval locomotor behavior.

Disclosures: **N.A. Velazquez Ulloa:** None. **G. Woods:** None. **E. Lowenstein:** None. **K. Corey:** None. **A. Wooten:** None. **A. Osgood:** None. **D. Mendoza Ortiz:** None. **I. Maxwell:** None. **S. Lee:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.13

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

Support: UK Medical Research Council (MR/N013700/1)

Title: Translating genetic susceptibility to neurobiology of disease - understanding the role of psychosis risk candidate ZNF804A during synaptic development

Authors: *L. SICHLINGER, L. DUTAN POLIT, A. C. VERNON, D. P. SRIVASTAVA; Kings Col. London, Kings Col. London, London, United Kingdom

Abstract: Variants of ZNF804A have been robustly linked to the risk of developing schizophrenia in genome wide association studies. Previously, ZNF804A has been implicated in multiple cellular processes including protein synthesis, synapse maintenance and formation, and activity-dependent signalling. During neurodevelopment, *ZNF804A* expression peaks during the second trimester suggesting a crucial role for the risk gene at this developmental stage. However, what role exactly that is, is not fully understood. In this study, human induced pluripotent stem

cell-derived immature glutamatergic neurons combined with a CRISPR/Cas9 approach were used to evaluate ZNF804A functioning during synaptogenesis. Bulk RNA sequencing comparing transcriptional profiles of ZNF804A-mutant neurons to wildtype cells revealed differential expression of genes associated with cell adhesion, synaptogenesis, and protein translation. Interestingly, a high-throughput imaging approach showed that not only pre- and postsynaptic protein expression was affected by ZNF804A mutations but also expression of ribosomal proteins. Furthermore, translational efficiency is decreased in mutation lines indicating a crucial role for ZNF804A in protein synthesis control. This seems to be governed by disruptions of the ERK1/2 pathway leading to dysregulation of translation initiation factors. These results indicate that ZNF804A governs protein translational processes via ERK1/2 signalling cascades downstream of cell adhesion molecules, which may lead to disruptions of translation of synaptic proteins. The results of this study contribute to our limited understanding of the neurobiological functioning of genetic risk factors for schizophrenia during neurodevelopment, which may help to decipher the underlying aetiology of this complex disorder and may provide targets for future therapeutic interventions.

Disclosures: L. Sichlinger: None. **L. Dutan Polit:** None. **A.C. Vernon:** None. **D.P. Srivastava:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.14

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

Support: NIH 1R01DA053372

Title: Mechanisms of deficits in synaptogenesis caused by methadone in human cortical organoids

Authors: *I. DWIVEDI, W. WU, H. YAO, G. G. HADDAD;
Dept. of Pediatrics, UC San Diego, La Jolla, CA

Abstract: Methadone is the most common pharmacological treatment for Opioid Use Disorder (OUD) during pregnancy. Prenatal exposure to this drug has been indicated to cause long term neurocognitive and behavioral sequelae. However, the underlying etiology of these deficits is not well understood, primarily due to limited access to human fetal tissues. To address these limitations, we utilized human iPSC-derived three-dimensional models of cortical development called cortical organoids (hCOs) that contain multiple, spatially organized, and functional cortical cell types, providing access to key aspects of cortico-genesis. First, we found that 2-months of chronic exposure to 1μM methadone in these hCOs led to significant changes in the expression of genes encoding canonical pre- and post-synaptic genes such as vesicular proteins, scaffolding proteins, receptors (Synaptic DEGs = 166, $|Confect| > Log_2(1.5)$, FDR < 0.05). These

were highly co-expressed and are known to physically interact with differentially expressed ciliary and extracellular matrix (ECM) proteins in the brain. To dissect methadone's impact on synaptogenesis, we have conducted both immunofluorescence (IF) and scanning electron microscopy (SEM) to identify structural synapses in our organoids. IF of 2-3-month-old hCOs revealed the presence of synapses using pre- and post-synaptic markers (SYN1 and PSD-95). Chronic methadone treatment markedly decreased pre- and post-synaptic labeling, congruent with our findings of methadone-induced transcriptional changes in hCOs. Our preliminary SEM studies also identified synapses as well as cilia-like projections in cells lining the ventricular zones of rosettes within the hCOs, enabling an investigation into methadone's effect on the architecture of these structures. These results are consistent with our prior-patch clamp experiments investigating the impact of methadone on synaptic function in hCOs. We found that the frequency and amplitude of spontaneous excitatory post-synaptic currents (sEPSCs) in the hCOs were significantly decreased following the 1-week treatment of 1μM or 10μM methadone. We believe the findings from this study will elucidate mechanisms leading to cognitive and behavioral deficits caused by prenatal methadone exposure and help improve methods of chemical intervention for maternal OUD.

Disclosures: I. Dwivedi: None. **W. Wu:** None. **H. Yao:** None. **G.G. Haddad:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.15

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

Support: NIH
NSF
W.M. Keck

Title: Synaptic protein levels and physiological activity in primary cortical neurons are influenced by time of day.

Authors: *J. WANG, B. L. ROBERTS, I. N. KARATSOREOS;
Psychological and Brain Sci., Univ. of Massachusetts, Amherst, Amherst, MA

Abstract: Prefrontal cortical (PFC) neurons are important in a wide array of cognitive and emotional behaviors. Notably, disruption of the circadian clock caused by our 24h "always on the go" society, including an unstable relationship between our internal clock and the external day, is thought to impair cognitive ability and increase risk of developing depression or anxiety. Previous work showed that environmental disruption of circadian rhythms in mice impacts gross morphology of neurons in the PFC. Moreover, growing evidence suggests that circadian rhythms are important in the formation and maintenance of synaptic connections, which can impact the integrity and structure of neuronal networks. Here, in an effort to develop an approach to probe

these mechanisms, we asked if primary cortical mouse neurons demonstrate rhythms in synaptic activity *in vitro* and if this is mediated by rhythms in synaptic protein density. To test this, we used primary cultures of cortical neurons from mixed male and female P0 pups on a C57BL/6N background. Cells were harvested every 6 hours at days *in vitro* (DIV) 14-15 to determine the presence and quantity of synaptic proteins synapsin and PSD-95. We observed that synaptic protein levels were dependent on the time of collection. To determine if these protein changes were associated with functional changes, we utilized patch-clamp recordings in cultures from DIV15-17 at two different circadian time (CT) bins, CT12-15 and CT18-21. We measured spontaneous excitatory postsynaptic currents (sEPSCs) to determine the impact of CT time on synaptic transmission. Our data suggest that presynaptic glutamate release may differ between the CT12-15 and CT18-21 bins. Further, the holding current of neurons at CT12-15 is decreased compared to CT18-21, suggesting that these neurons are more depolarized at CT12-15. Lastly, we found a time-of-day impact on multiple physiological properties, including membrane conductance. Together these data suggest that, *in vitro*, cortical neurons demonstrate both structural and functional rhythms, providing us with an important tool for understanding how the circadian clock impacts cortical function at the cellular level.

Disclosures: **J. Wang:** None. **B.L. Roberts:** None. **I.N. Karatsoreos:** None.

Poster

028. Synapse Formation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 028.16

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

Support: CrestOptics-IIT JointLab for Advanced Microscopy
Regione Lazio MARBEL Life2020
Regione Lazio Bio3DBrain FSE 2014-2020
Sapienza University
D-tails s.r.l.

Title: Intronic FTD-related tau mutation impairs cortical and retinal development in iPSC-derived 2D and 3D models

Authors: F. CORDELLA[1,3], L. MAUTONE[3], D. SALERNO[2], S. GHIRGA[3], G. DI GENNARO[1], E. PARENTE[1], Y. GIGANTE[3,4], M. PITEA[3,4], A. SOLOPERTO[3], ***S. DI ANGELANTONIO**[1,3,4];
[1]Fisiologia e Farmacologia, [2]Medicina Sperimentale, Sapienza Univ., Roma, Italy; [3]Cln2s, Inst. Italiano di Tecnologia, Roma, Italy; [4]D-TAILS, Roma, Italy

Abstract: Tauopathies, such as Alzheimer's Disease and Frontotemporal dementia, are characterized by several microtubule-associated protein tau gene (MAPT) mutations which lead to the hyperphosphorylation and accumulation of the microtubule-associated protein TAU,

leading to a synaptic and neuronal loss, together with a prominent neuroinflammatory state. The MAPT 10+16 intronic mutation causes frontotemporal lobar degeneration (FTLD) by increasing the expression of four-repeat (4R)-tau isoforms. We investigated investigate the impact of the IVS10+16 TAU mutation during cortical and retinal development and maturation exploiting 2D retinal culture and 3D patterned cortical organoids generated using both the hiPSC lines that carry that mutation and its isogenic control. Gene expression analysis revealed that IVS10+16 TAU mutation deeply affects neuronal, astrocytic, and synaptic maturation. Specifically, NanoString nCounter Analysis demonstrated that genes belonging to neuronal and synaptic maturation processes were upregulated at day 100 control organoids with respect to day 50. On the other hand, IVS10+16 TAU mutation strongly impaired neuronal and glial maturation, with specific downregulation at day 100 of glutamatergic and GABAregic synaptic genes, axon guidance, and ion transmembrane transport gene. Moreover, IVS10+16 TAU day 100 organoids displayed, with respect to control day 100 organoids, upregulation of cell proliferation, regulation of the apoptotic process, and Notch signaling pathway genes. Confocal analysis revealed that retinal and cortical neurons that carry the IVS10+16 TAU mutation displayed strong cytoskeleton alterations, characterized by small fragments with lower volume compared to control neurons. Functional analysis of Ca^{2+} oscillations of whole organoids and 2D neuronal cultures has shown that IVS10+16 TAU mutation impairs neuronal activity, reducing the frequency, synchronicity, and the number of active neurons. These results suggest that inherited intronic TAU mutation deeply affects neuronal and glial maturation already during neurodevelopment, highlighting the pathophysiological role of IVS10+16 TAU in the developing brain and paving the road for the identification of new therapeutical targets.

Disclosures: F. Cordella: None. **L. Mautone:** None. **D. Salerno:** None. **S. Ghirga:** None. **G. Di Gennaro:** None. **E. Parente:** None. **Y. Gigante:** A. Employment/Salary (full or part-time):; d-tails s.r.l. **M. Pitea:** A. Employment/Salary (full or part-time):; d-tails s.r.l.. **A. Soloperto:** None. **S. Di angelantonio:** F. Consulting Fees (e.g., advisory boards); D-Tails s.r.l..

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.01

Topic: A.07. Developmental Disorders

Support: NIH Grant U54 HD090256)

Title: Function of the Trinucleotide (CGG) Repeats in the FMR1 Gene in Neurons

Authors: *C. SIROIS, K. GUO, M. LI, T. KORABELNIKOV, S. SANDOVAL, Y. XING, B. LEVESQUE, A. BHATTACHARYYA, X. ZHAO;
Waisman Ctr., Univ. of Wisconsin-Madison, Madison, WI

Abstract: The 5’ UTR of the *FMR1* gene contains trinucleotide CGG repeats, that, when expanded, can lead to one of several disorders depending on the length of the repeats. Repeats that range from 55 to 200 CGGs lead to either Fragile X Tremor and Ataxia Syndrome (FXTAS) or Fragile X Primary Ovarian Insufficiency (FXPOI), while repeats beyond 200 CGGs lead to silencing of the *FMR1* gene and cause Fragile X Syndrome (FXS), a neurodevelopmental disorder that causes 2 to 5% of all autism cases. While CGG repeat lengths less than 55 repeats were previously considered to be “normal,” recent studies have led to the addition of further CGG repeat categories due to their association with certain health conditions: “low zone” (7-23 CGG repeats) and “gray zone” (41-54 repeats). One finding that has been replicated across multiple cohorts of patients is that CGG repeat number is associated with poorer health outcomes in individuals exposed to chronic life stress, both in premutation carriers and in individuals with repeats in the “low zone” range. To investigate the function of CGG repeats in FMR1 in human neurons, we generated two human embryonic stem cell (hESC) lines lacking the CGG repeats in FMR1 (H1ΔCGG, H13ΔCGG) using genome editing and differentiated these cells into neurons. Removal of the CGG repeats does not affect FMR1 expression at the mRNA or protein level in hESCs, neural progenitors, or early post mitotic neurons. However, neurons lacking these CGG repeats exhibit differential responses to cellular stress and altered localization of *FMR1* mRNA to their neurites. We have also used an alternative *in vitro* model to confirm these findings, explore potential cellular mechanisms, and determine whether these two phenotypes (cellular stress and RNA localization) are related. Our results demonstrate that CGG repeats in *FMR1* may have a function in early neural development, which could have important implications for CRISPR-based disease modeling and gene therapy approaches that aim to remove the expanded CGG repeats from the *FMR1* gene as a means to restore gene expression.

Disclosures: C. Sirois: None. **K. Guo:** None. **M. Li:** None. **T. Korabelnikov:** None. **S. Sandoval:** None. **Y. Xing:** None. **B. Levesque:** None. **A. Bhattacharyya:** None. **X. Zhao:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.02

Topic: A.07. Developmental Disorders

Support: FAPESP Grant 2022/07948-9
FAPESP Grant 2019/10868-4

Title: Further variability and combinatorial control of FMR1 transcripts with alternative expression of exons 14 and 15 in the rat forebrain

Authors: *L. A. HADDAD, A. M. LINARDI, D. K. TAKEMOTO, G. A. M. SUARDI, I. C. M. LIMA, T. GLASER, H. ULRICH, S. CHIAVEGATTO;
Univ. Sao Paulo, Sao Paulo, Brazil

Abstract: The *FMR1* gene is mutated in fragile X syndrome, the leading inherited cause of intellectual disability among men. *FMR1* encodes FMRP, an important RNA-binding protein in synaptic control and neurogenesis. Cerebral corticogenesis appears differentially sensitive to *FMR1* mRNA and protein amounts. Distinct post-transcriptional mechanisms regulate the stability of the *FMR1* transcript. As *FMR1* pre-mRNA may undergo alternative splicing, variable out-of-frame transcripts can potentially lead to auto-regulatory feedback loops onto its mRNA stability. We previously observed in the rat forebrain high amounts of total *Fmr1* mRNA and long FMRP isoforms on E11-E18 and P2-P7 developmental days, and their lowest levels on E19-E20 and beyond P14. *Fmr1* exon 14 can be alternatively skipped shifting the translational reading frame, and exon 15 has three splice acceptor sites (A, B and C). We formerly disclosed significant exon-14 skipping in E17-E20 rat forebrain and low levels of the resulting messages with exon 13-15 junctions. We show that the nonsense-mediated decay (NMD) pathway does not elicit the degradation of *Fmr1* mRNA without exon 14. We are now interrogating if the PAXT complex that adapts polyadenylated RNAs for decay by the nuclear exosome may trigger the reduction of those messages. Here, we unveil that, in E17-E20 rat forebrain, intron 14 is significantly retained in *Fmr1* mRNA, shifting the reading frame. Reducing UPF1, a major NMD protein, increases *FMR1* mRNA with intron 14 in HEK293T cells, indicating the relevance of this pathway for its control. Conversely, UPF1 knockdown decreases the number of junctions spanning splice site 15A in mRNA. In conclusion, we present evidence for increased *FMR1* exon 14 skipping and intron 14 retention in E17-E20 rat forebrain followed by a decrease of total mRNA, inferring a poor pre-mRNA splicing definition of exon/intron 14 at this developmental phase. While UPF1-mediated NMD downregulates *FMR1* messages with intron 14, UPF1 appears to stabilize mRNA produced by splice site 15A selection. UPF1 has been recently reported to directly interact with FMRP in the cytoplasm. As FMRP binds *FMR1* exon-15 RNA and our data suggest this exon should harbor UPF1 binding sites, UPF1 and FMRP should cooperate to stabilize the *FMR1* mRNA. The E19-E20 forebrain quantitative decline of the *Fmr1* mRNA and the long FMRP isoforms should be a combinatorial effect of multiple layers of regulation including the production of out-of-frame transcripts. They should ultimately dosage the *Fmr1* gene products contributing to proper cerebral corticogenesis.

Disclosures: L.A. Haddad: None. **A.M. Linardi:** None. **D.K. Takemoto:** None. **G.A.M. Suardi:** None. **I.C.M. Lima:** None. **T. Glaser:** None. **H. Ulrich:** None. **S. Chiavegatto:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.03

Topic: A.07. Developmental Disorders

Support: Rose F. Kennedy IDDRC Pilot and Feasibility Award sponsored by NICHD U54 HD090260

Title: Mis-localization of a nicotinic acetylcholine receptor auxiliary protein in a mouse model of Fragile X Syndrome

Authors: *S. GOEBEL, V. K. VERSELIS, A. FRANCESCONI;
Neurosci., Albert Einstein Col. of Med., Bronx, NY

Abstract: Fragile X Syndrome (FXS) is the most common form of inherited intellectual disability and often comorbid with autism. FXS arises from silencing of the *Fmr1* gene and loss of expression of FMRP protein, a condition mimicked in the *Fmr1* KO mouse, a pre-clinical model of FXS. FMRP binds many brain mRNAs to regulate their translation, trafficking, and stability but the predominant molecular pathologies leading to deficits in synaptic plasticity and circuit excitability in the *Fmr1* KO mouse remain unknown. Using unbiased quantitative iTRAQ proteomics, we discovered that Lymphocyte antigen 6H (Ly6H) is selectively decreased in lipid rafts isolated from the forebrain of adult *Fmr1* KO mice. Ly6H is GPI-anchored and acts as an auxiliary protein to α7 nicotinic acetylcholine receptors (α7 nAChRs), a function of particular relevance since α7 nAChRs contribute to many of the processes that go awry in FXS including hyperexcitability, seizures, and hyperarousal to sensory stimuli. Using a combination of biochemical and imaging techniques we tested whether Ly6H depletion from lipid rafts in the *Fmr1* KO mouse coincides with altered membrane expression or localization. Despite no significant difference in Ly6H total protein expression in the hippocampi of *Fmr1* KO mice compared to wild type (WT), we find increased Ly6H surface expression. This altered Ly6H surface expression was evident from increased abundance in hippocampal and cortical membrane fractions of adult *Fmr1* KO mice and elevated surface biotinylation in primary cortical neurons following *Fmr1* knock-down. Using high-resolution confocal microscopy, we find that Ly6H surface expression is selectively enhanced in the soma and proximal dendritic regions of *Fmr1* KO hippocampal neurons, areas in which α7 nAChRs reside. To investigate possible mechanisms underlying Ly6H mis-localization, we examined Ly6H posttranslational processing. Using enzymatic cleavage, we determined that Ly6H contains N-linked glycols, but that this glycosylation is unchanged in WT and *Fmr1* KO mice during hippocampal development (P4 through P14). These results indicate that mis-localization of Ly6H is not caused by aberrant glycosylation but whether posttranslational processing of the GPI anchor is altered remains to be shown. Given the established role of Ly6H in regulating α7 nAChR function, we propose that abnormal surface expression of Ly6H in *Fmr1* KO neurons will likely result in altered α7 nAChR activity and/or membrane expression. Overall, findings from these studies will contribute to a better understanding of the cellular pathophysiology of FXS and suggest Ly6H as a potential novel target for therapeutic intervention.

Disclosures: S. Goebel: None. **V.K. Verselis:** None. **A. Francesconi:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.04

Topic: A.07. Developmental Disorders

Support: NIH NICHD

Title: Fragile X syndrome alters ionome and whole-body composition in mice

Authors: *S. ALAM[1], C. PRATER[2], B. FATHEPURE[3], E. A. LUCAS[4], P. JEYASINGH[2], E. A. MCCULLAGH[5];
[2]Integrative Biol., [3]Microbiology and Mol. Genet., [4]Nutritional Sci., [1]Oklahoma State Univ., stillwater, OK; [5]Integrative Biol., Oklahoma State Univ., Prefer Not to Answer, OK

Abstract: Fragile X Syndrome (FXS) is the leading genetic form of autism characterized by intellectual disabilities, behavioral anomalies along with typical physical features. FXS has no cure, but therapeutic interventions can help mitigate the behavioral and physiological health complications caused by the disorder. A whole-body approach integrating phenotypes across systems is needed to not just treat neurological symptoms but understand the complexities of interactions between bodily systems. We performed experiments that measure ion abundance across the brain and gut, and bone and metabolite composition across tissues, to better understand and ultimately treat underlying imbalances in FXS. Ionomics is a new multidisciplinary field integrating the study of the ionome, or the composition of mineral and trace elements essential for homeostatic function found in all living organisms. Our preliminary data of the whole brain ionome showed no significant differences between the genotypes whereas the ionome of the cecum showed significant differences in ion abundance between genotypes and the sexes. These findings are consistent with altered microbiome findings in FXS. Initial results show significant increases in bone mineral density and decreased percent fat in FXS mice compared to wildtype that agrees with existing literature showing decreased fat percentage; however, the mice used for this experiment were not the same age. Future work is planned to measure gut microbiome and metabolite composition in the same animals as ionome and bone measurements are taken to characterize the whole-body phenotype of FXS mice. Lastly, we intend to use these results to inform a nutritional supplement to treat core symptoms of FXS. This research informs insights on whole body function in FXS and the underlying elemental, microbial, and organ level changes at the core of complex behaviors.

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Poster

029. Fragile X Syndrome

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.05

Topic: A.07. Developmental Disorders

Support: NIH Grant HD104558

Title: Molecular mechanisms of translation control during human neuronal development in Fragile X Syndrome

Authors: *V. G. SHANKAR[1], N. RAJ[2], O. KATSARA[3], M. KALINOWSKA[1], R. J. SCHNEIDER[3], G. J. BASSELL[2], E. KLANN[1,4];
[1]Ctr. for Neural Sciences, New York Univ., New York, NY; [2]Emory Univ., Atlanta, GA; [3]Dept. of Microbiology, New York Univ. Grossman Sch. of Med., New York, NY; [4]NYU Neurosci. Institute, New York Univ. Grossman Sch. of Med., New York, NY

Abstract: Fragile X Syndrome (FXS) is an inherited developmental disorder with a high incidence of intellectual disability and autism-spectrum disorder (ASD). Patients with FXS present display numerous neurodevelopmental issues that result in aberrant behavior. FXS is caused by a repeat expansion (CGG motif, repeated over 200 times) in the upstream element of the *FMR1* gene that results in methylation and silencing of the gene, and a subsequent loss in expression of the protein product, Fragile X messenger ribonucleoprotein 1 (FMRP). Various studies have identified roles of FMRP in regulating translation, as well as neuronal function and development, but the mechanisms connecting altered translation with abnormal neuronal development in FXS remain unclear. It is known that human neuronal development requires various stages of translational programming, with differentiation leading to progressively less actively translating cells. This reduced translational load appears to be through a switch to alternate, non-canonical translation initiation machinery, evident in changes in levels of various translation initiation factors over the course of neuronal development, such as eIF3, eIF4E, eIF4G, as well as effectors of the S6K and 4E-BP pathways. Using differentiated excitatory neurons from control and FXS patient-derived induced pluripotent stem cells (iPSCs), we have explored the developmental changes in translational regulation in neurons from normal humans and in FXS patients. We have found that in FXS neurons, there is dysregulation in the developmental shift to non-canonical forms of translation, and consequently translation control is lost, and growth of neurons is slower. Our findings suggest that neuronal development requires utilizing cap-dependent, but eIF4E-independent, translational control instead of the canonical eIF4E-based translation initiation, and that the developmental shift to these alternate, non-canonical forms of translation is mediated by FMRP. This work was supported by NIH grant HD104558 (G.B. and E.K.).

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Poster

029. Fragile X Syndrome

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.06

Topic: A.07. Developmental Disorders

Support: FRAXA Research Foundation

Title: Dysregulated cholesterol homeostasis in human forebrain astrocytes modeling fragile X

Authors: K. TALVIO[1], R. MINKEVICIENE[3], A. O. KULINICH[4], V. WAGNER[5], J. S. KIRKWOOD[6], R. KÄKELÄ[2], I. M. ETHELL[7], ***M. L. CASTRÉN**[3];
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Abstract: Fragile X syndrome (FXS) is the most common inherited intellectual disability syndrome and a monogenic cause of autism spectrum disorder. FXS results from the absence of FMR1 protein (FMRP) that is essential for normal synapse formation and plasticity. Deficits of astrocytes are shown to contribute to impaired neuronal function in the brain of *Fmr1* knockout (KO) mice, the mouse model of FXS. Astrocytes maintain cholesterol homeostasis in the brain and produce cholesterol to the demand of neurons. Treatment with an inhibitor of cholesterol biosynthesis, lovastatin, has been shown to produce beneficial effects in the *Fmr1* KO mouse and in clinical trials of FXS, suggesting a role for cholesterol in the pathogenesis of FXS. Since brain cholesterol forms a separate cholesterol compartment in the body, understanding astrocytic regulation of cholesterol homeostasis in FXS is important for design of treatment targeting cholesterol function. We have studied cholesterol balance in human forebrain astrocytes generated from patient-specific human FXS (N=4) and control (N=4) induced pluripotent stem cells (iPSCs) and in astrocytes derived from the *Fmr1* KO and wild type mice. We found reduced cholesterol efflux transporter expression in FXS astrocytes. Using mass spectrometry, we observed that cholesterol content in astrocyte conditioned medium (ACM) collected from FXS and control astrocytes did not differ, but the ratio of cholesterol to cholesterol esters was increased in FXS astrocytes compared with controls. Lipidome analysis of *Fmr1* KO astrocytes showed accumulation of cholesterol and molecular species changes in several classes of lipids. Especially, polyunsaturated phospholipid species were reduced in *Fmr1* KO astrocytes. Abnormalities in the cytokine secretion profile and altered responses to cytokine exposure suggested contribution of anti-inflammatory cytokines to changes of cholesterol homeostasis in KO astrocytes. Altogether, our results demonstrated abnormal cholesterol balance combined with changes in phospholipidome potentially interfering with function of transporters and channels on the membrane, directly or by altering bilayer stiffness and fluidity.

Disclosures: K. Talvio: None. **R. Minkeviciene:** None. **A.O. Kulinich:** None. **V. Wagner:** None. **J.S. Kirkwood:** None. **R. Käkelä:** None. **I.M. Ethell:** None. **M.L. Castrén:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.07

Topic: A.07. Developmental Disorders

Title: Cell type-specific transcriptional dysregulation in the sensory cortices of Fragile X knockout mice.

Authors: *A. SURESH, J. BUTH, M. GANDAL, C. PORTERA-CAILLIAU;
Univ. of California Los Angeles, Univ. of California Los Angeles, Los Angeles, CA

Abstract: Fragile X Syndrome (FXS) is a prototypical neurodevelopmental disorder (NDD) characterized by intellectual disability, autistic traits, and atypical sensory processing. FXS arises from transcriptional silencing of the *FMR1* gene, which leads to the near-complete loss of the RNA binding protein fragile X messenger ribonucleoprotein 1 (FMRP). Functionally, FMRP is a repressor of protein translation and regulates the expression of several hundred genes. Exactly how the loss of FMRP and the resulting dysregulation of molecular signaling pathways impacts brain circuit function, has not yet been elucidated. Recent studies have implicated changes in excitatory and inhibitory circuits in the etiology of FXS, including reduced firing and density of parvalbumin (PV) neurons, the major subtype of inhibitory interneurons in the cerebral cortex. To investigate whether loss of FMRP similarly affects the transcriptome of excitatory and inhibitory neurons, we used a Ribo-Tag approach to isolate mRNA from Ca^{2+}/calmodulin-dependent protein kinase II (CAMK2) and PV neurons in primary somatosensory (S1) and visual (V1) cortices of adult *Fmr1*$^{-/-}$ mice and wild-type controls. Differential gene expression analysis identified 3046 and 537 dysregulated genes in CAMK2 and PV neurons respectively. Gene enrichment and pathway analysis identified several dysregulated pathways, including many unique to each cell type. Intriguingly, although down-regulated pathways such as *small GTPase signaling* were equally dysregulated in both PV and CAMK2 transcriptomes, up-regulated pathways such as *Autophagy, RNA processing, and Chromatin Remodeling* were exclusive to CAMK2 cells. These findings indicate that loss of FMRP has cell type-specific effects on the transcriptome of cortical sensory neurons, with CAMK2 neurons being more vulnerable than PV interneurons.

Disclosures: **A. Suresh:** None. **J. Buth:** None. **M. Gandal:** None. **C. Portera-Cailliau:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.08

Topic: A.07. Developmental Disorders

Support: National Agency for Research and Development (ANID) / Scholarship Program / DOCTORADO NACIONAL/2020 – 21200657
Project FONDECYT Regular 1210069

Title: Atypical anatomical traits in the early stages of the sensory pathways of the Fmr1 KO mice

Authors: *M. NAVARRETE, M. RUIZ-FLORES, A. DEICHLER, A. NUNEZ-PARRA, J. MPODOZIS;
Dept. de Biología, Facultad de Ciencias, Univ. de Chile, Santiago, Chile

Abstract: Fragile X syndrome (FXS) is produced by the silencing of the *FMR1* gen resulting in the absence of expression of the Fragile Mental Retardation Protein (FMRP). FMRP regulates the translation of several proteins involved in synaptic function, neuronal plasticity and neurodevelopment, which underpins a variety of neurological symptoms observed in individuals with FXS, including intellectual disability and atypical sensory perception. Indeed, FXS animal models show atypical social, olfactory and visual behaviors, as well as hyperexcitability and neural immaturity traits in sensory cortical areas. At present, whether and how the lack of FMRP affects early stages of sensory processing remains poorly explored. Here we compared the main anatomical traits of the olfactory bulbs and the retina between *Fmr1* KO and WT male adult mice by means of cytoarchitectonical and stereological conventional methods. Specifically, we performed volumetric measurements as well as cell counts at the different layers of the main (MOB) and accessory (AOB) olfactory bulbs. The AOB, located posterior to the MOB, is specialized in the processing of pheromonal cues mediating socio-sexual behaviors. At the retina we estimated the number and retinal distribution of cells in the ganglion cell layer (RGCs). In the MOB we did not find appreciable volumetric or cytoarchitectonic differences between WT and *Fmr1*-KO mice. The overall volume of the AOB was also not significatively different. However, the volumetric ratio between the anterior and posterior division of the AOB (aAOB/pAOB) was significantly smaller in the *Fmr1 KO* compared to the WT, a difference that was related to a decrease in the volume of the aAOB glomerular layer and to an increase in the volume of the pAOB granular cells layer. These results suggest that the *Fmr1* KO mouse may have a decreased sensibility to volatile pheromones, and thus an impaired discriminatory ability of the socio-sexual status of its conspecifics. At the retina we found that *Fmr1* KO and WT mice present similar numbers of RGCs, and, to our surprise, a large interindividual variation in the pattern of RGs retinal distribution. Nevertheless, retinal distribution of RGCs appear be more homogeneous in the retina of *Fmr1 KO* than in the WT. Retinal specializations with higher density of RGCs were less conspicuous and ill-defined in *Fmr1* KO, which hints to a loss of visual acuity and visual reactivity in these animals. In conclusion, our results suggest that the *Fmr1* mutation leads to specific morphological abnormalities at the initial stages of the sensory pathways, which could partially explain the atypical social and visual behaviors described in the *Fmr1* KO mice.

Disclosures: M. Navarrete: None. **M. Ruiz-Flores:** None. **A. Deichler:** None. **A. Nunez-Parra:** None. **J. Mpodozis:** None.

Poster

029. Fragile X Syndrome

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.09

Topic: A.07. Developmental Disorders

Support: NIH Grant R01NS109381

Title: Astrocytic contribution to sensory hypersensitivity in a mouse model of fragile X syndrome

Authors: *L. BERGDOLT, K. HOFFMAN, M. DOUCHEY, A. ANDING, P. RAGUNATHAN, **A. DUNAEVSKY**;
Univ. of Nebraska Med. Ctr., Omaha, NE

Abstract: Fragile X syndrome (FXS) is caused by mutations in the fragile X messenger ribonucleoprotein 1 (*Fmr1*) gene, which result in substantial or complete loss of expression of its protein product, FMRP. Patients with FXS are often diagnosed with autism spectrum disorder (ASD) and exhibit additional symptoms which may include intellectual disability, seizures, anxiety, and sensory hypersensitivity. Sensory hypersensitivity may contribute to other symptoms of FXS including learning difficulties, anxiety, and sleep disturbances, underscoring the importance of understanding the mechanisms underlying this symptom. FMRP regulates expression and function of hundreds of proteins, many of which are genes associated with ASD. Neuronal impairments in the absence of FMRP have been extensively characterized, but much less is known about the impact that loss of astrocytic FMRP has on behavior, especially sensory hypersensitivity. One form of sensory hypersensitivity exhibited by *Fmr1* knockout (KO) mice is tactile hypersensitivity in response to repeated whisker stimulation. We confirmed that *Fmr1* KO mice exhibit greater avoidance behavior, characterized by longer and faster movement away from the stimulus, during whisker stimulation compared to sham stimulation while wild-type littermate controls do not. To determine how loss of astrocytic FMRP contributes to this phenotype, we are using the inducible, astrocyte-specific cre line Aldh1l1-cre/ERT2 and administering tamoxifen during the first postnatal week to generate mice with astrocyte-specific deletion of *Fmr1* (*Fmr1* conditional KO, cKO). Analysis of avoidance behavior in *Fmr1* cKO mice is ongoing. Another form of sensory hypersensitivity exhibited by *Fmr1* KO mice is auditory hypersensitivity. A robust phenotype in *Fmr1* KO mice is susceptibility to audiogenic seizures, manifested as wild running and tonic-clonic seizures following exposure to a 120 dB siren. We found that loss of astrocytic FMRP confers susceptibility to audiogenic seizures in three-week-old male mice. Moreover, selective restoration of *Fmr1* expression in astrocytes decreases the propensity for audiogenic seizures. Reduced expression of inositol 1,4,5-trisphosphate type 2 receptor (IP3R2), a main contributor to astrocytic calcium signaling, prevents audiogenic seizures in *Fmr1* cKO mice, suggesting that altered calcium signaling may contribute to this phenotype. Taken together, our results indicate that loss of astrocytic FMRP contributes to both auditory and tactile hypersensitivity in mice.

Disclosures: L. Bergdolt: None. **K. Hoffman:** None. **M. Douchey:** None. **A. Anding:** None. **P. Ragunathan:** None. **A. Dunaevsky:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.10

Topic: A.07. Developmental Disorders

Support: NIH Grant 1U54 HD082008
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USAMRDC Grant W81XWH-15-1-0434
NIH Grant 1F31NS117178-01

Title: Astrocytes dysregulate GABA expression and transmission in Fragile X Syndrome affecting cortical responses to sound

Authors: *V. A. WAGNER[1], M. RAIS[1], A. O. KULINICH[1], W. WOODWARD[2], X. S. SHUAI[2], S. SUTLEY[3], J. KOKASH[2], T. P. PIEPPONEN[4], M. L. CASTRÉN[5], K. A. RAZAK[6], I. M. ETHELL[1];
[1]Biomed. Sci., [2]Univ. of California, Riverside, Riverside, CA; [3]Biomed. Sci., Univ. of California Riverside, Riverside, CA; [4]Univ. of Helsinki, Helsinki, Finland; [5]Helsinki Univ., Helsinki, Finland; [6]Univ. California, Riverside, Riverside, CA

Abstract: Fragile X syndrome (FXS) is a leading genetic cause of autism-like symptoms associated with sensory hypersensitivity and cortical hyperexcitability, resulting from silencing of the Fragile X messenger ribonucleoprotein (*Fmr1*) gene. Recent observations in humans and *Fmr1* knockout (KO) animal models of FXS suggest symptoms are mediated by abnormal GABAergic signaling. As most studies have focused on neuronal mechanisms, astrocytes' contribution to defective inhibition is largely unknown. We used high-performance liquid chromatography (HPLC), western blotting, and immunostaining to assess GABAergic components in human male FXS astrocytes derived from patient-specific induced pluripotent stem cells (iPSCs) and P28 male mouse (Mus musculus) astrocytes (n=4/group) following astrocyte-specific *Fmr1* KO during the postnatal period (cKO) compared to control iPSC lines and mice lacking the *Fmr1* flox (Ctrl WT). We found abnormally increased GABA levels in our human and mouse models, and altered synaptic $GABA_A$ receptor levels and parvalbumin (PV) cell development in the hippocampus and cortex of cKO mice. In EEG recordings obtained from P60-P70 adult male Ctrl WT (n = 10) and cKO (n = 9) mice, we observed altered communication between excitatory neurons and PV cells, where cKO mice had impaired cortical sound-evoked gamma synchronization, with enhanced baseline and on-going sound-evoked EEG power. Adult cKO mice had increased locomotor activity and altered social behaviors in an elevated plus maze and social novelty test (n=8/group) scored blind to condition. Preliminary data show upregulation of GABA-synthesizing enzymes GAD65/67 levels in KO mouse astrocytes. Sex differences were not assessed. These results demonstrate a profound role of astrocytic FMRP in the development of inhibitory circuits and are the first to address astrocyte-mediated mechanisms of abnormal inhibition in FXS.

Disclosures: V.A. Wagner: None. **M. Rais:** None. **A.O. Kulinich:** None. **W. Woodward:** None. **X.S. Shuai:** None. **S. Sutley:** None. **J. Kokash:** None. **T.P. Piepponen:** None. **M.L. Castrén:** None. **K.A. Razak:** None. **I.M. Ethell:** None.

Poster

029. Fragile X Syndrome

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.11

Topic: A.07. Developmental Disorders

Support: NIH Grant R01 MH116500

Title: Impaired visual experience-dependent synaptic inter-areal connectivity in the visual cortex of Fmr1 KO mice

Authors: *X. CHENG, S. NAREDDULA, P. A. EDENS, A. A. CHUBYKIN;
Dept. of Biol. Sci., Purdue Univ., West Lafayette, IN

Abstract: Fragile X syndrome (FXS) is the most common form of inheritable autism spectrum disorder (ASD), associated with hypersensitivity, difficulty habituating to novel sensory stimuli, as well as intellectual disability. Visual perception and learning deficits were reported in FX patients. Previous studies have described disrupted excitatory to inhibitory (E/I) balance, impaired short-term (STP) and long-term plasticity, and aberrant functional connectivity in Fmr1 KO mice, the model of FXS. The reciprocal projections connecting the primary visual cortex (V1) and higher visual areas, including both the ventral and dorsal pathways, have been implicated in regulating cognitive processes such as prediction, attention, and visual learning. However, how the inter-areal connectivity is affected in FX is poorly understood. To gain more insight into this process, we developed a new perceptual experience paradigm leading to the emergence of familiarity-specific theta oscillations. We measured the strength and properties of the long-range synaptic connections between V1 and the higher visual area, lateromedial area (LM), as the part of the ventral pathway, in wildtype (WT) and Fmr1 KO mice, pre-and post-visual experience, using channelrhodopsin-2-assisted circuit mapping (CRACM) in acute brain slices. CRACM of feedforward projections reveals increased synaptic strength from V1 onto pyramidal cells (PCs) in all cortical layers of LM after visual experience in WT, while only a mild increase in the superficial layer II/III in Fmr1 KO mice. CRACM of feedback projections reveals decreased synaptic strength from LM onto PCs in layer II/III of V1 and increased strength in deep layer V after experience in WT, but no changes in Fmr1 KO mice. E/I ratio measurement reveals hyperexcitation post-experience of the feedforward synaptic connections in Fmr1 KO, primarily attributed to attenuated inhibition. Interestingly, some of the synaptic properties of the feedback projections in Fmr1 KO mice appeared to improve following visual experience, such as the paired-pulse ratios as a measurement of STP. Our findings provide the first measurements of the inter-areal synaptic connectivity before and after visual experience in WT and Fmr1 KO mice and indicate that visual training may serve as a promising therapeutic intervention for ASD.

Disclosures: X. Cheng: None. **S. Nareddula:** None. **P.A. Edens:** None. **A.A. Chubykin:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.12

Topic: A.07. Developmental Disorders

Support: NIH Grant R01MH050047
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Kelvin Foundation
Canel Family Fund

Title: Aberrant neural response and eye gaze pattern in girls with fragile X syndrome

Authors: *R. LI, A. PICCIRILLI, A. A. LIGHTBODY, A. L. REISS;
Stanford Univ., Stanford, CA

Abstract: Fragile X syndrome (FXS) is the most common inherited cause of intellectual disability, affecting approximately 1 in 4000 males and 1 in 7000 females worldwide. Individuals with FXS often exhibit significant symptoms of avoidance, anxiety, and hyperarousal, particularly in response to daily social interaction. However, little is currently known about the aberrant patterns of brain neural response and biobehavioral such as eye gaze movement in girl with FXS, an important but understudied clinical population. The present study sought to characterize brain network properties and eye gaze patterns in girls with FXS during natural face-to-face conversation. We recruited 35 girls with FXS (11.35±3.07 years) as the FXS group, and 31 age- and developmentally matched (i.e., verbal IQ, autism-related social behavior, executive function) girls as the control group (11.41±2.32 years). A portable brain imaging technique called functional near-infrared spectroscopy (fNIRS) and an eye gaze tracker were used to measure the hemodynamic response and eye gaze movement of each participant during a structured face-to-face conversation with a researcher, respectively. We found that, compared to the control group, girls with FXS exhibit significantly increased inter-regional functional connectivity and greater nodal strength within the prefrontal cortex (PFC), frontal eye field (FEF) and superior temporal gyrus (STG) during the conversation. Moreover, girls with FXS showed significantly less eye contact with their conversational partner and more unregulated eye gaze behavior compared to the control group. Within the FXS group, correlational analysis was performed between the aberrant brain network measures and eye gaze behaviors. We found that greater nodal strength at left and right STG were significantly associated with higher eye gaze fixations on research's eye region in girls with FXS. We also found that nodal strength at the left FEF, and functional connectivity between the left and right FEF, were negatively correlated with mean saccade duration when the FXS girls were engaging in the conversation. Through the simultaneous brain-eye gaze measurement, for the first time, we provide preliminary evidence for linking aberrant neural response of girls with FXS to their abnormal eye gaze behaviors during naturalistic social interaction. The findings here thus expand our current knowledge of the neural mechanisms and eye gaze behaviors underlying naturalistic social interaction in girls with FXS. We expect that these results could be further evaluated and developed as intermediate phenotypic endpoints for the evaluation of treatment trial in FXS.

Disclosures: R. Li: None. **A. Piccirilli:** None. **A.A. Lightbody:** None. **A.L. Reiss:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

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Program #/Poster #: 029.13

Topic: A.07. Developmental Disorders

Title: Social Preference Learning in Young & Adult Fmr1 KO Mice

Authors: A. HUSSAIN, K. A. RAZAK;
Univ. of California Riverside, Riverside, CA

Abstract: BackgroundFragile X Syndrome (FXS) is a leading known genetic cause of autism spectrum disorder, caused by a gene mutation that disrupts the production of fragile X messenger ribonucleoprotein (FMRP). Disruption of FMRP can lead to debilitating symptoms in the cognitive, social, and communication domains. The *Fmr1* knockout (KO) mouse model is a well-studied animal model of FXS to characterize pathophysiological mechanisms. Young mice (~P30) show learned preference for social environment (vs isolation), but this social conditioned place preference (sCPP) learning decreases into adulthood (>P60). In this study, the hypothesis is that the developmental plasticity of sCPP is abnormal in KO mice. MethodsWe utilized the sCPP task that uses a 3-chambered apparatus to measure social and isolation-avoidant behaviors. Wildtype (WT) and KO mice (FVB strain) were tested at two ages, p30 – p40 (young) and p60 – p70 (old). This task uses a 4-day protocol with a 30 min pretest on the first day and a posttest on the last day where the test mouse has access to the entire chamber with two different bedding types in two of the chambers, and with a neutral middle chamber. Test mouse is conditioned to a social setting in one chamber, with two littermates, and one of the bedding types for 24 hr, followed by a 24 hr isolation conditioning in the opposite chamber with the other bedding. After the posttest, the mouse was left in the apparatus for an another 15 mins to induce cFos activation. Test mouse brains were collected to measure cFos density in several brain areas connected to social and avoidant behaviors.
ResultsSplit 15 min analysis showed social preference in the last half of the test for young WT and KO. Sex differences were seen with old WT females showing no social preference throughout the whole task, while the old KO females showed social preference only in the last half of the test. Lastly, old KO males showed no avoidance of the isolation chamber in the last half of the test.
A negative correlation was observed between cFos density and social preference index as well as avoidance index in old WT and KO in the NAcc and VMH. This suggests that adult KO mice may have different underlying mechanisms, allowing them to reach the same behavioral outcome as WT.
ConclusionsThe results partially support our hypothesis with only older female KO's exhibiting an abnormal social preference. Different relationships were seen between cFos density and social preference in older WT and KO mice. This is a novel finding in several aspects, FMRP deficit does not initially affect social preference learning. However, there is an altered developmental experience that results in differences from WT mice in adulthood.

Disclosures: **A. Hussain:** None. **K. A. Razak:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.14

Topic: A.07. Developmental Disorders

Support: NIH Grant T32 MH016804-41

Title: Hyperexcitability and increased excitatory synaptic input in D1R MSNs in the dorsomedial striatum of the Fmr1 KO mouse

Authors: *L. NELSON[1], M. JANECEK[3], L. CHEN[2], R. PEIXOTO[2];
[2]Dept. of Psychiatry, [1]Univ. of Pittsburgh, Pittsburgh, PA; [3]Ctr. for Neurosci., Univ. of Pittsburgh Ctr. For Neurosci., Pittsburgh, PA

Abstract: Fragile X Syndrome is a monogenic disorder caused by a decrease in Fmr1 gene and FMR1 protein expression. Loss of function of Fmr1 causes hyperactivity, motor coordination problems, anxiety and sensory over-reactivity throughout life. These behaviors are strongly regulated by the striatal network however, there is very little research investigating changes in striatal cell connectivity and function due to loss of Fmr1. Studies in humans with Fragile X Syndrome have found decreased corticostriatal connectivity suggesting that striatal neurons may have decreased synaptic input. Structural and functional characteristics of medium spiny neurons (MSNs) in the dorsal striatum were measured using patch clamp electrophysiology in Fmr1 -/y (knockout) and Fmr1+/y (wildtype) adult male mice. The frequency of mEPSCs, a measure of synaptic input, and active cell properties such as firing frequency, rheobase, and firing rate were measured in MSNs. Additionally, we assessed whether there are differences in direct pathway, dopamine receptor 1 (D1R) positive, and indirect pathway, D1R negative, MSNs since these distinct pathways work together to during normal striatal function. There was increased variability in mEPSC frequency in the Fmr1-/y MSNs suggesting that loss of Fmr1 causes variability in the number of excitatory synaptic connections onto MSNs. A separate experiment found that when MSNs were sorted into D1R positive and negative cells, we found that D1R positive, but not negative cells, had an increased number of mEPSCs, suggesting an MSN subtype specific increase in excitatory synaptic innervation. We measured function properties of D1R and D2R MSNs using current clamp electrophysiology. FMR1 D1R MSNs had a decreased spike threshold and rheobase, and had voltage dependent changes in membrane resistance compared to WT mice. Loss of Fmr1 expression had no effect on D2R MSNs. Loss of Fmr1 leads specific changes in D1R MSNs suggesting a role for the direct pathway MSNs in Fragile X Syndrome. Assessing cell function during a striatal specific task will help determine how changes in cell properties affect *in vivo* function of D1R MSNs.

Disclosures: **L. Nelson:** None. **M. Janecek:** None. **L. Chen:** None. **R. Peixoto:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.15

Topic: A.07. Developmental Disorders

Support: NIH NS112706-01

Title: From abnormal metabolism to behavioral deficits-early postnatal intervention in Fragile X mice.

Authors: *P. LICZNERSKI, V. K. GRIBKOFF, E. A. JONAS;
Yale Univ. Sch. of Med., New Haven, CT

Abstract: Loss of function of the gene (Fmr1) encoding Fragile X mental retardation protein (FMRP) results in unregulated, elevated mRNA translation and aberrant synaptic morphology. We have recently described a completely novel function of a "leak channel" termed ATP synthase c-subunit leak channel (ACLC), formed by the C-subunit octamer within the ATP-synthase in the mitochondrial inner membrane. We discovered that in Fragile X syndrome the pharmacological inhibition of ACLC by the ATP synthase modulator Dexpramipexole (Dex) normalizes the elevated protein translation rates and attenuates autistic behaviors, which raises the spectrum of new possibilities for therapeutic intervention. Moreover, ACLC closure normalized elevated lactate and key glycolytic and tricarboxylic acid (TCA) cycle enzyme levels in Fragile X and triggered synapse maturation. In the current study we are examining the effects of ACLC closure in early postnatal Fragile X mice, during a key peak of synaptogenesis at P9-10. We hypothesize that modulation of ACLC at this time speeds metabolic development of the synapse with effects on specific protein synthesis, synaptic structure and connectivity, and this may normalize circuitry and produce behavioral benefits into adulthood. We are now testing this hypothesis.

Disclosures: P. Licznerski: None. **V.K. Gribkoff:** None. **E.A. Jonas:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.16

Topic: A.07. Developmental Disorders

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R01MH094681 (NIH-NIMH)
FRAXA foundation

Title: Improvement of sensory deficits in Fragile X mice by increasing cortical interneuron activity after the critical period.

Authors: *N. KOURDOUGLI[1], A. SURESH[2], B. LIU[2], P. JUAREZ[3], A. LIN[2], D. T. CHUNG[2], A. GRAVEN SMITH[4], M. GANDAL[2], V. MARTINEZ-CERDENO[3], D. BUONOMANO[2], B. HALL[4], C. MOMBEREAU[4], C. PORTERA-CAILLIAU[2];
[1]UCLA, [2]UCLA, Los Angeles, CA; [3]Univ. of California Davis, Univ. of California Davis, Carmichael, CA; [4]Lundbeck A/S H, Copenhagen, Denmark

Abstract: Inhibitory interneurons (INs) play a critical role in shaping the activity of excitatory neurons in the mature brain. Although recent studies have reported on the origin of different IN subclasses and how they functionally integrate into circuits, less is known about how INs modulate excitatory neurons in the developing brain, or about how this process might be affected in neurodevelopmental disorders (NDDs). For example, hypofunction of cortical INs has been implicated in the pathophysiology of various NDDs, but the nature of deficits within GABAergic populations throughout neonatal development remains unexplored. Here, we sought to identify when IN hypofunction is first apparent in the developing neocortex of Fmr1 knockout (*Fmr1*$^{-/-}$) mice, the best studied animal model of Fragile X Syndrome (FXS). Using all-optical in vivo approaches in early postnatal somatosensory cortex (S1), we find that parvalbumin (PV) IN precursors are hypoactive and decoupled from excitatory neurons in *Fmr1*$^{-/-}$ mice. This leads to excessive loss of PV-INs in both mice and humans with FXS. Increasing the activity of future PV-INs in neonatal *Fmr1-/-* mice with Gq DREADDs restores PV density and ameliorates transcriptional dysregulation in S1, but not circuit dysfunction. Critically, administering a novel allosteric modulator of Kv3.1 channels after the S1 critical period (from P15 to P20) does rescue circuit dynamics and tactile defensiveness. These suggests that circuit changes and sensory symptoms in FXS and other NDDs could be ameliorated with interventions that target PV-INs

Disclosures: N. Kourdougli: None. **A. Suresh:** None. **B. Liu:** None. **P. Juarez:** None. **A. Lin:** None. **D.T. Chung:** None. **A. Graven Smith:** None. **M. Gandal:** None. **V. Martinez-Cerdeno:** None. **D. Buonomano:** None. **B. Hall:** None. **C. Mombereau:** None. **C. Portera-Cailliau:** None.

Poster

029. Fragile X Syndrome

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Program #/Poster #: 029.17

Topic: A.07. Developmental Disorders

Title: Conditional restoration of FMRP in PV interneurons partially rescues visual familiarity coding in FX mice

Authors: *S. NAREDDULA, A. A. CHUBYKIN;
Purdue Univ., Purdue Univ., West Lafayette, IN

Abstract: Autism spectrum disorder (ASD) is a neurodevelopmental disorder that widely affects information processing in the brain resulting in deficits in learning and memory. One of the prevalent forms of ASD is Fragile X Syndrome (FXS), which results from a mutation in the FMR1 protein (FMRP). Previous studies have shown alterations in cell morphology, synaptic connections, and neural circuits pertaining to sensory perception in FXS model systems. Consistent with this, our lab has identified significant differences in the visual response of FX mice to a visual perceptual experience paradigm. Visual experience evokes low-frequency (4-8 Hz) theta oscillations in the primary visual cortex of wild-type mice, suggesting these oscillations are a possible mechanism for visual memory encoding. In FX mice, however, these oscillations are attenuated in duration, amplitude, and frequency, potentially leading to the learning disability symptomatic of the disorder. We propose a novel model that predicts the specific interaction between excitatory pyramidal cells and fast spiking inhibitory neurons in V1 which forms the circuitry responsible for the oscillations. Parvalbumin (PV) interneurons are reported to be developmentally impaired in FX mice and have reduced functionality associated with visual perception. To better understand the role of PV interneurons in the observed visual experience evoked oscillations and the subsequent impairments in FX mice, we used a novel Fmr1 conditional restoration mouse strain (Fmr1 cON/PV-Cre) to restore the expression of FMRP specifically and only in PV interneurons. While blinded to the genotype of the mice, we performed an experiment using Fmr1 cON/PV-Cre strain with WT and FX strains as controls. Silicon probe recordings of V1 were taken before and after the visual experience training paradigm for all three strains. We found that visual experience evoked oscillations in the Fmr1 cON/PV-Cre strain showed improvements from the FX strain. The conditional restoration strain showed a higher number of oscillation cycles, and a shift towards a higher frequency in LFPs. Further, the oscillations had more pronounced peaks and troughs than FX, indicating sharper inhibition in the circuit. This partial rescue of phenotypes seen in Fmr1 cON/PV-Cre strain emphasizes the importance of the role PV interneurons play in the cellular mechanisms of the visual experience evoked oscillations and could provide a potential therapeutic avenue of treatment for FXS.

Disclosures: S. Nareddula: None. **A.A. Chubykin:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.18

Topic: A.07. Developmental Disorders

Support: NIH R01MH118827
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NIH R01AG067025
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U54HD090256

Title: Sustained correction of hippocampal neurogenic and cognitive deficits after a brief treatment by Nutlin-3 in a mouse model of Fragile X Syndrome

Authors: *S. JAVADI[1], Y. LI[2], J. SHENG[3], L. ZHAO[3], Y. FU[3], D. WANG[3], X. ZHAO[3]; [1]UW-Madison, Univ. of Wisconsin - Animal Sci. Program, madison, WI; [2]Inst. of Traditional Chinese Med., Tianjin, China; [3]Univ. of Wisconsin-Madison, madison, WI

Abstract: Fragile X syndrome (FXS), the most prevalent inherited intellectual disability and one of the most common monogenic form of autism, is caused by a loss of FMRP translational regulator 1 (FMR1). FMR1 suppresses the levels of ubiquitin ligase MDM2 and its active form, phosphorylated-MDM2 (P-MDM2) ,in young adult FMR1-deficient mice and treatment by an MDM2 inhibitor Nutlin-3 rescues both hippocampal neurogenic and cognitive deficits in FXS mice when analyzed shortly after the administration. However, it is unknown whether Nutlin-3 treatment can have long-lasting therapeutic effects. We injected 2-month-old young adult FMR1-deficient male mice with Nutlin-3 every other day for 10 days (10 mg/kg body weight) and then assessed the persistent effect of Nutlin-3 on both proliferation and neuronal differentiation of hippocampal progenitor cells (n = 3 - 4) and cognitive functions (evaluated by novel object recognition and novel location recognition tests, n = 8 -11 mice) when mice were 6-month-old mature adults. To investigate the mechanisms underlying the persistent effects of Nutlin-3, we analyzed proliferation and differentiation of neural stem cells isolated from hippocampal dentate gyrus of treated male and assessed the transcriptome of the hippocampal tissues of treated mice (n = 3 mice). Two tailed and unpaired t-test was used to compare two conditions. Two-way ANOVA with Tukey's post hoc analysis was used for analyzing multiple groups. Probabilities of $P < 0.05$ were considered as significant.We found that transient treatment with Nutlin-3 of 2-month-old young adult FMR1-deficient mice prevents the emergence of neurogenic and cognitive deficits in mature adult FXS mice at 6-month of age. We further found that the long-lasting restoration of neurogenesis and cognitive function might not be mediated by changing intrinsic properties of adult neural stem cells. Transcriptomic analysis of the hippocampal tissue demonstrated that the short-term Nultin-3 treatment leads to significant changes in the expression levels of genes related to extracellular matrix, secreted factors, and cell membrane proteins in FMR1-deficient hippocampus with no significant effects on control animals treated with vehicle. Our data indicates that transient Nutlin-3 treatment in young adults leads to long-lasting neurogenic and behavioral changes likely through modulating adult neurogenic niche that impact adult neural stem cells. Our results demonstrate that cognitive impairments in FXS may be prevented by an early intervention through Nutlin-3 treatment.

Disclosures: **S. Javadi:** None. **Y. Li:** None. **J. Sheng:** None. **L. Zhao:** None. **Y. Fu:** None. **D. wang:** None. **X. zhao:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.19

Topic: A.07. Developmental Disorders

Support: NCATS UL1TR002373
NIA P50-AG033514
NIEHS R25 ES020720
FRAXA Research Foundation

Title: Ketogenic diet affects sleep architecture in a mouse model of fragile X

Authors: P. R. WESTMARK, A. GHOLSTON, R. K. MAGANTI, ***C. J. WESTMARK**; Neurol., Univ. of Wisconsin, Madison, WI

Abstract: Nearly half of children with fragile X syndrome experience sleep problems such as trouble falling asleep and frequent nighttime awakenings. The goals here were to assess sleep-wake cycles in mice in response to *Fmr1* genotype and an intervention that reduces hyperactivity. Electroencephalography results were compared with published rest-activity patterns to determine if actigraphy is a viable surrogate for sleep electroencephalography. Specifically, sleep-wake patterns in adult wild type and $Fmr1^{KO}$ littermate mice were recorded after electroencephalography electrode implantation and the recordings manually scored for vigilance states. The data indicated that $Fmr1^{KO}$ mice, which are hyperactive in actigraphy assays, exhibited sleep-wake patterns similar to wild type littermates when maintained on a control diet. Treatment with a high fat, low carbohydrate ketogenic diet increased the percentage of non-rapid eye movement sleep in both wild type and $Fmr1^{KO}$ mice during the dark cycle, which corresponded to decreased activity levels. Treatment with ketogenic diet flattened circadian sleep periodicity in both wild type and $Fmr1^{KO}$ mice. Differences in several sleep microstructure outcomes (number and length of wake and sleep bouts) supported the altered sleep states in response to ketogenic diet and were correlated with altered rest-activity cycles. While actigraphy may be a less expensive, reduced labor surrogate for sleep EEG during the dark cycle, daytime resting in mice did not correlate with sleep states.

Disclosures: P.R. Westmark: None. **A. Gholston:** None. **R.K. Maganti:** None. **C.J. Westmark:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.20

Topic: A.07. Developmental Disorders

Support: LouLou Foundation Grant CDKL5-21-D-101-01

Title: Thinkrare - biomarker analysis to bridge preclinical and clinical research in rare neurodevelopmental disorders (ndds) like cdkl5 deficiency disorder (cdd) and fragile x syndrome

Authors: *M. BIANCHI[1], J. KEALY[1], A. THORNTON[1], C. CALLAGHAN[1], A. FREEBURN[1], D. DISHA[1], C. MCGURK[1], C. CONNOLLY[1], F. TRAINI[1], C. KILSTRUP-NIELSEN[2], I. BARBIERO[2], B. GARRONE[3], C. MILANESE[3], F. DI GIORGIO[3];
[1]Ulysses Neurosci. Limited, Dublin, Ireland; [2]Univ. of Insubria, Insubria, Italy; [3]Angelini, Rome, Italy

Abstract: Neurodevelopmental disorders (NDDs) are chronic, heterogeneous, underdiagnosed conditions estimated to affect around 1.5% of the world population. NDDs can be rare and ultrarare conditions characterised by severe intellectual and physical disabilities impacting the daily life of both patients and families. Our #ThinkRare research currently focuses on the pathogenesis and treatment of the rare genetic NDDs CDKL5 Deficiency Disorder (CDD) and Fragile X Syndrome (FXS), both of which are due to mutations of specific genes located on the X chromosome namely *Cdkl5* and *Fmr1*, respectively. An important aspect of our research is the patient-centricity value; thus patients/family and associations actively participate in the design of our preclinical and clinical research with the aim to improve the translational aspect of our projects. The #ThinkRare team is exploring central and peripheral biomarkers including microtubule proteins (i.e. alpha-tubulin post-translational modifications (PTMs)), synaptic markers (i.e. synaptophysin, SV2A, PSD-95 and spinophilin) as well as inflammatory molecules (i.e. a panel of cytokines /chemokines). Preclinical data show that expression of alpha-tubulin PTMs is significantly altered in *Cdkl5*-knock out (KO) mice (CDD model) and *Fmr1*-KO mice (FXS model) in both brain regions and plasma. These alterations are rescued by pharmacological treatment with steroid derivatives and gene therapy in the *Cdkl5*-KO mice. Pharmacological treatment with protein kinase modulators was efficacious in rescuing both behavioural (i.e. marble burying, locomotor activity and social discrimination) and biomarker alterations in *Fmr1-KO* mice. Our next step was to bridge our preclinical and clinical research by analysing the expression of alpha-tubulin PTMs in the plasma of patients affected by CDD and FXS. Plasma from CDD patients and healthy controls was obtained from US and Italian cohorts, while plasma samples from FXS patients and healthy controls were obtained from an Argentinian cohort. The results showed significant alterations in alpha-tubulin PTMs in both CDD and FXS patients compared to healthy controls. These alterations show a similar pattern to the ones observed in the plasma of *Cdkl5*-KO mice and *Fmr1*-KO mice. In conclusion, our translational data indicate new potential avenues for the identification of biomarkers of disease progression in CDD, FXS and other NDDs.

Disclosures: M. Bianchi: None. **J. Kealy:** None. **A. Thornton:** None. **C. Callaghan:** None. **A. Freeburn:** None. **D. Disha:** None. **C. McGurk:** None. **C. Connolly:** None. **F. Traini:** None. **C. Kilstrup-Nielsen:** None. **I. Barbiero:** None. **B. Garrone:** None. **C. Milanese:** None. **F. Di Giorgio:** None.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.21

Topic: A.07. Developmental Disorders

Title: Functional Phenotypic Screening of Small Molecules in a human patient-derived cell model for Fragile X Syndrome

Authors: *K. JÜGELT[1], L. SCHULTZ[1], J. CHARLWOOD[2], **O. H.-U. SCHROEDER**[1]; [1]NeuroProof Systems GmbH, Rostock, Germany; [2]Enterprise Therapeut., Falmer, United Kingdom

Abstract: Fragile X syndrome, FXS, is the most common form of inherited cognitive disability. FXS is a neuro-developmental disease with a prevalence of one per 5000 to 7000 males and one per 4000 to 6000 females (Hunter, Rivero-Arias et al. 2014). There is no effective treatment for FXS available today.
Functional phenotypic screening with iPSC-derived neuronal cell cultures from patients is an extremely powerful method to identify new leads in drug development. Diseases with a monogenetic cause such as fragile x syndrome are well suited for disease modeling with human patient iPSC-derived neurons. Such cell culture models, combined with a functional readout of electrical activity patterns by microelectrode array recordings, helps to identify best drug candidates with a high likelihood of success. We describe a functional phenotypic screening campaign for a new FXS treatment, which successfully identified the phosphodiesterase 10A inhibitor balipodect (TAK-063) as a potential new treatment of FXS. We compare the potential therapeutic effects of balipodect, mavoglurant, arbaclofen, and lovastatin in this model. We used a well-characterized iPSC cell line from an FXS patient and differentiated it into a glutamatergic neuronal cell culture. The neuronal cell cultures were cultivated on and studied using microelectrode array plates.

Disclosures: K. Jügelt: A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **L. Schultz:** A. Employment/Salary (full or part-time):; NeuroProof Systems GmbH. **J. Charlwood:** 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 UK Ltd. **O.H. Schroeder:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); NeuroProof Systems GmbH.

Poster

029. Fragile X Syndrome

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 029.22

Topic: A.07. Developmental Disorders

Support: FRAXA

Title: Adiponectin Modulation as a Novel Neuroendocrine Therapeutic Approach to Fragile X Syndrome

Authors: *I. SHKOLNIKOV, J. THACKER, L. BETTIO, S. LIANG, B. CHRISTIE;
Dept. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada

Abstract: Fragile X Syndrome (FXS) is a leading cause of monogenic inherited intellectual disability and aprevalent genetic cause of autism. To date, the only FDA approved therapies for FXS are antipsychotics and further exploration is imperative to propose therapeutics which better address the molecular sources of FXS. In the present study we investigate neuroendocrine modulation through adiponectin receptor agonism as a potential therapeutic approach to the molecular etiology of FXS. As a lipid signaling hormone, adiponectin regulates insulin sensitivity, metabolic homeostasis, and cell metabolism which are all systems known to be dysregulated in FXS.

We used molecular and behavioral techniques to explore therapeutic effects of AdipoRon, a synthetic adiponectin receptor agonist, on disease pathogenesis in a mouse model of FXS (FMR1KO). We measured weight and blood serum adiponectin concentration among adult male FMR1KO mice to find that these animals maintain a higher average body mass and lower serum adiponectin than WT animals (n= 8 animals, 23% ± 1.10 WT, p<0.05 t-test). These findings confirm adiponectin signaling perturbation in FMR1KO animals and corroborate suitability as a target for exploration.

Furthermore, learning and memory deficits are a key presentation of FXS in both patients and animal models. Electrophysiological interrogation of synaptic plasticity is a useful proxy for pharmacological effectiveness in restoring altered synaptic activity. In ex vivo electrophysiology, we found that a twenty minute incubation with AdipoRon restored long-term potentiation in the dentate gyrus (DG) of FMR1KO mice to control levels (n=8 animals/treatment group, 31% ± 14.07 FXS-vehicle, p<0.05 One-way ANOVA). These promising ex vivo results with adiponectin treatment prompted in vivo pharmacology to further validate AdipoRon as a potential therapeutic.

Eight-week-old FXS and Control (CO) adult male mice were treated with AdipoRon for fifteen days (n=5 animals/treatment group), then performed a series of behavioral tasks: open field (OF), novel object recognition (NOR), and three chamber sociability (TCS). In OF, there was a moderate decrease in hyperlocomotion and reduced anxiety behavior in AdipoRon-treated FMR1KO mice relative to vehicle and cage control mice. There were no changes in NOR, and a moderate improvement in TCS among AdipoRon-treated FMR1KO mice. Together, these findings suggest that neuroendocrine alterations and cell metabolism are promising targets for FXS therapeutic development and that adiponectin-dependent modulation of these systems is a novel therapeutic angle for FXS which requires further explorative attention.

Disclosures: **I. Shkolnikov:** None. **J. Thacker:** None. **L. Bettio:** None. **S. Liang:** None. **B. Christie:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.01

Topic: A.07. Developmental Disorders

Support: Pitt Hopkins Research Foundation
NINDS grant R01NS114086

Title: Regional and cellular organization of the intellectual disability and schizophrenia-associated transcription factor TCF4 in the developing rhesus macaque brain

Authors: *C. GONZALEZ RAMIREZ[1], S. SALVADOR[1], H. VIHMA[1], D. G. AMARAL[2], A. C. BURETTE[1], B. D. PHILPOT[1];
[1]Cell Biol. and Physiol., UNC-CH, Chapel Hill, NC; [2]Univ. of California Davis, Sacramento, CA

Abstract: TCF4, a member of the basic helix-loop-helix family of transcription factors, differentially controls the expression of hundreds of genes and plays a critical role in brain development. TCF4 has been linked to human neurodevelopmental disorders such as intellectual disability, schizophrenia, and Pitt-Hopkins syndrome. TCF4 expression has been studied mainly in the mouse brain, but to identify potential pathophysiological mechanisms and targets for therapeutic interventions in TCF4-linked disorders, we must understand where TCF4 is expressed in human brain development. Rhesus macaque monkey (Macaca mulatta) represents a close primate relative to humans. Here, we compared the spatiotemporal expression of TCF4 in the mouse and the rhesus macaque brain. Combining high-resolution immunohistochemistry with hybridization chain reaction in situ, we mapped TCF4 regional and cellular expression in normal prenatal (gestational day 50, 100, and 150), neonatal (2 and 4 weeks old), and adult (3 months and 5 years) macaque brains. At the gross anatomical level, TCF4 expression resembles that observed in mice, with the strongest expression in the neocortex, hippocampus, and cerebellum. However, within a given brain region, we found unexpected interspecies differences. This was most notable in the neocortex, where in the macaque, TCF4 expressing cells concentrate in layers 2 and 4, while in the mouse, TCF4 is uniformly expressed across all layers of the neocortex. Focusing on the neocortex, we found that TCF4 expression levels widely vary in neurons: a small population of GABAergic and glutamatergic neurons (~20%) expressed high TCF4 levels while most other neurons (~80%) have much lower TCF4 levels. This range of heterogeneous pattern of expression was not observed in mice. We observed the strongest TCF4 expression in neuronal precursors cells in the subventricular zone. Surprisingly, unlike what we observed in mice, little to no TCF4 mRNA or protein was detected in astrocytes in the macaque brain.
Our results indicate a more complex regulation of TCF4 expression in primates than in rodents

and help to define critical parameters, such as TCF4 biodistribution and cell type, for eventual therapeutic interventions.

Disclosures: C. Gonzalez Ramirez: None. **S. Salvador:** None. **H. Vihma:** None. **D.G. Amaral:** None. **A.C. Burette:** None. **B.D. Philpot:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.02

Topic: A.07. Developmental Disorders

Support: JSPS KAKENHI 22K06441

Title: Cell proliferation is increased in the dentate gyrus of adult mice overexpressing motopsin that cause hyperactivity

Authors: *S. MIYATA[1], M. TSUDA[2], S. MITSUI[1];
[1]Gunma Univ., Maebashi, Japan; [2]Kochi Univ., Nankoku, Japan

Abstract: Motopsin (PRSS12) is a secreted serine protease that is highly expressed in the hippocampus and cortex during the early postnatal period. Motopsin deficiency causes neuropsychiatric disorders in humans, and impaired spatial memory with decreased filopodia density in mice, suggesting crucial roles in synaptogenesis. We generated the double transgenic (DTG) mice overexpressing motopsin in the brain to explore the function of motopsin in vivo. Adult DTG mice showed hyperactivity in a novel environment and the number of cFos-positive cells was increased in the dentate gyrus after a cognitive behavior test. It has been reported that hippocampal neural precursor cell proliferation was increased by physical activity (Fabel et al., 2009). Overexpression of motopsin in vitro increased cleavage of agrin, an extracellular substrate of motopsin, and administration of the agrin fragments restored filopodia formation in motopsin-deficient hippocampal slices (Matsumoto-Miyai et al., 2009). Therefore, we investigated the promotion of cell proliferation and the density of immature or mature neurons in DTG mice. DTG mice were injected with 5-bromo-2'-deoxyuridine (BrdU), a thymidine analog which is selectively incorporated into actively proliferating cells. Seven days after the BrdU administration, brains were immunostained with anti-BrdU and anti-neurofilament antibodies. DTG mice had significantly increased the number of BrdU-positive cells in the dentate gyrus compared with control littermates, in spite that motopsin was overexpressed in the pyramidal neurons in the CA1 regions but not dentate gyrus. Our preliminary studies showed no change in protein revels of the mature synaptic makers, synaptophysin and PSD95. We are analyzing the fluorescence intensity of neurofilaments and the neurite maturation in DTG mice using immature neurite makers. The abnormal behavior of DTG mice was not restored by the suppression of motopsin overexpression after adulthood. Therefore, we predict that motopsin is important for the establishment of neuronal circuits in the developing brain, which results in proper neural

activities modulating behaviors. In Neuroscience 2022, we will discuss the results and invite your input on the tracing of hippocampal neurons that we plan to undertake in the future.

Disclosures: S. Miyata: None. **M. Tsuda:** None. **S. Mitsui:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.03

Topic: A.07. Developmental Disorders

Support: NICHD Grant P01HD083157

Title: Nociceptive versus mechanoreceptive balance in the trigeminal pathway in a mouse model of perinatal dysphagia

Authors: C. FLINN[1], C. WOOD[2], Z. ERWIN[2], T. M. MAYNARD[3], *A.-S. LAMANTIA[4]; [1]Virginia Tech. Carilion Sch. of Medicine-Fralin B, Fralin Biomed. Res. Inst. Virginia Tech., Roanoke, VA; [2]The Fralin Biomed. Res. Institute, Virginia Tech., Roanoke, VA; [3]Fralin Biomed. Res. Inst., Virginia Tech. Carilion, Roanoke, VA; [4]Fralin Biomed. Res. Institute, Virginia Tech., Roanoke, VA

Abstract: In typically nursing infants, nociceptive afferents, relayed from the trigeminal ganglion via the trigeminal nerve (CN V) to the spinal trigeminal nucleus (SpV), and mechanosensory inputs, relayed via CN V to the principal sensory trigeminal nucleus (PrV) detect S/F/S-related stimuli in the perioral region and anterior oropharynx. Disruption of the balance between CN V nociceptive and mechanosensory relays may contribute to S/F/S difficulties—perinatal dysphagia—in infants with neurodevelopmental disorders, including those with 22q11.2 Deletion Syndrome (22q11DS), most of whom have some degree of perinatal dysphagia. Our previous work indicates that in CN V differentiation is disrupted by diminished dosage of 22q11 genes, leading to an increase in nociceptive neurons in the trigeminal ganglion. To assess whether this apparent shift in peripheral innervation is reflected in changes in central nociceptive versus mechanosensory relays during S/F/S, we are mapping activation of SpV versus PrV relay neurons using cFOS in P7 WT and *LgDel* mice, a genomically accurate 22q11DS model. As we began this work, we recognized that there was no reliable, single atlas of sensory and cranial nerve nuclei in the P7 mouse brainstem. We used a combination of mechanosensory, nociceptive, and motor-neuron selective reporters to generate this resource in WT mice to guide our subsequent analyses. We then compared c-Fos immunolabeling in the SpV and PrV, identified securely based upon our new P7 brainstem atlas, of nursing WT and *LgDel* P7 pups challenged with graded concentrations of mustard oil, a chemical irritant, applied to perioral skin to quantify nociceptive versus mechanosensory activation. In parallel, we are quantifying nociceptive and mechanosensory glutamatergic and GABAeric terminals in the SpV and PrV using a combination of reporter mice and immunolabeling. The proportion of activated

SpV versus PrV relay neurons may be larger in *LgDel* in response to noxious peripheral stimulation, suggesting a potential change in the balance of nociceptive input during S/F/S due to 22q11 deletion. In parallel, the frequency of *LgDel* SpV nociceptive excitatory terminals may increase, and local inhibitory inputs decrease. Changes in the balance of CN V nociceptive versus mechanosensory function and circuitry may contribute significantly to disrupted S/F/S in *LgDel* 22q11-deleted mice.

Disclosures: C. Flinn: None. **C. Wood:** None. **Z. Erwin:** None. **T.M. Maynard:** None. **A. LaMantia:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.04

Topic: A.07. Developmental Disorders

Support: R01AI147496
1S10OD028515-01

Title: Knockout of Selenoprotein I in neurons results in reduced balance and motor coordination

Authors: *L. G. A. NUNES[1], C. MA[2], F. W. HOFFMANN[2], M. W. PITTS[2], P. R. HOFFMANN[2];
[1]Anatomy, Biochemistry, and Physiol., [2]Cell and Mol. Biol., John A. Burns Sch. of Med., Honolulu, HI

Abstract: Selenoprotein I (SELENOI; EPT1), a member of the Kennedy Pathway, is a phosphotransferase that is involved in the synthesis of phosphatidylethanolamine (PE) and plasmenyl PE. SELENOI catalyzes the addition of ethanolamine from CDP-ethanolamine to 1,2-diacylglycerol (DAG) for PE production and 1-alkyl-2-acyl-glycerol for plasmenyl PE in the endoplasmic reticulum (ER) membrane. Global SELENOI knockout in a murine model resulted in embryonic lethality early in embryogenesis (~E6), making it the fifth essential selenoprotein. Interestingly, in humans, rare cases of SELENOI loss-of-function mutations have been associated with hereditary spastic paraplegia (HSP) that involves a multitude of neurological deficits such as upper or lower limb spasticity, sensorineural-deafness, blindness, and seizures. This led us to investigate the role of SELENOI in the brain. Single-cell RNA seq data from the Allen Brain Atlas indicate that SELENOI is expressed preferentially in neuronal cell types, which was supported by in situ hybridization showing SELENOI mRNA colocalized with multiple neuronal markers, including Tubulin 1-alpha (Tuba1a) mRNA. We therefore developed a murine neuron specific KO of SELENOI (Tuba1a-Cre: SELENOIFL/FL) and observed deficits that reflect some symptoms observed in patients. We characterized our nervous system SELENOI KO model utilizing multiple behavioral tests such as rotorod, inverted grid hang, grip strength, vertical rod, elevated plus maze, open field, and startle tests. Nervous system SELENOI

KO mice performed poorly on the rotorod and vertical rod tests, suggesting lack of balance and motor coordination. Furthermore, KOs had a consistently lower weight compared to wildtypes throughout development. Collectively, these data demonstrate that SELENOI is primarily expressed in neuronal cell types and that deficiency in SELENOI in the nervous system results in neurological phenotypes similar to those observed in patients with HSP.

Disclosures: L.G.A. Nunes: None. **C. Ma:** None. **F.W. Hoffmann:** None. **M.W. Pitts:** None. **P.R. Hoffmann:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.05

Topic: A.07. Developmental Disorders

Title: Phenotyping the CLN3(Deltaex7/8)mouse model of Batten disease: fine motor kinematics and retinal function

Authors: *K. LEHTIMÄKI[1], T. BRAGGE[1], S. BÄCK[1], J. OKSMAN[1], D. MISZCZUK[1], T. W. ROSAHL[2], R. DROLET[2];
[1]Charles River Discovery Services, Kuopio, Finland; [2]Merck Res. Labs., Kenilworth, NJ

Abstract: CLN3 Batten Disease, juvenile NCL (neuronal lipofuscinosis), is an ultra-rare condition which becomes symptomatic between 5 to 15 yrs and is typically fatal by the late teens or early twenties. Earliest and most progressive symptoms are related to the vision, but affected children suffer from problems with speech, cognition, behavioral changes, and motor decline. $CLN3^{\Delta ex7/8}$ mice have been used to model the human disease with established phenotype including retinal dysfunction and neurological deficits. Here we studied $CLN3^{\Delta ex7/8}$ mice for longitudinal changes in gait and retinal function that could provide an early window for novel treatments.
Eighteen $CLN3^{\Delta ex7/8}$ homozygous and age matched wildtype mice (WT, n=17) were used. Kinematic gait analysis (KGA) and flash electroretinogram (fERG) were conducted at 4, 8, 12 and 16 months of age. For the KGA testing, the movements of the animals were captured from 3 directions using a high-speed camera. The analyzed parameters included general gait, body posture and balance, and fine motor skills. For scotopic fERG, mice were anesthetized, and 6 light intensities were tested: 0.003, 0.01, 0.1, 1, 3 and 10 $cd.s/m^2$. The responses were identified for the amplitudes and latencies for both a-wave and b-wave.
The phenotype model for fine motor performance was created for common gait characteristics over the study period (4-16 mo). The changes in the gait were found in the interlimb coordination with increased double support and subsequent reduction in single support in $CLN3^{\Delta ex7/8}$ mice. Diagonal trot mode was reduced with subsequent change in homologous mode and three/four supports. Vertical movement was increased (hip height range, tail tip range) in $CLN3^{\Delta ex7/8}$ mice, and tail was positioned higher than for WT mice. In the fERG, most

pronounced changes were seen in the b-wave, composed mainly by the responses of bipolar and Müller cells; reduced amplitudes already at 4 months with progressive profile at 12 and 16 months. The photoreceptor function (a-wave) was less impaired with increased latencies for 3 lowest intensities at 12 and 16 months.
Current data show that there is clear CLN3$^{\Delta ex7/8}$ model effect in KGA and fERG, allowing studies with parameters that are readily translational. Parallel experiments utilizing OCT (retinal layer thickness), fundus autofluorescence, biochemical protein aggregation and autophagy biomarkers in the retina and brain were performed in separate cohorts (adjoining poster). Together, the data depict a model that recapitulates key aspects of retinal and motor dysfunction that occur in CLN3 Battens Disease that can be utilized to assess therapeutic interventions into the disease process.

Disclosures: K. Lehtimäki: None. **T. Bragge:** None. **S. Bäck:** None. **J. Oksman:** None. **D. Miszczuk:** None. **T.W. Rosahl:** None. **R. Drolet:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.06

Topic: A.07. Developmental Disorders

Title: Identifying translatable retinal biomarkers of lysosome storage disorders through phenotypic characterization of CLN3 Batten's disease mice

Authors: *L. MA[1], X. PING[2], L. YANG[3], W. LUO[4], C. N. NUNES[2], M. E. AULT[2], B. CULP[2], D. E. METZGER[3], S. BELLUM[5], W. O. COOK[5], X. SHEN[2], N. LI[3], M. COSDEN[1], C. A. GRETZULA[1], J. M. USLANER[1], J. MARCUS[1], S. M. SMITH[1], R. DROLET[1];
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Abstract: Lysosomes are membrane-bound organelles involved in degrading and recycling cellular waste, cellular signaling and energy metabolism. Lysosome dysfunction is implicated in numerous neurodegenerative disorders and lysosome storage diseases, and retinal dysfunction is a shared symptom in these diseases. CLN3 Batten disease is an autosomal recessive, neurodegenerative, lysosomal storage disease caused by mutations in CLN3, which encodes a lysosomal membrane protein. CLN3$^{\Delta ex7/8}$ mice harbor the most common genetic defect causing juvenile Batten's disease. Visual dysfunction is often the presenting symptom of Batten's disease, which typically progresses to complete vision loss within the first decade of diagnosis. Yet, retinal dysfunction remains poorly characterized in the most common mouse model of the disorder. The goals of the present study were to characterize retinal pathology in the CLN3$^{\Delta ex7/8}$ mouse and to identify translatable biomarkers of retinal lysosome dysfunction that may be applied to other neurodegenerative disease models. To identify translational biomarkers of lysosome dysfunction, we explored longitudinal and cross-sectional studies to phenotype the

$CLN3^{\Delta ex7/8}$ mouse retina compared to wild-type littermate controls. Optical coherence tomography (OCT) and fundus autofluorescence (FAF) imaging showed a progressive reduction in retinal thickness and increased lipofuscin deposits in homozygous $CLN3^{\Delta ex7/8}$ mice up to 14 months old. In addition, homozygous $CLN3^{\Delta ex7/8}$ mice display functional impairment in retinal conductivity as assessed via full field scotopic electroretinogram (fERG). These pathological defects correlated with biochemical alterations in lysosome and autophagy biomarkers including elevated p62, LAMP1 and CatB as well as age-dependent protein aggregation. These data demonstrate the utility of the CLN3 in vivo mouse model for interrogating lysosome biology and identify translatable retinal biomarkers that can be used to support efficacy trials for therapeutic mechanisms targeting lysosome dysfunction.

Disclosures: L. Ma: None. **X. Ping:** None. **L. Yang:** None. **W. Luo:** None. **C.N. Nunes:** None. **M.E. Ault:** None. **B. Culp:** None. **D.E. Metzger:** None. **S. Bellum:** None. **W.O. Cook:** None. **X. Shen:** None. **N. Li:** None. **M. Cosden:** None. **C.A. Gretzula:** None. **J.M. Uslaner:** None. **J. Marcus:** None. **S.M. Smith:** None. **R. Drolet:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.07

Topic: A.07. Developmental Disorders

Support: Program in Neuroscience and Behavior, Mount Holyoke College
Harap scholarship fund for student research
Vorwerk scholarship fund for student research
Curtis-Smith Neuroscience award
Department of Psychological Sciences, University of San Diego
McNair scholarship

Title: Memory deficits and pro-inflammatory cytokines in the hippocampus in a rat model of ADHD

Authors: L. G. ANDERSON[1], E. VOGIATZOGLOU[1], S. LUIZ[1], J. P. GHALY[2], T. DUQUE[2], M. M. WYATT[2], S. TANG[1], J. B. HALES[2], *M. SABARIEGO[1];
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Abstract: Attention deficit hyperactivity disorder (ADHD) is a heterogeneous behavioral disorder characterized by hyperactivity, impulsivity, and inattention, as well as deficits in both working memory (WM) and sense of time. Although animal models cannot fully reflect human psychiatric disorders, they can provide insight into the disorder that cannot be obtained from human studies. The spontaneously hypertensive rat (SHR) is one of the best-studied animal models of ADHD. In comparison to their control, the Wistar-Kyoto (WKY) rat, male SHRs

show multiple behavioral phenotypes characteristic of ADHD, including hyperactivity, impulsivity, and poor sustained attention. In addition, there is increasing evidence that SHRs show impairments in learning and memory. The hippocampus has long been known to be a key brain region for learning and memory, and hippocampal lesions or inactivations in rats cause poorer performance in multiple memory tasks, including elapsed time discrimination and spatial WM tasks. Inflammatory signaling is also a critical contributor to memory and cognitive deficits; however, the precise role that cytokines play in the modulation of ADHD behavioral symptoms remains unknown. To investigate this question, we first examined the behavior of SHRs and WKY rats in multiple hippocampal-dependent memory tasks: the delayed alternation task, time discrimination task, objects in updated locations task, and traveling salesman problem. We used rats of both sexes in an effort to rectify the current sex imbalance in knowledge about ADHD and to directly examine sex as a biological variable. To better understand the impact of inflammatory signaling on spatial and temporal memory in SHR and WKY rats, animals were sacrificed after behavioral testing and hippocampal samples were collected. We analyzed the levels of several inflammatory cytokines (IL-1a, IL-1b, IL-4, IL-6, IL-10, IL-18, and TNFa) in the dorsal and ventral hippocampus of female and male SHRs and WKY rats. SHRs showed worse memory performance in all memory tasks relative to the WKY rats. Specifically, SHR showed the greatest impairment under the longest delay condition in both the delayed alternation and the time discrimination task. Moreover, SHRs made more memory errors in the traveling salesman problem. Combined, our data are consistent with impairments seen in rats with hippocampal lesions. These results will be discussed in relation to the levels of cytokines in the hippocampus of SHRs and WKY rats and the impact of inflammation on memory and cognition in ADHD.

Disclosures: L.G. Anderson: None. **E. Vogiatzoglou:** None. **S. Luiz:** None. **J.P. Ghaly:** None. **T. Duque:** None. **M.M. Wyatt:** None. **S. Tang:** None. **J.B. Hales:** None. **M. Sabariego:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.08

Topic: A.07. Developmental Disorders

Support: NIGMS Grant 5P20GM103427 and NOT-GM-21-016

Title: Investigation of CPT2 Deficiency Using A Zebrafish Model System

Authors: *C. BAKER[1], A. MARTA[2], N. ZIMMERMAN[2], R. WICKRAMASKARA[3], H. STESSMAN[3], Z. KORADE[4], T. MATTOS[4], K. MIRNICS[4], A. SHIBATA[2];
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Abstract: Carnitine palmitoyltransferase (CPT) 2 is an inner mitochondrial membrane protein and part of a carnitine shuttle involved in beta-oxidation of long chain fatty acids. During periods of early development and starvation, beta-oxidation provides an alternative pathway of energy production. CPT2 deficiency is a rare genetic disorder. Our published case study of a proband with the myopathic form of CPT2 deficiency reveals novel episodes of psychosis (Wickramasekara, 2020). We hypothesize that CPT2 deficiency contributes to CNS dysfunction resulting in abnormal beta-oxidation in early brain development. A zebrafish model system was used to examine the hypothesis. CPT2 was knocked down in zebrafish at the single cell stage using translation blocking (TB) and splice blocking (SB) morpholinos. Scrambled morpholino and uninjected zebrafish were used as controls. Embryos were raised to five days post fertilization (5dpf) for analyses. PCR and western blot confirmed knockdown of CPT2. LC-MS/MS was performed for acylcarnitine species and showed an increase in TB and SB larvae compared to controls. Morphological studies showed average standard length of TB and SB fish significantly decreased by 128.2± 17.9µm and 316.7± 48.2µm respectively as compared to controls ($p<0.0001$, ±=SEM). Deformed Meckel's cartilage, ceratohyal cartilage, and ceratobranchials cartilage were observed in Alcian blue stained TB and SB fish and not in controls. Decreased lipid deposition was present in TB and SB fish stained with Oil Red O. Distance between eyes measuring brain and telencephalon development in SB fish significantly decreased by 48.5±8.4µm compared to controls ($p<0.001$, ±=SEM). Behavioral assays were performed using Zebrabox to quantify and track movement to stimuli. Normal movement count was significantly decreased in SB by 874.3 ± 191.2 ($p< 0.0001$, N=96, ±=SEM) compared to scrambled control. Duration of small distance movement was significantly decreased in SB by 204.3s± 47.40s ($p=0.0001$, N=106, ±=SEM) compared to controls. Large movement distance significantly decreased in SB by 11722 nm ± 2411 nm ($p< 0.0001$, N=106, ±=SEM) compared to controls. qRT-PCR analysis was performed for genes regulating neurotransmitter release and synthesis. A significant decrease of ~0.4fold in *Htr1b* gene expression was measured in SB fish compared to control fish ($p< 0.05$, N=30, ±=SEM). These studies suggest that CPT2 function and beta-oxidation play a role in normal vertebrate CNS development, gene expression and function. Continued work will focus on understanding how CPT2 and the carnitine shuttle affect neurological function and behavior.

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Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.09

Topic: A.07. Developmental Disorders

Support: NIGM RL5GM118969
NIDCR 1R03DE029517-01A1

NIGM TL4GM118971
NIGM R25GM069621
NIGM UL1GM118970

Title: Characterizing the role of HCFC1 in anxiety disorders using adult zebrafish

Authors: *B. E. PINALES, D. PAZ, A. M. QUINTANA;
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Abstract: Mutations in the HCFC1 transcriptional cofactor cause *cblX* syndrome or X-linked intellectual disability, two disorders with hallmark neurodevelopmental phenotypes. Multiple model systems have been developed to characterize the function of HCFC1 in brain development and disease, but they cause embryonic or peri-lethality and therefore, the role of HCFC1 in adult neuropsychiatric disorders has not been characterized. Here we developed an adult viable homozygous missense mutation in the zebrafish *hcfc1a* gene ***to investigate the role of HCFC1 in adult neuropsychiatric disorders.*** We implemented the novel tank diving test to measure anxiety like phenotypes in zebrafish. The novel tank paradigm is based on a zebrafish's instinct to remain at the bottom of a new environment until safety is established. The paradigm measures the latency to explore upper zones of the tank, the number of entries into designated zones, and the total swimming distance in each zone. Male or female adult zebrafish were placed in a 1.8 L tank, which was divided into three equal zones: top, center, and bottom. Each fish was independently introduced to the "novel" tank, with fresh water for each trial, and video captured for 5 minutes. The test is accompanied by ImageJ tracking software to track fish and follow their swimming path to generate time, distance, and velocity with set parameters to each zone. Preliminary analysis demonstrated an increase in the distance swam within the bottom region in male carriers and a decrease in the distance swam in the top region in female carriers, both indicative of anxiety like phenotypes. Thus, our study establishes the first homozygous viable model to determine the anxiety phenotypes associated with mutations in HCFC1.

Disclosures: B.E. Pinales: None. **D. Paz:** None. **A.M. Quintana:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.10

Topic: A.07. Developmental Disorders

Support: MRC Sackler studentship

Title: A reverse genetic approach in zebrafish to model genes associated with Major Depressive Disorder

Authors: *O. SIMMONDS[1,2], R. HINDGES[1,2], J. RIHEL[3], J. LARSCH[4], K. LAGOGIANNIS[1,2];

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Abstract: Major Depressive Disorder (MDD) affects approximately 350 million people worldwide and is associated with increased co-morbidities and mortality. The precise aetiology of MDD remains unknown, however the heritability is estimated to be ~40%. Genome wide association studies (GWAS) aim to deduce the genetic contribution of complex disorders and a large GWAS recently identified 44 loci associated with MDD. A constraint of GWAS is that they do not identify causal variants and only provide a signal to the genomic regions associated with the disorder. With this in mind, our work has two aims: 1) validation of candidate genes inferred from GWAS findings associated with MDD and 2) use post-GWAS analysis to understand the functional link between the genetics and phenotypes of MDD to better discern the molecular underpinnings of the disorder. To do this, we applied a reverse genetic approach using CRISPR-Cas9 gene editing technology to create a number of candidate gene knockout zebrafish mutant lines and crispants. These lines were then assessed in a battery of MDD-relevant behavioural and biochemical assays informed by the DSM-IV criteria and previously hypothesised pathophysiology of MDD, respectively. Behavioural phenotypes, more specifically; sleep, appetite, locomotion and anhedonia, were assessed in larval or juvenile zebrafish offspring from in crossed heterozygous mutant lines, which were genotyped following the assay providing sibling controlled and fully blinded studies. Preliminary results for our Lrfn5a mutant show no changes in appetite, assessed via hunting events and prey intake or anhedonia, measured through social interaction. However, the same mutant line exhibits a trend for increased locomotion and shows a significant sleep phenotype. Homozygous fish initiate sleep significantly more than sibling-controls but overall show a significant reduction in total time spent sleeping insinuating that Lrfn5a is involved in sleep structure. These findings enable a more focused inquiry into how a given gene leads to an MDD-relevant endophenotype, thereby helping to expand our understanding of the disorder. Importantly, the methodology also offers a high-throughput screening platform to develop potential therapeutic strategies.

Disclosures: O. Simmonds: None. **R. Hindges:** None. **J. Rihel:** None. **J. Larsch:** None. **K. Lagogiannis:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.11

Topic: A.07. Developmental Disorders

Support: NIH P50MH112491-01
NIH AR072965

Title: Aav9 expression of anti hc4 nanobody (hc4nb8) prevents schizophrenia-like phenotype in hc4a mice

Authors: *E. YALCIN[1], A. ZARANTONELLO[2], M. MA[1], C. CASTRILLION[1], G. R. ANDERSEN[3], M. CARROLL[1];
[1]Harvard Med. School/ Boston Children's Hosp., Boston, MA; [2]Ctr. de Recherche des Cordeliers:, Paris, France; [3]Dept. of Mol. Biol. and Genetics, Aarhus University,, Aarhus, Denmark

Abstract: Schizophrenia is a heritable neurodevelopmental psychiatric disorder of unknown etiology that affects 1% of the adult population. Pathology studies of postmortem tissues identify excessive loss of gray matter and reduced dendritic spine density suggesting that the loss of synaptic connections might contribute to the behavioral and cognitive deficits, primarily in the frontal cortex. Recent large-scale genetic studies identified elevated expression of the human complement C4A allele as a significant risk factor for schizophrenia. In parallel, BAC transgenic mice that express increased copy number of hC4A (hC4Atg) are susceptible to medial prefrontal cortex (mPFC) synaptic over-pruning during adolescence; excessive microglial engulfment of presynaptic terminals during this period results in decreased synapse density in the adult mPFC, and behavior change. However, a treatment study to target hC4A overexpression to prevent disease progression in schizophrenia remains unaddressed. We hypothesized that generating a viral vector expressing anti-hC4 nanobody (hC4Nb8) can be an effective rescue strategy for schizophrenia-like symptoms in hC4Atg mice. The results showed that hC4Nb8 blocks the classical pathway activity in hC4Atg mouse serum and cerebrospinal fluid *in vitro*. Moreover, intraparenchymal hC4Nb8 injections at P4 reversed the increase in microglial uptake of presynaptic terminals in mice. Moreover, sustained expression via the AAV9 vector between P1-P10 and P10 - P60 proved that hC4Nb8 rescues the hC4A overexpression-mediated synaptic pruning defects in the developing visual system and medial prefrontal cortex, respectively. Supporting our hypothesis, hC4A overexpressing mice no longer demonstrated cognitive deficits upon hC4Nb8-AAV treatment. Taken together, our data show that nanobody expressing viral vectors provide a potential approach to prevent disease progression in neuropsychiatric disorders like schizophrenia.

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Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.12

Topic: A.07. Developmental Disorders

Support: NIMH Grant R01MH115027-04
NIMH Grant R01MH048404-29
NIAAA T32 Training Grant

Title: Effect of a dopamine neuron-specific manipulation of Grin2a on behaviors and physiological measures related to schizophrenia

Authors: M. L. KIELHOLD[1], A. TORRADO PACHECO[1], D. S. JACOBS[1], J. T. WILLIAMS[1], L. S. ZWEIFEL[2], B. MOGHADDAM[1];
[1]Oregon Hlth. & Sci. Univ., Portland, OR; [2]Univ. of Washington, Seattle, WA

Abstract: Effect of a dopamine neuron-specific manipulation of Grin2a on behaviors and physiological measures related to schizophrenia Michelle Kielhold, Alejandro Torrado-Pacheco, David Jacobs, John Williams, Larry Zweifel, and Bita MoghaddamBackground: Multiple lines of evidence have demonstrated that glutamatergic mechanisms are associated with the pathophysiology of schizophrenia, but the mechanistic link between glutamate and dopamine neurotransmission in the context of schizophrenia susceptibility and symptom expression remains elusive. Here we focused on the role of gene encoding the GluN2A subunit of the NMDA receptor (*Grin2a*) in mediating dopaminergic effects relevant to schizophrenia. Recent genome-wide association and whole exome/genome sequencing studies have linked variants of *GRIN2A* to schizophrenia. Critically, this subunit is developmentally regulated and expression levels change in adolescence through early adulthood, when symptoms of schizophrenia typically manifest. Here we report on the impact of a dopamine cell-specific disruption of GluN2A during adolescence on behaviors and related functions that are relevant to symptoms of schizophrenia. Methods: Cre-dependent CRISPR/SaCas9 viruses for *Grin2a* (cKO) or *Rosa26* (control) were injected in the VTA of Th:Cre juvenile male and female rats. Adolescent animals were tested on a battery of behavioral tasks and for sensitivity to psychostimulants. Postmortem tissue was analyzed by IHC or slice electrophysiology.Results: Intra-VTA viral infusion led to a significant reduction of GluN2A in the VTA, with IHC showing dopamine neuron-specific virus expression. The cKO animals were seemingly normal and did not show differences, as compared to control, in spontaneous motor behavior in the open field or simple action-outcome associative learning. Differences were observed, however, in more complex paradigms such as the progressive ratio schedule of reinforcement and the flexible contingency learning task with cKO animals showing disruptions that were indicative of disrupted salience attribution. Additionally, we observed a significant effect of treatment with propsychotic drugs producing exaggerated locomotor effects selectively in *Grin2a* cKO animals. Discussion: These results suggest that selective manipulation of the GluN2A subunit in adolescent dopamine neurons may provide a useful model for some aspects of genetic vulnerability to schizophrenia.

Disclosures: M.L. Kielhold: None. **A. Torrado Pacheco:** None. **D.S. Jacobs:** None. **J.T. Williams:** None. **L.S. Zweifel:** None. **B. Moghaddam:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.13

Topic: A.07. Developmental Disorders

Support: This work was supported by the Eunice Kennedy Shriver NICHD Intramural Award to CJM.

Title: Developmental Grin1-ablation driven interneuronopathy precipitates paradoxical neuropsychiatric abnormalities

Authors: *V. MAHADEVAN, D. ABEBE, C. MACKENZIE-GRAY SCOTT, C. ESNAULT, Y. ZHANG, D. MARIC, K. A. PELKEY, R. CHITTAJALLU, R. DALE, T. J. PETROS, C. J. MCBAIN;
NIH, NIH, Bethesda, MD

Abstract: Interneuron-centric aberrant signaling mechanisms are thought to incite circuit-wide abnormalities underlying neuropsychiatric disorders. However, a detailed molecular and behavioral mapping of such interneuronopathies is currently nascent. Here, we establish that a developmental ablation of NMDA-type glutamate receptor (NMDAR) signaling within medial ganglionic eminence (MGE)-derived GABAergic interneurons is sufficient to trigger a conjunction of robust paradoxical anti-depressant and psychosis-like states. We also serendipitously uncover that the MGE-NMDAR-ablated mice exhibits profound behavioral resilience in the face of an acute stressor, which is further exacerbated with ketamine treatment. By employing multi-omic investigations we catalogue the cell-specific transcriptional and mRNA translation abnormalities triggered by interneuronal NMDAR ablation in the hippocampus and frontal cortex. In addition, we demonstrate that developmental-NMDAR ablation within MGE interneurons is sufficient to trigger global impairments in a range of growth factor signaling including hippocampal BDNF expression.Our study hence provides a road map for the systematic examination of global molecular abnormalities underlying anti-depressant psychosis, revealing potential genetic targets that could separate the beneficial and pathological molecular impact of manipulating interneuronal NMDAR signaling.
Keywords: GABAergic interneurons, Medial ganglionic eminence, PV, SST, NGFC, NMDAR, Transcription, mRNA translation, Schizophrenia, Major depression, Psychosis, Ketamine, NMDA-hypofunction, Hippocampus, Frontal Cortex, scRNAseq, Ribotag

Disclosures: V. Mahadevan: None. **D. Abebe:** None. **C. Mackenzie-Gray Scott:** None. **C. Esnault:** None. **Y. Zhang:** None. **D. Maric:** None. **K.A. Pelkey:** None. **R. Chittajallu:** None. **R. Dale:** None. **T.J. Petros:** None. **C.J. McBain:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.14

Topic: A.07. Developmental Disorders

Support: 1R15DA046926

Title: Metabotropic glutamate receptor type 5 (mGlu5) as a novel target for alleviating deficits in sensorimotor gating and the enhanced rewarding effects of nicotine in a heritable model of drug abuse vulnerability in psychosis

Authors: *L. D. PEETERS[1], L. J. WILLS[1], S. E. TURNEY[1], W. D. GILL[1], S. R. MASSEY[1], J. T. GASS[2], C. VIED[3], R. W. BROWN[1];
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Abstract: Heritable and environmental factors contribute to an individual's risk of comorbid substance use disorder and psychosis. Our laboratory has established that rats neonatally treated with quinpirole (NQ), a dopamine (DA) D_2-like receptor agonist, display a significant increase in DAD_2 receptor sensitivity throughout the animal's lifetime. Increased DAD_2 receptor sensitivity is common in schizophrenia (SZ), but is also observed in bipolar disorder, obsessive-compulsive disorder, panic disorder and major depression. In addition, increased DAD_2 sensitivity is thought to contribute to substance abuse comorbidity commonly found in all of these behavioral disorders. Increases in cigarette smoking, at a rate 3 to 4x greater than the general population, often complicates treatment success. We bred NQ-treated male and female rats with their NQ-treated or neonatal saline (NS)-treated counterparts to determine whether increases in DAD_2 sensitivity could be passed to the next generation. Offspring of at least one NQ-treated founder demonstrated increases in DAD_2 receptor sensitivity both behaviorally and neurobiologically. RNASeq preliminary data revealed an increase in cortisol synthesis and release in F1 generation animals, demonstrating an enhanced response to stress, consistent with a model of drug abuse vulnerability in psychosis. Consistent with this finding, F1 generation rats demonstrated enhanced nicotine conditioned place preference (CPP) and had an enhanced brain derived neurotrophic factor (BDNF) response to nicotine in the nucleus accumbens (NAcc), a brain area critical to drug reward. The DAD_2 receptor forms a triple heteromer with the adenosine A(2A) and metabotropic glutamate type 5 (mGlu5) receptor, such that stimulation of either receptor results in a decrease in DAD_2 activity. Therefore, we analyzed whether use of a positive allosteric modulator (PAM) of mGlu5 in the F1 generation would block nicotine CPP and improve sensorimotor gating deficits, which is a hallmark of psychosis. The mGlu5 PAM effectively blocked the enhanced rewarding effects of nicotine and alleviated sensorimotor gating deficits in this model. CDPPB is expected to produce these effects via normalization of G protein-dependent and -independent signaling of the DAD_2 receptor in F1 generation animals, consistent with previous findings in F0 generation founders. In essence, we demonstrate in results reported here that the DAD_2-mGlu5-A(2A) heteromer is a potential therapeutic target for substance abuse comorbidity and psychosis.

Disclosures: L.D. Peeters: None. **L.J. Wills:** None. **S.E. Turney:** None. **W.D. Gill:** None. **S.R. Massey:** None. **J.T. Gass:** None. **C. Vied:** None. **R.W. Brown:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.15

Topic: A.07. Developmental Disorders

Support: NIH RO1 ES032270

Title: Latrophilin-3 knock-out versus heterozygous Sprague Dawley rats: effects on activity, startle, learning and memory

Authors: S. L. REGAN[1], C. SUGIMOTO[3], **M. T. WILLIAMS**[4], *C. V. VORHEES[2]; [1]Pediatrics, Div. of Neurol., [2]Div. of Neurol., Cincinnati Children's Hosp & Univ. of Cincinnati, Cincinnati, OH; [3]Physiol., Michigan State Univ., East Lansing, MI; [4]Pediatrics, Div. of Neurol., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: *Latrophilin-3* knock-out versus heterozygous Sprague Dawley rats: effects on activity, startle, learning and memory

Samantha L. Regan[1,2], Chiho Sugimoto[1,3], Hannah E. Dawson[1], Michael T. Williams[1], and Charles V. Vorhees[1]*

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Latrophilin-3 (LPHN3) is a brain specific adhesion G-protein coupled receptor associated with increased risk of attention deficit hyperactivity disorder (ADHD) and cognitive impairment. CRISPR/Cas9 was used to generate a constitutive knockout (KO) rat of *Lphn3* by deleting exon 3. This this model was used to investigate how LPHN3 disruption affected ADHD-related behaviors. Previously we showed that *Lphn3* KO rats are hyperactive, have attenuated response to d-amphetamine, and exhibit cognitive deficits. Here, we tested KO, heterozygous (HET), and wildtype (WT) *Lphn3* rats for gene-dosage effects in in home-cage activity on postnatal day (P)35 and P50, egocentric learning (Cincinnati water maze (CWM)), spatial learning (Morris water maze (MWM)), working memory (radial water maze (RWM)), incidental learning (novel object recognition (NOR)), acoustic startle (ASR) habituation, tactile startle (TSR) habituation, prepulse inhibition of acoustic startle (PPI), one-way passive avoidance, conditioned freezing, and mirror image CWM. KO and HET rats were hyperactive. KO and HET rats had egocentric (CWM; Figure) and spatial deficits (MWM) and increased startle response. There were no effects on working (RWM), contextual, or cued conditioned freezing. The gene-dosage effect observed in *Lphn3* HET rats indicates that *Lphn3* exhibits dominate expression on functions where the gene and protein are most abundantly expressed (striatum, hippocampus). The data add to evidence on the effect of this trans-synaptic protein on brain function and behavior.
[Supported by University of Cincinnati Dean's Dissertation Completion Award (SLR) and NIH grant R01 ES032270 (CVV and MTW)]

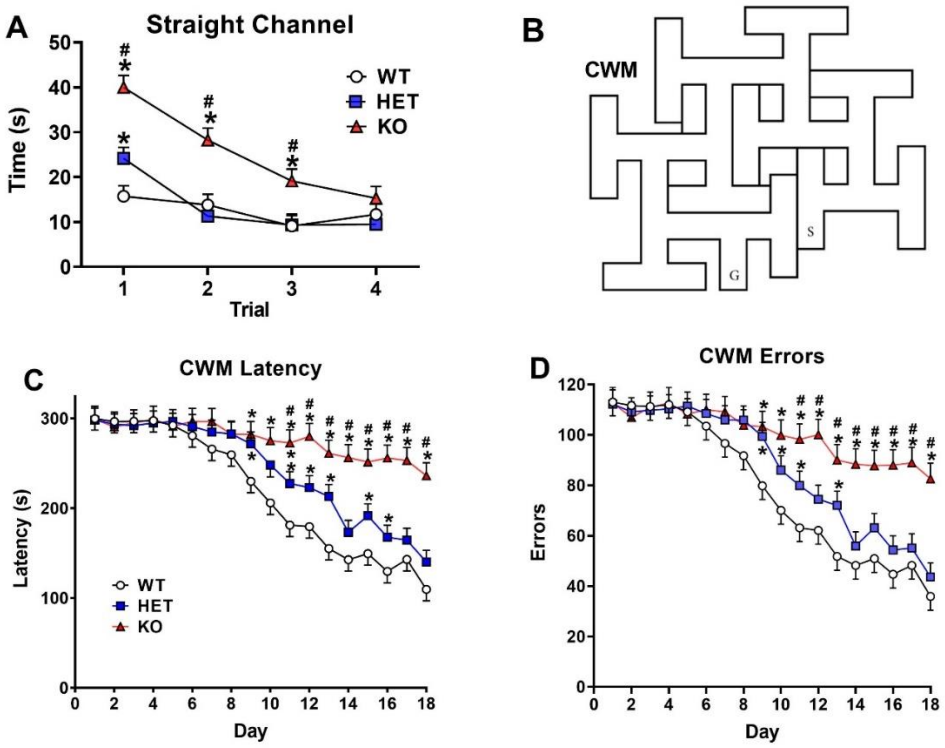

Figure 2

Disclosures: S.L. Regan: None. **C. Sugimoto:** None. **M.T. Williams:** None. **C.V. Vorhees:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.16

Topic: A.07. Developmental Disorders

Support: 1R15DA046926

Title: An adenosine A(2A) agonist alleviates enhanced associative rewarding aspects of nicotine and sensorimotor gating deficits in a heritable model of drug abuse vulnerability

Authors: *R. W. BROWN[1], L. D. PEETERS[1], L. J. WILLS[1], W. D. GILL[1], S. E. TURNEY[1], O. A. JENNINGS[2], C. VIED[3];
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Abstract: Neonatal treatment of the dopamine (DA)D_2-like receptor agonist quinpirole (NQ) to rats induces an increase in DAD_2 receptor sensitivity throughout the animal's lifetime, which has validity to schizophrenia (SZ) and a number of clinical conditions. These clinical conditions demonstrate increased drug abuse vulnerability, especially to cigarette smoking, but all of these disorders are also heritable. Based on this permanent change in DAD_2 sensitivity, we bred NQ-treated male and female rats with their NQ-treated or neonatal saline (NS)-treated counterparts. F1 generation offsrping demonstrated enhanced behavioral responding to nicotine on the conditioned place preference (CPP) paradigm in adolescence. These F1 offspring of NQ-treated rats also demonstrated increases of G-protein dependent and G-protein independent DAD_2 signaling, and enhanced brain-derived neurotrophic factor (BDNF) response to nicotine in the nucleus accumbens (NAcc). RNASeq analysis revealed that F1 generation offspring of two NQ-treated founders demonstrated an increase in gene expression associated with cortisol synthesis and an increased expression of genes associated with response to steroid hormones. These data suggest that the F1 generation is enhanced in sensitivity to stress, consistent with drug abuse vulnerability. A major focus is on the adenosine A(2A) receptor as a novel pharmacological treatment target. The A(2A) receptor forms a mutual inhibitory heteromer with the DAD_2 receptor, and CGS 21680, an A(2A) agonist, is known to decrease DAD_2 signaling. Data here demonstrate that CGS 21680 blocked enhanced nicotine CPP, alleviated deficits in sensorimotor gating present in the F1 generation, and reversed changes in DAD_2 G-protein independent signaling in F1 generation rats, Interestingly, CGS 21680 did not affect G-protein dependent signaling in F1 generation rats, suggesting a specific G-protein independent mechanism underlies these effects. This is the first study to show the adenosine A(2A) receptor to be a therapeutic target to both enhanced nicotine abuse and psychosis in a heritable rodent model of drug abuse vulnerability.

Disclosures: R.W. Brown: None. **L.D. Peeters:** None. **L.J. Wills:** None. **W.D. Gill:** None. **S.E. Turney:** None. **O.A. Jennings:** None. **C. Vied:** None.

Poster

030. Developmental Disorders: Genetic Models I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 030.17

Topic: A.07. Developmental Disorders

Support: ERC grant 715508 (REVERSEAUTISM)

Title: A non-canonical mTOR pathway regulates S6 phosphorylation state in the adult brain.

Authors: *L. GARCIA-RABANEDA, E. HAIMERL, B. BASILICO, M. BAREL, L. SCHWARZ, G. NOVARINO;
ISTA Inst. of Sci. and Technol. Austria, Klosterneuburg, Austria

Abstract: During the last decade, the mTOR pathway has been unraveled as a critical biological pathway, which its dysfunction has been observed in an important number of neurodevelopmental disorders. mTOR signalling regulates, differentially, cell shape, migration and differentiation during brain development, and synaptic plasticity during adulthood, but how the principal targets of mTORC1 are specifically regulated during different stages of development in the nervous brain is unknown currently. Genes encoding proteins forming the KICSTOR and GATOR complexes, the effectors of the amino acid-sensing arm of the mammalian target of rapamycin complex 1 (mTORC1), have been implicated in neurodevelopmental disorders. However, an understanding of the role of these proteins in the brain is still largely missing. We discovered that Kaptin, a component of KICSTOR, has an essential role in the brain, regulating S6 phosphorylation through a S6 kinase-independent form. Kaptin becomes increasingly more important in regulating the mTORC1-signaling cascade from birth on, while the canonical regulation of S6 by S6K becomes progressively less prominent. Surprisingly, our data shown a new molecular partner of Kaptin, which it has a fundamental function in vivo and in vitro. With this work, and through the specific function of Kaptin, we show a new mechanism of regulation of ribosomal protein S6 in the brain. This new mechanism should bring new pharmacological strategies to treat specific neurodevelopmental mTORopathies.

Disclosures: L. Garcia-Rabaneda: None. **E. Haimerl:** None. **B. Basilico:** None. **M. Barel:** None. **L. Schwarz:** None. **G. Novarino:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.01

Topic: A.10. Development and Evolution

Support: Endowment 7501371
DP2 DP2NS122550
Weill 2017270

Title: Regionally Distinct Populations of GABAergic Inhibitory Neurons Migrating in the Developing Gyrencephalic Cortex.

Authors: *J. KIM[1], K. SANDOVAL GUITERREZ[1], J. CHU[1], M. MUI[1], A. PODDAR[1], Y. HE[1], E. HORTON[1], P. JI[2], E. MAGA[2], B. W. KRAMER[3], T. BARTELS[4], C. WOOD[4], N. ROBERTSON[4], A. ROBERTS[5], M. F. PAREDES[1];
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Abstract: The ongoing incorporation of GABAergic-inhibitory neurons (INs) into the early postnatal cortex offers a mechanism for brain plasticity and adaptation to environmental stimuli. However, the cellular substrate underlying the late migration of GABAergic-IN and its functional significance is unknown. Furthermore, disruption in IN function in the brain has been frequently linked to the emergence of neurodevelopmental disorders (NDDs). We have previously shown that the human neonatal brain harbors a population of doublecortin (DCX)-expressing cells, at the dorsolateral border of the lateral ventricle. This region, termed the Arc, contributes to populations of IN that migrate to forebrain targets such as the anterior cingulate cortex (CC). To understand the cellular and molecular properties of the Arc population, we have investigated the postnatal DCX+ population across gyrencephalic brains. We found that the Arc population is present in developing gyrencephalic brains, such as in pigs, macaques, and humans, but not in the lissencephalic non-human primate brain, such as marmosets. Furthermore, we identified distinct migratory streams originating from the Arc including a dorsal migratory stream (DMS) targeting the CC and superior frontal gyrus, as observed in the human, and a ventral migratory stream (VMS) into the temporal cortex in the developing pig and human brains; these migratory populations were not found in the marmoset brains. A large proportion of neurons emanating from both the pig and human Arc were composed of caudal ganglionic eminence (CGE)-derived young GABAergic-IN, supporting this as a conserved feature of "late" IN migration. Using a single-cell spatial transcriptomic approach, we profiled the molecular identity of the migratory neurons from the DMS and VMS in the pig brain. These two spatially separated collections appear to be molecularly distinct with a heterogeneous group of DCX+ cells expressing Nkx2.1, Tshz1, and Prox1 in the DMS and a more homogenous IN-subpopulation expressing VIP and CXCR4 in the VMS. Our data support that Arc and its associated migratory streams are an evolved feature in the developing gyrencephalic brain. The regionally and molecularly distinct population of migratory GABAergic-IN could serve as a cellular substrate underlying the acquisition of distinct functionalities of cortical areas. This study expands our view for understanding the contribution of GABAergic-INs in the developing gyrencephalic brains and will reveal new developmental processes that when disrupted lead to the emergence of NDDs.

Disclosures: J. Kim: None. **K. Sandoval Guiterrez:** None. **J. Chu:** None. **M. Mui:** None. **A. Poddar:** None. **Y. He:** None. **E. Horton:** None. **P. Ji:** None. **E. Maga:** None. **B.W. Kramer:** None. **T. Bartels:** None. **C. Wood:** None. **N. Robertson:** None. **A. Roberts:** None. **M.F. Paredes:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.02

Topic: A.10. Development and Evolution

Support: NIH Grant U01-NS123972
NIH Grant U01-NS108637

Title: Neuronal cell type mapping in the ring and rhinophore ganglia of a gastropod mollusc using single cell transcriptomics

Authors: *M. D. RAMIREZ[1], T. N. BUI[2], P. S. KATZ[3];
[1]Univ. of Massachusetts Amherst, Univ. of Massachusetts Amherst, Amherst, MA; [2]Whitehead Inst. for Biomed. Res., Cambridge, MA; [3]Univ. of Massachusetts - Amherst, Univ. of Massachusetts, Amherst Neurosci. and Behavior Program, Amherst, MA

Abstract: Although neuroscience research on gastropod molluscs has provided important insights into the neural circuit function, it has generally been limited to the large identifiable neurons in the central ring ganglia (CRG). Here we take a transcriptomics approach to map neurons from ganglia in the nudibranch, *Berghia stephanieae*. The CRG (cerebropleural, pedal, and buccal ganglia) were separately dissected from the relatively unstudied rhinophore ganglion (RhG). Neurons were dissociated and their RNA sequenced using the 10X Genomics and Illumina platforms. Transcriptomes from about 2000 cells were recovered and subjected to standard clustering gene expression analyses. Neuronal and non-neuronal cell types were distinguished based on gene expression. Two clusters were composed almost exclusively of RhG neurons, however RhG neurons were also found throughout other clusters. Some clusters represented expected cell types such as mechanosensory afferents, differentially expressing Brn3, Drgx, Islet, and VGlut, and somatic efferents, expressing Mnx1, Lhx3/4, and ChAT. Other clusters included putative neuroblasts, expressing genes such as Sox2 and Sox6, Scratch1, LMO4, and neurogenin. Neurons expressing particular sets of genes were mapped by multiplexing fluorescence in-situ hybridization chain reaction. Neurotransmitter and neuropeptide classes were identified, uncovering general rules of neurotransmitter phenotypes, such as cholinergic neurons do not express particular peptides. Some genes were found to have widespread expression including CCWamide; others, including the transcription factor Six6, defined particular zones of neurons or specific ganglia. Previously unannotated genes were expressed in specific neuron types. The results provide insight in the organization and identity of gastropod neurons and provide the basis for future large-scale neural research on *Berghia*.

Disclosures: **M.D. Ramirez:** None. **T.N. Bui:** None. **P.S. Katz:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.03

Topic: A.10. Development and Evolution

Support: Ruth K. Broad Foundation Postdoctoral Fellowship,Duke University

Title: Evolutionary modifications in a FZD8 enhancer influence cortical neurogenesis and brain size

Authors: *J. LIU[1], F. MOSTI[1], J. S. FONSECA[2], D. LOLLIS[1], A. J. MASSRI[2], J. SHENG[3], A. M. M. SOUSA[3], G. A. WRAY[2], D. L. SILVER[1];
[1]Mol. Genet. and Microbiology, Duke Univ. Med. Ctr., Durham, NC; [2]Genomic and Computat. Biol., Duke Univ., Durham, NC; [3]Neurosci., Univ. of Wisconsin-Madison, Madison, WI

Abstract: The striking increase in the size of the human neocortex, accompanied by increased numbers and complexity of neural cells, is considered the basis for the higher cognitive ability in human. Brain expansion is thought to be due in part to divergent spatiotemporal gene expression in the developing human neocortex. Human Accelerated Regions (HARs) are loci which show rapid nucleotide changes in the human lineage, a large fraction of which function as cis-regulatory elements during brain development. One example is *HARE5*, which includes 4 nucleotide changes over ~600 nt human sequence, compared with chimpanzee. We previously showed *HARE5* promotes cortical expansion by acting as an enhancer of the WNT receptor, Frizzled8 (FZD8). However, the cellular and molecular mechanisms by which *HARE5* impacts cortical development remain unclear. To address this, we generated a new mouse model in which we replaced the mouse locus with human *HARE5*. The cortical size of *Hs-HARE5* mice was significantly enlarged postnatally, along with a significant increase of neurons at P0 and P21. Deleting *HARE5* sequence by crossing with *Emx*-Cre mice showed a reciprocal decrease in cortical area, indicating *Ms-HARE5* itself is important for cortical development. In comparison to WT mice, *Hs-HARE5* mice showed pronounced accumulation of Neural Progenitor Cells (NPCs). *HARE5* promoted self-renewal of neural epithelial progenitors (NEs) at E12.5 while promoting radial glial progenitor (RGC) differentiation at E14.5. Single-cell RNA sequencing suggest *Hs-HARE5* may also promote proliferative changes in basal progenitors. In order to understand the contribution of individual mutations to enhancer activity, we used luciferase assays in human NPCs; Two Hs-specific mutations drove higher enhancer activity, including one adjacent to an autism-associated mutation. We are currently using CRISPR/Cas9 to assess the role of these evolutionary modifications in human NPCs. Our findings indicate new cellular and molecular mechanisms by which human-specific genetic changes alter enhancer activity and modify cell fate during human brain development.

Disclosures: J. Liu: None. **F. Mosti:** None. **J.S. Fonseca:** None. **D. Lollis:** None. **A.J. Massri:** None. **J. Sheng:** None. **A.M.M. Sousa:** None. **G.A. Wray:** None. **D.L. Silver:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.04

Title: WITHDRAWN

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.05

Topic: A.10. Development and Evolution

Support: HFSP RGP0060/2017

Title: Signaling in aneural animals and preneural larvae highlight potential roles of cilia and their sensory-communicataion systems in early brain evolution

Authors: A. WU[1], L. C. DENG[3], L. CROSSLEY[1], K. BARKER[1], S. FULTON[2], R. NGUYEN[1], R. JACKSON[4], E. PRICE[2], S. CHALASANI[5], L. L. MOROZ[6], ***E. EDSINGER**[5];
[1]Biol., Univ. of Washington, Seattle, WA; [2]Interdisciplinary Arts and Sci., Univ. of Washington, Tacoma, WA; [3]Inst. Polytechnique de Paris, Palaiseau, France; [4]Western Washington Univ., Bellingham, WA; [5]Salk Inst. for Biol. Studies, La Jolla, CA; [6]The Whitney laboratory for Marine Biosci., Univ. of Florida Dept. of Neurosci., Saint Augustine, FL

Abstract: The brain expresses a surprising diversity of genes, with many having additional roles outside the nervous system. Comparative genomics suggests “neuroid” type communications in organisms lacking nervous systems. Here, we explore and functionally test for the presence of sensory and signaling components in aneural animals and preneural vs. neural larval stages. To do this, we developed an in-house pipeline, GIGANTIC, and performed phylogenomic gene family analyses of transmitters, receptors, and ciliome components in animals. We further performed differential expression analyses that focused on these components in embryonic and larval development of various marine invertebrates, including the marine snail *Lottia scutum* (Mollusca). Ongoing work in *L. scutum* includes genome assembly and annotation to enable comparative analyses and development of genetic tools, including CRISPR, and RNA-seq. We initially established baseline ethograms of cilia-based larval swimming and characterized cilia and nervous system development in *L. scutum* using scanning electron microscopy and immunohistochemistry with confocal microscopy. We then tested the effect of candidate transmitters, including acetylcholine, serotonin, dopamine, glutamate, glycine, GABA, histamine, ATP, and also of secondary messengers on cilia-based swimming behavior in our model preparations. Finally, we developed particle image velocimetry-based rigs, behavioral assays, and analysis pipelines to quantify ciliary activity in preneural vs neural swimming. We find that different transmitters and messengers modulate ciliary activity in preneural and neural stages, often in distinct ways. For example, serotonin increases swimming speed in both preneural and neural larval stages. ATP similarly increases swimming speed in preneural larvae but results in much tighter spiral circling, and unusually tight non-spiral circles, or “donuts”, in comparison to serotonin. Importantly, a cilia-based larval swimming system in *L.scutum*, consisting of an apical tuft of non-motile cilia and a prototroch band of motile cilia, is under preneural and then neural control. How the two control systems operate and either replace or integrate with one another in larval swimming is of future interest, with *Lottia* offering a unique experimental platform to understand cilia sensory-communication systems and their potential role in the origins, evolution, and function of brains and nervous systems, including human.

Disclosures: **A. Wu:** None. **L.C. Deng:** None. **L. Crossley:** None. **K. Barker:** None. **S. Fulton:** None. **R. Nguyen:** None. **R. Jackson:** None. **E. Price:** None. **S. Chalasani:** None. **L.L. Moroz:** None. **E. Edsinger:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.06

Topic: A.10. Development and Evolution

Support: NIH Grant U01MH114819
Stanley Center for Psychiatric Research at the Broad Institute of MIT and Harvard
James and Patricia Poitras Center for Psychiatric Disorders Research at MIT
Hock E. Tan and K. Lisa Yang Center for Autism Research at MIT

Title: Marmosets have birth siblings's microglia

Authors: *R. C. DEL ROSARIO[1,2], F. M. KRIENEN[2,1], Q. ZHANG[3,1], C. MELLO[2,1], M. GOLDMAN[2,1], A. LUTSERVITZ[2,1], J. NEMESH[1,2], A. WYSOKER[1,2], G. FENG[3,1], S. A. MCCARROLL[2,1];
[1]Stanley Ctr. for Psychiatric Res., Broad Inst., Cambridge, MA; [2]Genet., Harvard Med. Sch., Boston, MA; [3]Dept. of Brain and Cognitive Sci., McGovern Inst. for Brain Research, Massachusetts Inst. of Technol., Cambridge, MA

Abstract: Chimerism is the presence in the same animal of cells from multiple animals. Marmosets, which most frequently develop as fraternal twins or triplets (birth siblings) with connected circulatory systems *in utero*, harbor persistent chimerism in the blood. The presence of Y-chromosome DNA sequences in the organs of female marmosets with male birth siblings has long suggested that other organs might also exhibit chimerism, but whether this arises from blood or other cell types has been unknown. Here we show by single-cell RNA-seq that, in liver and kidney, chimerism arises from infiltrating macrophages and other blood-derived monocytes. Intriguingly, though, in addition to macrophage chimerism, marmosets harbored multiple populations of brain microglia: one population with the animal's own genome, and other population(s) with the genomes of birth siblings. Across 137 tissue samples from 11 animals, cells from birth siblings comprised 20-52% of an animal's microglia. Sibling microglial populations inhabited different brain areas in distinct relative proportions, suggesting that these sibling microglial populations were differentially responsive to local recruitment or proliferation cues. Sibling microglia exhibited clear gene-expression differences, but analyses of two siblings' microglia in both siblings' brains indicated that context (host brain) played a larger role in shaping their gene expression. Chimerism will offer powerful, well-controlled ways to study the effects of genes, mutations and brain contexts on microglial biology and to distinguish between effects of microglia and other cell types on brain phenotypes.

Disclosures: **R.C. del Rosario:** None. **F.M. Krienen:** None. **Q. Zhang:** None. **C. Mello:** None. **M. Goldman:** None. **A. Lutservitz:** None. **J. Nemesh:** None. **A. Wysoker:** None. **G. Feng:** None. **S.A. McCarroll:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.07

Topic: A.10. Development and Evolution

Title: Characterization of PCM1isoforms in the mammalian brain

Authors: *C. CHANG[1], N. GHANI[2], F. VANDERFORD[1], A. HARRINGTON[1], V. VO[3], E. OH[1];
[2]NIPM, [3]Nevada Inst. of Personalized Med., [1]Univ. of Nevada, Las Vegas, Las Vegas, NV

Abstract: The pericentriolar material 1 protein (PCM1) is a component of the centriolar satellites and regulates the biogenesis and function of the centrosome and cilium. Here, we show that PCM1 spliced isoforms are localized to discrete cellular compartments and are expressed differentially during development. Using mass spectrometry, we identified protein networks that complex with PCM1 spliced isoforms, and we show how each isoform can coordinate protein post-translational modifications in tubulin. Deletion of the PCM1 isoforms results in the modulation of neuronal activity and protein trafficking. Electron tomography analyses of synaptic terminals in the adult hippocampus highlight how PCM1 isoform products can modify synaptic vesicle size and density. Taken together, our data characterize a new role for centriolar satellite protein isoforms during development and identify a potential mechanism for ciliary proteins in human neurological disease states.

Disclosures: **C. Chang:** None. **N. Ghani:** None. **F. Vanderford:** None. **A. Harrington:** None. **V. Vo:** None. **E. Oh:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.08

Topic: A.10. Development and Evolution

Support: The Tiny Blue Dot Foundation

Title: Identifying cell-type specific actions of psychedelic drugs psilocybin and psilocin on mouse and human neocortical neurons

Authors: *L. NG[1], D.-W. KIM[1], M. KIM[1], B. E. KALMBACH[1], S. F. OWEN[2], D. KEENE[3], M. FERRERIA[4], A. KO[4], J. G. OJEMANN[4], J. HAUPTMAN[5], C. COBBS[6], H. ZENG[1], E. LEIN[1], J. T. TING[1], C. KOCH[1];
[1]Allen Inst. For Brain Sci., Seattle, WA; [2]Stanford Univ., Palo Alto, CA; [3]Pathology, [4]Neurolog. Surgery, Univ. of Washington, Seattle, WA; [5]Neurosurg., Seattle Children's Hosp., Seattle, WA; [6]Swedish Neurosci. Inst., Seattle, WA

Abstract: Psilocybin and other serotonergic hallucinogenic drugs can profoundly alter consciousness and have recently come to the forefront in brain research regarding their therapeutic potential for treating a range of debilitating psychiatric conditions. Although it is known that psilocybin and its psychoactive metabolite psilocin bind to and activate specific serotonin receptors, relatively little is known in the rodent and virtually nothing in human about cell type specific actions of these drugs in the brain. Furthermore, analysis of Allen mouse and human single cell RNA-seq datasets revealed marked species divergence in genes for serotonergic signaling pathways. These findings underscore the importance of testing drug action on human brain cell types directly rather than assuming conservation. To establish potency of our psilocin and psilocybin we performed *in vivo* drug administration in mice and analyzed the frequency of head-twitch response (HTR)—an established behavioral response to serotonergic hallucinogenic drugs dependent on 5-HT_{2A} receptor activation. Robust, dose dependent HTR was observed with both drugs but not saline control. We then performed Patch-seq in acute brain slices to measure electrophysiology, morphology, and transcriptomes of single mouse and human neurons in layers 2/3 and 5 following bath or focal application of psilocin or psilocybin (50 µM). We utilized RNA-seq data from the Patch-seq recorded neurons to map against our Allen Institute mouse and human cortical cell type taxonomies, respectively, to determine the transcriptomic cell types that responded to drug application. As a complementary approach, we monitored neuronal firing with the genetically encoded calcium indicator GCaMP8s during drug application to mark responding cells for sequential targeted Patch-seq recording. We find that ~20% of neocortical pyramidal neurons respond with a modest hyperpolarization for 10s of seconds while less than 10% of pyramidal neurons respond with strong depolarization leading to action potential firing lasting roughly equally long. These findings, together with Patch-seq mapping results, are strongly suggestive of cell type specific actions of psilocybin and psilocin. Additionally, we are now conducting scRNA-seq profiling and analysis of immediate early gene expression after psilocybin administration in mice to map the cell type and brain region-specific response profiles. Continued data generation and analysis will confirm the transcriptomic cell types most involved in psychedelic drug actions and the extent to which cell type specific mechanisms in the neocortex are conserved (or not) from mouse to human.

Disclosures: L. Ng: None. **D. Kim:** None. **M. Kim:** None. **B.E. Kalmbach:** None. **S.F. Owen:** None. **D. Keene:** None. **M. Ferreria:** None. **A. Ko:** None. **J.G. Ojemann:** None. **J. Hauptman:** None. **C. Cobbs:** None. **H. Zeng:** None. **E. Lein:** None. **J.T. Ting:** None. **C. Koch:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.09

Topic: A.10. Development and Evolution

Support: NIH Grant UG3MH120095
NIH Grant 1RF1MH114126

Title: Characterization of AAV-based genetic tools for marking somatostatin and chandelier cells across species

Authors: *D. MACHEN[1], J. MICH[1], M. LEYTZE[1], B. KALMBACH[1], M. KIM[1], N. WEED[1], C. RADELII[1], L. NG[1], V. OMSTEAD[1], N. TASKIN[1], A. HUNKER[1], R. MARTINEZ[1], L. GRAYBUCK[1], K. SMITH[1], R. CANFIELD[2], Y. KOJIMA[2], G. D. HORWITZ[2], H. ZENG[1], T. DAIGLE[1], B. TASIC[1], E. LEIN[1], J. TING[1], B. LEVI[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Washington Natl. Primate Res. Center, Univ. of Washington, Seattle, WA

Abstract: Viral genetic tools that label cell populations with high specificity across species will enable researchers to study cell type function and treat circuit dysfunction. In this study, we developed a collection of new enhancers that selectively drive gene expression in populations of Somatostatin+ (SST) and Chandelier cell (ChC) GABAergic interneurons in mouse, rat, and macaque cortex. We identified subclass-specific regulatory elements with mouse and human snATAC-seq data to construct reporter enhancer-adeno-associated viruses (AAVs). We established the specificity of reporter expression after systemic delivery of these enhancer-AAVs in the mouse using a combination of molecular approaches including multiplexed fluorescent in situ hybridization (mFISH), immunohistochemistry (IHC), and scRNA-seq. Enhancers that showed selective expression in mouse were then tested by intraparenchymal injection in macaque cortex. Cell selectivity was determined through a combined IHC and mFISH method. We report several enhancer-AAVs that drive gene expression in the SST cell subclass and the transcriptionally-defined ChC population with similar specificity across species. Lastly, we characterized the physiological properties and molecular identity of virus-labeled neurons using the Patch-seq technique and mapping to the Allen Institute human temporal cortex cell type taxonomy. Ultimately, this collection of tools will be key to the functional characterization of SST and ChC interneurons across species.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.10

Topic: A.10. Development and Evolution

Support: NIH 1U01MH121282-01

Title: Comprehensive analysis of single-cell chromatin accessibility in the human brain

Authors: *Y. E. LI[1], M. MILLER[2], N. D. JOHNSON[3], W. TIAN[3], H. JIAO[2], A. WANG[2], O. POIRION[2], S. PREISSL[2], A. PINTO-DUARTE[3], K. SILETTI[4], N. EMERSON[3], J. OSTEEN[3], J. LUCERO[3], L. LIN[2], Q. YANG[2], R. HODGE[5], S. LINNARSSON[4], T. BAKKEN[6], E. LEIN[7], M. BEHRENS[8], J. R. ECKER[9], B. REN[1];
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Abstract: The human brain is a complex organ that controls a wide range of behaviors, which are coordinated by diverse neuronal and glial cell types. To better understand the transcriptional regulatory programs that are responsible for the unique identity and function of each cell type, we have probed the open chromatin landscape at single cell resolution in more than 560,000 individual nuclei from 31 brain regions from cerebral cortex, hippocampus, basal nuclei, amygdala, thalamus, midbrain, and hindbrain. We identified >120 distinct cell types and delineated their regional specificity and gene regulatory landscapes. We further identified the state of >440,000 candidate *cis*-regulatory DNA elements (cCREs) in these brain cell types. Joint profiling of histone modifications and transcriptome in the motor cortex reveal the active and repressive chromatin state for 34.8% of cCREs. Comparative analyses of single cell atlases of open chromatin between human and mouse brains identified ~35% of the cCREs as conserved in sequence as well as cell-type specificity in both species, and uncovered a significant degree of evolutionary changes involving both sequence turnovers and regulatory divergence. We further identified significant associations between 79 GWAS traits with at least one of the brain cell types, linking multiple neuronal and glial cell types to Autism spectrum disorder and other neurological diseases. Finally, our results also facilitated functional interpretation of >1.3 million non-coding risk variants associated with various neurological and psychiatric traits at cell-type resolution.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.11

Topic: A.10. Development and Evolution

Support: NIH R01NS123959
NIH P51OD010425
NIH U01MH114812

Title: Areal specializations in L5 extratelencephalic-projecting neuron morpho-electric and transcriptomic properties in the primate neocortex

Authors: C. RADAELLI[1], L. ALFILER[1], D. BERTAGNOLLI[1], R. DALLEY[1], M. HUDSON[2], V. OMSTEAD[1], X. OPITZ-ARAYA[1], C. RIMORIN[1], N. TASKIN[1], M. TIEU[1], N. J. WEED[1], J. WILSON[1], T. BAKKEN[1], K. SMITH[1], S. A. SORENSEN[1], E. LEIN[1], S. I. PERLMUTTER[3], W. J. SPAIN[2], J. T. TING[1], N. C. DEMBROW[2], ***B. E. KALMBACH**[1];
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Abstract: Single cell RNA-sequencing atlases have revealed cell subclasses in the primate neocortex that correspond to anatomically-defined extratelencephalic- (ET) and intratelencephalic-(IT) projecting layer 5 (L5) pyramidal neurons from rodents. These atlases indicate that there are substantial differences in gene expression between clades (primates vs rodents) and neocortical areas (e.g., motor/MCx vs temporal cortex/TCx). How gene expression diversity relates to phenotypic diversity and cell type identity is poorly understood, especially in the primate neocortex. To bridge this gap, we examined i) whether transcriptomically-defined L5 ET and IT neurons are physiologically distinct, ii) whether areal specializations in L5 ET subclass properties are predicted by transcriptomic differences, and iii) whether transcriptomically-defined L5 ET neurons project to sub-cerebral regions. To address these questions, we performed Patch-seq (combined patch clamp physiology and single cell sequencing) experiments from L5 neurons in acute and cultured *ex vivo* brain slices prepared from MCx and TCx. Multiple intrinsic membrane properties differentiated L5 ET and IT neurons in both MCx and TCx. Notably, L5 ET neurons had a lower input resistance, narrower spikes and more pronounced HCN channel related properties compared with L5 IT neurons. A linear classifier trained to predict transcriptomic cell subclass identity based solely on physiology features achieved ~95% accuracy, highlighting the dissimilarity of the subclasses. We also performed Patch-seq experiments on L5 neurons labeled by an injection of AAV-retro-CAG-tdTomato in the spinal cord. These anatomically identified corticospinal neurons mapped to the L5 ET transcriptomic subclass and/or were predicted to be L5 ET neurons based on physiology.

Interestingly, L5 ET neurons exhibited considerable areal specialization. Compared with TCx, L5 ET neurons in MCx had short, fast action potentials. L5 ET neurons in TCx, but not MCx, responded to suprathreshold current injections with a high frequency burst of action potentials. Dendritic morphology of L5 ET neurons was also distinct across areas. In TCx L5 ET neurons had elaborate apical tuft dendrites, whereas in MCx they were defined by long (up to 1.5 mm) basal dendrites. These results demonstrate that L5 ET and IT neurons have distinctive transcriptomic, physiological, and morphological properties in multiple primate brain regions. In addition, L5 ET neurons have distinct properties in TCx vs MCx. Combined, these data may provide mechanistic insight into the highly specialized functional architecture of the primate neocortex.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.12

Topic: A.10. Development and Evolution

Support: Telethon Grant ggp19226

Title: The decoupling of brain and face development in Homo sapiens through selection of epigenetic switches

Authors: *A. VITRIOLO[1,2,3], F. MORETTINI[1], F. DOSSENA[1], V. FINAZZI[1], D. RAFFAELLI[1], D. CAPOCEFALO[1], O. LEONARDI[1], C. BOECKX[4], G. TESTA[5]; [1]Human Technopole Fndn., Milano, Italy; [2]Univ. of Milan, Milano, Italy; [3]IEO, European Inst. of Oncology IRCCS, Milan, Italy, Milano, Italy; [4]ICREA (Catalan Inst. for Advanced Studies and Research), Barcelona, Italy; [5]Fndn. Human Technopole, Milano, Italy

Abstract: Domestication experiments suggest that the selection of pro-human behaviours results in smaller breeds, a process likely rooted in a reduced neural crest activity. This reduction is enough to explain the physical adaptations entailed by domestication and might partially explain its behavioural features. Through the observations that modern humans are smaller than archaics, and their social groups have been larger since multiple human species coexistence on the planet, a self-domestication hypothesis has been put forward, stating that modern humans were selected through a process analogous to domestication. Basically, similarly to what humans do with animal breeds, self-selection of higher pro-sociality resulted in phenotypes that are analogous to domesticates. BAZ1B is a gene that has already been associated with domestication, located in

the Williams Beuren Syndrome Critical Region (WBSCR). We recently outlined the molecular basis of its contribution to the Williams Beuren syndrome phenotype. In fact, we showed that it regulates neural crest induction and migration and we placed it upstream of several genes causing craniofacial and intellectual disabilities. Incidentally, craniofacial phenotypes associated with WBS clearly recall the evolution of human facial traits. Following this lead, we identified a handful of BAZ1B downstream mediators and defined their centrality for craniofacial and cognitive development with respect to human evolution. We did it by integrating Psychencode, and large publicly available -omics data, with the mutational landscape pictured by all available genomics data from archaic humans spanning the last 2 million years. Moreover, by leveraging single-cell RNA-seq of several neurodevelopmental organoid systems, we disentangled BAZ1B's contribution to neural epithelium formation, neural crest induction, and central nervous system differentiation trajectories in humans. By doing so, we systematize how BAZ1B is involved in human evolution and its contribution to human-specific brain development, beyond shaping the modern face.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.13

Topic: A.10. Development and Evolution

Support: NIH grant P51OD010425
NCATS grant UL1TR000423

Title: Cross species comparison of short-term synaptic dynamics in mammalian cortex

Authors: *M. KIM[1], C. RADAELLI[1], T. CHARTRAND[2], B. E. KALMBACH[3], L. CAMPAGNOLA[1], S. C. SEEMAN[1], N. DEE[1], N. TASKIN[1], N. J. WEED[2], T. CASPER[1], M. CLARK[1], J. GLOE[1], W. HO[1], A. KO[4], J. G. OJEMANN[5], D. L. SILBERGELD[7], R. P. GWINN[8], C. COBBS[8], C. D. KEENE[6], T. JARSKY[2], H. ZENG[2], J. T. TING[3], E. LEIN[3]; [1]Allen Inst. for Brain Sci., Seattle, WA; [3]Human Cell Types, [2]Allen Inst. For Brain Sci., Seattle, WA; [5]Dept Neurosurg., [6]Pathology, [4]Univ. of Washington, Seattle, WA; [7]Dept Neurosurg., Univ. Washington, Seattle, WA; [8]Swedish Neurosci. Inst., Seattle, WA

Abstract: Properties of cortical neuron short-term synaptic dynamics are essential for shaping functional cortical microcircuit motifs such as recurrent excitation and feedback inhibition. However, it is not well understood yet whether these circuit dynamics within a cortical column are conserved from mouse to non-human primate and human. To understand primate conservation and specialization of short-term synaptic dynamics, we extended prior studies of mouse V1 (VISp) and human temporal cortex (Seeman, Campagnola et al 2018; Campagnola,

Seeman et al., 2021) to temporal cortex of mouse (TEa) and inferior temporal cortex of Southern pig-tailed macaque (Macaca nemestrina), and Rhesus macaque (Macaca mulatta). To access cell type-selective analysis we utilized multiple patch-clamp recordings in monkey organotypic slice cultures with a parvalbumin (PVALB) neuron specific enhancer adeno-associated virus (AAV) vector that allowed efficient targeting of fast spiking interneurons in addition to some neighboring pyramidal neurons (Mich et al., 2021). Here, we analyzed short-term synaptic dynamics between excitatory neurons, and from excitatory to fast-spiking inhibitory interneurons in layer 2/3 and 5 of human cortex compared to mouse (TEa and VISp) and non-human primate (inferior temporal gyrus) cortices. We find evidence for both conserved and species specialized features of short-term synaptic dynamics that vary by cell type. This direct cross-species comparative study of short-term synaptic dynamics and their recovery time courses will contribute to a better understanding of the functional properties of cortical column computations in each mammalian species.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.14

Topic: A.10. Development and Evolution

Support: NIH Grant U01MH114812

Title: Morphoelectric and transcriptomic divergence of the layer 1 interneuron repertoire in human versus mouse neocortex

Authors: *T. CHARTRAND[1], R. DALLEY[1], B. E. KALMBACH[2], J. A. MILLER[1], J. T. TING[2], G. MOLNAR[3], N. GORIOUNOVA[4], B. R. LEE[1], K. SMITH[1], A. MUKORA[1], T. BAKKEN[1], A. GALAKHOVA[4], T. S. HEISTEK[4], R. MANN[1], M. TIEU[1], A. KO[5], J. G. OJEMANN[6], C. COBBS[7], R. GWINN[7], C. D. KEENE[5], A. PATEL[5], D. L. SILBERGELD[6], R. G. ELLENBOGEN[5], J. S. HAUPTMAN[5], S. A. SORENSEN[1], H. ZENG[1], C. P. DE KOCK[4], G. TAMAS[3], H. MANSVELDER[4], E. LEIN[2];
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Abstract: Neocortical layer 1 (L1) is a site of convergence between pyramidal cell dendrites and long-range axons where inhibitory signaling can profoundly shape higher order cortical

processing. Single cell transcriptomic analyses of human L1 have revealed high interneuron diversity, but the characteristics of these transcriptomically defined cell types and their conservation or specialization compared to mouse remains underexplored. Single cell RNA-seq demonstrates that four coarse interneuron subclasses (LAMP5, MC4R, PAX6 and VIP/SNCG) are conserved from mouse to human, but significant differences in relative proportions and molecular phenotypes hinder alignment of finer cell types between species. Patch-seq analysis using human neurosurgically resected tissues identified distinctive morphological and physiological characteristics of human transcriptomic subclasses, including axonal arbor shape, spike shape adaptation, and sag, differing from the features that distinguish subclasses in mouse. Finer transcriptomic cell types generally showed similar morphoelectric properties within subclasses, but two human types emerged with strongly distinct phenotypes: the compact, irregular-spiking MC4R 'rosehip' type and the large, burst-spiking PAX6 TNFAIP8L3 type. Similar electrophysiological signatures were observed in a small subset of homologous mouse neurons, but these neurons were less distinct across all modalities. Human and mouse neurons also showed consistent differences in certain morphological and physiological properties across all subclasses, despite a general similarity in cell size. These results indicate a general conservation of L1 inhibitory neuron types, but with significant specializations in cell proportions and properties, likely leading to differences in the regulation of higher order input to the human cortical circuit.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.15

Topic: A.10. Development and Evolution

Support: NIH Grant U01MH121282

Title: Single nucleus methylome and 3D chromatin heterogeneity reveals cell-type identity, spatial location and regulatory genome complexity in the human brain

Authors: *W. TIAN[1], A. BARTLETT[1], J. ZHOU[1], H. LIU[1], R. G. CASTANON[1], M. KENWORTHY[1], J. ALTSHUL[1], J. NERY[1], H. CHEN[1], Y. LI[2], K. SILETTI[3], R. D. HODGE[4], N. JOHNSON[1], S. LINNARSSON[6], E. LEIN[5], B. REN[2], M. BEHRENS[7], J. R. ECKER[8]; [1]Salk Inst. for Biol. Studies, La Jolla, CA; [2]Univ. of California, San Diego, CA; [3]Dept. of Med.

Biochem. and Biophysics, Karolinska Institutet, Solna, Sweden; [4]Cell Types Program, [5]Human Cell Types, Allen Inst. For Brain Sci., Seattle, WA; [6]Karolinska Inst., Stockholm 17177, Sweden; [7]CNL-B, The Salk Inst., La Jolla, CA; [8]PBIO-E, Salk Inst., La Jolla, CA

Abstract: The brain is the most complex organ in the human body consisting of billions of neuronal and non-neuronal cells with extensive diversity in gene expression, anatomy and functions. Elucidating the genome regulatory landscape underlying such cellular complexity is critical for understanding normal and dysfunctional brain states. Here we describe a comprehensive examination of human brain cell epigenomes by probing single-nucleus cytosine DNA methylation in ~ 400,000 individual cells from 45 brain structures, including basal forebrain, cerebral nuclei, cerebral cortex, hippocampal formation, thalamus, midbrain and hindbrain. We identify hundreds of epigenetically distinct cell types, and characterize their molecular features including marker genes, candidate regulatory elements, and signature transcription factors, allowing construction of a cell-type taxonomy. Single nucleus methylome profiles revealed remarkable epigenomic heterogeneity associated with specific cell-types and spatial locations in both cortical excitatory neurons, subcortical inhibitory neurons, and in glia. Using these deep datasets, an artificial neural network model was developed that precisely predicts a cell's identity and location within the brain. Strikingly we found that epigenomic information predicting cell type identity was distributed throughout the genome, and assignment of single cells to major types is encoded in as little as only a few hundred CpG sites, suggesting these epigenetic sites could serve as cell-type identity barcodes. Finally, simultaneous profiling of DNA methylation and chromatin 3D conformation in over 30,000 single cells covering 20 major cerebral cortical cell types revealed remarkable cell type specificity of topologically associating domains (TADs) and chromatin loops and putative regulatory elements enriched in genetic variants for a variety of neurological disorders. This multimodal brain cell atlas provides the basis for understanding the cellular landscape and underlying regulatory genome complexity in the adult human brain.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.16

Topic: A.10. Development and Evolution

Support: NIH Grant U19MH114830
NIH Grant U01MH114812

Title: Cross-species taxonomies of primate basal ganglia cell types defined by single nucleus RNA-sequencing

Authors: M. WIRTHLIN[1], N. JOHANSEN[1], J. A. MILLER[1], A. YANNY[1], R. FERRER[1], K. SILETTI[2], R. CANFIELD[3], J. GOLDY[1], N. GUILFORD[1], J. GUZMAN[1], D. HIRSCHSTEIN[1], V. OMSTEAD[1], T. PHAM[1], N. SHAPOVALOVA[1], S. SOMASUNDARAM[1], N. TASKIN[1], A. TORKELSON[1], N. J. WEED[1], C. D. KEENE[4], S. LINNARSSON[7], Y. KOJIMA[5], G. D. HORWITZ[6], B. P. LEVI[1], J. T. TING[1], K. SMITH[1], R. D. HODGE[1], T. E. BAKKEN[1], E. LEIN[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Dept. of Med. Biochem. and Biophysics, Karolinska Institutet, Solna, Sweden; [3]Dept. of Bioengineering, [4]Pathology, [5]Otolaryngology – Head and Neck Surgery, [6]Physiol. and Biophysics, Univ. of Washington, Seattle, WA; [7]Karolinska Inst., Stockholm, Sweden

Abstract: The human basal ganglia (BG) are composed of approximately 800 million neurons engaged in a variety of behaviors and implicated in a broad range of neurological disorders. These neurons have been extensively characterized by their morphological, molecular, and electrophysiological properties. Based on shared properties, BG neurons have been grouped into types, which facilitate deeper examination of their specific roles in health and disease. The advent of high-throughput transcriptomic profiling of single cells has enabled building comprehensive censuses of mammalian cell types in many brain regions, and we have applied this technology to refine our understanding of cellular diversity in primate basal ganglia. We performed multi-omic (RNA and ATAC) sequencing of over 850,000 nuclei isolated from multiple structures of the adult human and macaque basal ganglia, including caudate, putamen, nucleus accumbens, internal and external segments of the globus pallidus, subthalamic nucleus, and substantia nigra. Nuclei were stained for NeuN and OLIG2 antibodies and captured with fluorescence-activated nuclear sorting (FANS) to enrich for neurons (NeuN+) while also capturing non-neuronal (OLIG2+ and OLIG2-) nuclei. Sequencing libraries were generated with the 10x Genomics Single Cell Multiome kit, and nuclei were sequenced to a depth of approximately 120,000 reads per sample. Individual species cell type taxonomies were generated for human and macaque using iterative clustering of the RNA-seq data, and integrated both between primates and with existing mouse taxonomies to develop a consensus cross-species cell type taxonomy. This consensus cell type taxonomy was corroborated using marker gene expression and agreement with previously published taxonomies. Linked RNA and ATAC data for conserved cell types facilitated the identification of robust cell type-specific enhancer elements, including major populations of medium spiny neurons (MSNs) and rare populations such as striatal cholinergic interneurons. Building a comprehensive cross-species cell type taxonomy enables development of novel genetic tools that can be used across species and characterization of divergent gene expression and regulation that may drive the evolution of species-specific traits.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.17

Topic: A.10. Development and Evolution

Support: U01MH114812
ALTF 1015-2018

Title: Transcriptomic diversity of cell types across the adult human brain

Authors: *K. SILETTI[1], A. MOSSI-ALBIACH[1], R. D. HODGE[2], T. BAKKEN[2], L. HU[1], C. MANNENS[1], S.-L. DING[2], A. YANNY[2], Y. E. LI[3], W. TIAN[4], J. R. ECKER[4], B. REN[3], E. LEIN[2], S. LINNARSSON[1];
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Abstract: The human brain executes an enormous range of complex behaviors. However, the cellular diversity underlying this ability remains poorly understood. We therefore used high-throughput single-nucleus RNA sequencing to survey the entire adult human brain. As part of the Brain Initiative for Cell Census Network, we isolated and sequenced more than three million nuclei from approximately 100 precisely dissected regions in three postmortem donors. The regions were sampled from major brain structures within the forebrain, midbrain, and hindbrain, and fluorescence-activated cell sorting was used to enrich for neurons. Clustering analysis of the dataset identified over 500 cell types and revealed surprisingly high neuronal diversity in non-cortical regions like the midbrain and hindbrain. Glial cells also exhibited regional diversity at multiple scales: astrocytes and oligodendrocyte precursors clustered into subtypes specific to telencephalic and non-telencephalic regions of the brain, and astrocytes exhibited additional regional diversity. Our findings therefore suggest a unique cellular composition of the telencephalon with respect to major brain cell types. As the first single-cell transcriptomic census of the human brain, the data form a basis for future census efforts and a critical resource for investigating the human brain in health and disease.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.18

Topic: A.10. Development and Evolution

Support: 1RF1MH114126-01
UG3MH120095
UF1MH128339

Title: A collection of enhancer-AAVs reveals distinct glial populations across the brain in multiple species

Authors: *B. P. LEVI[1], J. K. MICH[1], M. LEYTZE[1], R. A. MARTINEZ[1], N. JOHANSEN[1], X. OPITZ-ARAYA[1], J. T. MAHONEY[1], L. LOFTUS[1], V. OMSTEAD[1], N. TASKIN[1], N. J. WEED[1], N. DEE[1], T. CASPER[1], J. GOLDY[1], Y. BISHAW[1], D. M. MACHEN[1], B. B. GORE[1], E. MORIN[1], R. CANFIELD[2], K. A. SMITH[1], J. WATERS[1], S. YAO[1], T. BAKKEN[1], Y. KOJIMA[2], G. D. HORWITZ[2], H. ZENG[1], T. L. DAIGLE[1], B. TASIC[1], E. S. LEIN[1], J. TING[1]; [1]Allen Inst. for Brain Sci., Seattle, WA; [2]Washington Natl. Primate Res. Ctr., Univ. of Washington, Seattle, WA

Abstract: Selective transgene expression is critical for studying brain cell types including their roles in brain function and disease, and their targeting for therapeutic access. However, few tools exist that can selectively drive gene expression in defined cell populations and can be applied across mammalian species. To enable specific brain cell population labeling across species, we have generated a collection of enhancer-AAV vectors that drive transgene expression in defined cell subclasses. We undertook a systematic screen to find enhancer elements capable of targeting gene expression from AAV vectors to major neocortical cell populations. In previous work, we have described enhancers for several different populations of cortical neurons, and we demonstrated the maintenance of specificity in mouse and primate (Mich et al., Cell Reports 2021; Graybuck and Daigle et. al., Neuron 2021). Here, we show our progress generating a new collection of enhancer-AAV tools to target glial cell populations. We present sets of astrocyte-selective and oligodendrocyte-selective enhancer-AAV vectors and characterize their diverse patterns of activity across brain regions in mouse, rat, and macaque. Our growing toolbox of enhancer-AAVs for selective transgene expression will be important to dissect the roles of cell populations in brain function across species.

Disclosures: **B.P. Levi:** 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. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Patch Biosciences. **J.K. Mich:** None. **M. Leytze:** None. **R.A. Martinez:** None. **N. Johansen:** None. **X. Opitz-Araya:** None. **J.T. Mahoney:** None. **L. Loftus:** None. **V. Omstead:** None. **N. Taskin:** None. **N.J. Weed:** None. **N. Dee:** None. **T. Casper:** None. **J. Goldy:** None. **Y. Bishaw:** None. **D.M. Machen:** None. **B.B. Gore:** None. **E. Morin:** None. **R. Canfield:** None. **K.A. Smith:** None. **J. Waters:** None. **S. Yao:**

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.19

Topic: A.10. Development and Evolution

Support: 1U01MH114812
UG3MH120095

Title: Signature morpho-electric properties of GABAergic interneuron subclasses and types in the human neocortex

Authors: *R. DALLEY[1], B. R. LEE[1], J. A. MILLER[1], T. CHARTRAND[1], B. E. KALMBACH[1], L. NG[1], R. MANN[1], A. MUKORA[1], J. GLOE[1], A. ABDELHAK[1], W. HO[1], T. BAKKEN[1], N. JOHANSEN[1], R. D. HODGE[1], N. DEE[1], K. A. SMITH[1], N. GORIOUNOVA[2], R. P. GWINN[3], D. L. SILBERGELD[4], C. COBBS[3], J. G. OJEMANN[4], A. L. KO[4], G. TAMAS[5], H. D. MANSVELDER[2], C. P. DE KOCK[2], B. P. LEVI[1], J. BERG[6], S. A. SORENSEN[1], E. LEIN[1], J. T. TING[1];
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Abstract: A reductionist approach to understanding neocortical function is to start with identifying the constituent cell types and characterize their defining properties across multiple modalities. Exploration of human brain cell types has been hindered by the lack of mature brain tissue platforms and tools to support targeted cell type analysis. The ex vivo adult human neocortical slice culture paradigm enables extended use of human neurosurgically-derived acute brain slices over days to weeks, thereby enabling application of viral genetic tools. We used rapid onset AAV vectors with cell class specific enhancers to prospectively label and then target human neocortical interneurons for multimodal Patch-seq analysis, and then assign cell types based on gene expression (“t-types”). This multimodal approach allowed for the evaluation of the effect of the culture paradigm and the use of genetic tools on the electrophysiological, transcriptomic, and morphological features of recorded cells. We found subtle shifts in select electrophysiological, morphology, and transcriptomic features between acute versus culture conditions, but these do not preclude the ability to identify cell types. This culture paradigm and viral targeting has provided an avenue to define the constituents of neuronal cell types and enabled the most comprehensive examination of the morpho-electric properties of human GABAergic interneurons in layers 2-6 of neocortex to date. We characterized the transcriptomic and intrinsic physiological properties of 780 such interneurons and reconstructed the morphology of 140 of these cells and use this to describe the morpho-electric properties of cells belonging to

VIP, LAMP5/PAX6, PVALB and SST interneuron subclasses. Viral targeting of human neocortical interneurons also enabled a more extensive examination of SST+ and other under-represented types. We found a high proportion of classical double bouquet cell (DBC) morphologies within the SST-CALB1 and SST ADGRG6 t-type. Additionally, we find the SST FRZB t-type to have morpho-electric properties resembling PVALB-positive basket cells, matching observations from homologous mouse cell types. The combination of these approaches provides a robust method to characterize genetically-defined human neuron types, including diverse neocortical GABAergic neurons that have previously been difficult to reliably target.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.20

Topic: A.10. Development and Evolution

Support: NIH Brain Initiative U01MH114819
Broad Institute's Stanley Center for Psychiatric Research
Hock E. Tan and K. Lisa Yang Center for Autism Research at MIT
Poitras Center for Psychiatric Disorders Research at MIT
McGovern Institute for Brain Research at MIT

Title: A marmoset brain cell atlas reveals effects of local context on neurons

Authors: *F. M. KRIENEN[1,2], K. LEVANDOWSKI[3], H. ZANIEWSKI[3], R. C. H. DEL ROSARIO[2], Q. ZHANG[3], M. WIENISCH[3], T. SHIN[3], A. LUTSERVITZ[1], M. SCHROEDER[3], K. X. LI[3], M. R. GOLDMAN[2], A. WYSOKER[2], J. NEMESH[2], E. S. BOYDEN[3,4], S. MCCARROLL[1,2], G. FENG[3,2];
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Abstract: Marmosets are New World monkeys that share fundamental aspects of brain cell-type composition with other primates including humans. Marmosets are also an emerging model for translational neuroscience, due to their small size, rapid reproductive cycle and evolutionary relationship to humans. We used single-nucleus RNA sequencing to analyze 2.4 million brain cells sampled from 11 cortical and 7 subcortical locations in the adult marmoset brain,

identifying cell types and their properties across these brain areas. Analysis of the resulting 288 neuronal types revealed unexpected relationships across phylogenetically distinct brain structures. The relatively small size and lower myelination levels in the marmoset brain also facilitate quantitative spatial mapping and reconstruction of cells' morphology. We take advantage of the small size of the marmoset brain to image and quantify spatial locations of GABAergic interneuron types using single-molecule FISH across whole sagittal sections, and reconstruct the morphology of a subset of these types with GFP AAV labeling, including the recently-discovered, primate-specific TAC3+ striatal interneurons.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.21

Topic: A.10. Development and Evolution

Support: NIH P51OD010425
NIH NS123959

Title: Intrinsic oscillatory properties of supragranular pyramidal neuron types in the motor and temporal cortex of the macaque

Authors: C. RADAELLI[1], M. HUDSON[3], V. OMSTEAD[1], X. OPITZ-ARAYA[1], N. TASKIN[1], N. J. WEED[2], K. SMITH[2], C. RIMORIN[2], M. TIEU[2], D. BERTAGNOLLI[2], T. BAKKEN[2], E. LEIN[1], S. I. PERLMUTTER[3], W. J. SPAIN[3,4,5], J. T. TING[1], B. E. KALMBACH[1], *N. C. DEMBROW[3,5];
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Abstract: Robust oscillatory activity is observed in both the superficial and deep layers of neocortex of primates performing behavioral tasks, and specific frequency bands (theta, beta and gamma) have been implicated to reflect behavioral state. While such oscillations emerge from synaptically-driven network activity, the complement of ion channels expressed by individual neurons within the network also shape these patterns. With the advent of single cell RNA-sequencing atlases of both neocortex across many species, it has become evident that there is a greater diversity of transcriptomically-defined subclasses of supragranular pyramidal neurons in primates than in rodents. How this cellular diversity relates to oscillatory activity is poorly understood. Here we examined the intrinsic membrane properties of supragranular pyramidal

neurons and tested whether these neurons have innate frequency preferences. In recordings from layers 2 and 3 from *ex vivo* slices of the macaque primary motor cortex (**MCx**) and the superior temporal gyrus (**TCx**) we tested the intrinsic frequency selectivity across several voltage states: subthreshold, near rheobase, and during action potential firing. In a subset of recordings, the nuclei from these neurons were collected and their transcriptomes sequenced and mapped within Allen Institute cell type taxonomies.Here we report that many supragranular pyramidal neurons had HCN-dependent properties that preferentially amplified subthreshold oscillations at high delta (2 - 4 Hz) and low theta (3 - 6 Hz) frequencies - a property previously reported in human, but not rodent supragranular pyramidal neurons. These HCN-dependent properties varied with depth from pia in both MCx and TCx. To assess the frequency selectivity in the suprathreshold regime, we drove supragranular neurons to fire at ~ 10 Hz for thousands of action potentials with exponentially filtered (5 ms tau) white noise current injections and analyzed which current frequencies exhibited the highest gain response. Supragranular pyramidal neurons from both MCx and TCx exhibited high responsivity to beta-gamma (30-200 Hz) frequency bands of input. Combined, our data suggest that the intrinsic properties of a subset of supragranular pyramidal neurons in the macaque preferentially amplify specific frequencies during subthreshold and suprathreshold regimes.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Program #/Poster #: 031.22

Topic: A.10. Development and Evolution

Support: NIH Grant U01MH124602
NIH Grant F30AG069446
Fidelity Brain Research Institute

Title: An integrative single-cell genomics analysis of Alzheimer’s disease identifies cellular state alterations and neuronal vulnerabilities

Authors: *T. KAMATH[1], V. GAZESTANI[1], S. BURRIS[1], N. NADAF[1], C. R. VANDERBURG[1], A. ABDULRAOUF[3], B. ROONEY[4], S. E. MARSH[5], T. RAURAMAA[6], V. LEINONEN[6], B. A. STEVENS[7], E. MACOSKO[2];
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Abstract: Alzheimer's disease (AD) is associated with the buildup of protein aggregates of beta-amyloid and hyper-phosphorylated tau, the induction of reactive glial cell populations, and the death of neurons. Several studies have employed single-nucleus RNA-sequencing (snRNA-seq) to better understand these disease-associated cellular alterations in postmortem AD brains. Yet, there exist discrepancies in the nominated populations and pathways between these studies, which could arise from postmortem artifacts, computational challenges, and/or biological differences amongst individual study cohorts. To identify consistent cell-type-specific changes in AD, we performed a meta-analysis of 32 published snRNA-seq datasets of postmortem AD and mouse models of amyloidosis. We combined these datasets with an additional 1,002,705 nuclei profiled from 58 frontal cortex brain biopsies of individuals presenting with idiopathic normal pressure hydrocephalus (iNPH) and concomitant AD pathology, allowing for the ascertainment of cellular alterations devoid of postmortem and agonal state. Our meta-analysis identified five significantly (p-adjusted < 0.05) differentially abundant cell types in association with early and late stages of AD. One population, marked by the expression of *GPNMB* and *LPL* and recurrently induced in mouse models of amyloidosis, was significantly increased in abundance (p-adjusted < 0.01) in association with AD pathology. Comparison of this population Parkinson's disease microglia identified AD-specific pathological alterations, including an induction of lipoprotein-responsive and interferon-associated genes. We additionally identified the early loss of one *NDNF*-expressing inhibitory interneuron population residing in layer 1 of the neocortex. Consistent with the loss, we found the induction of genes associated with hypermetabolism/hyperexcitability in upper-layer excitatory neurons specifically in the earliest stages of disease and the up-regulation of glutathione-related genes in cortical astrocytes. Finally, we identified significant (p-adjusted < 0.05) dysregulation of beta-amyloid-processing genes in oligodendrocytes, including the up-regulation of *APP* at similar levels as excitatory neurons. In stem cell (SC)-derived oligodendrocyte cultures, beta-amyloid peptide levels were found at similar levels compared to SC-derived mature neurons. Taken together, our work identified consistently altered cellular populations and differentially expressed pathways associated with AD pathogenesis, and offers a strategy for nominating a gestalt of AD-associated molecular changes.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.23

Topic: A.10. Development and Evolution

Support: U01MH114812
U19MH114830

Title: Cellular and molecular specialization of functional areas in human neocortex

Authors: N. JORSTAD[1], ***N. JOHANSEN**[1], E. BARKAN[1], D. BERTAGNOLLI[1], T. CASPER[1], K. CRICHTON[1], N. DEE[1], S. DING[1], J. GOLDY[1], D. HIRSCHSTEIN[1], M. KROLL[1], D. MCMILLEN[1], T. PHAM[1], C. RIMORIN[1], N. SHAPOVALOVA[1], S. SHEHATA[1], K. SILETTI[2], S. SOMASUNDARAM[1], J. SULC[1], M. TIEU[1], A. TORKELSON[1], H. TUNG[1], A. M. YANNY[1], C. D. KEENE[3], B. P. LEVI[1], S. LINNARSSON[2], K. SMITH[1], R. D. HODGE[1], T. E. BAKKEN[1], E. S. LEIN[1];
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Abstract: Cytoarchitecture defines cortical regions (e.g. Brodmann areas) based on histological staining of cells. Single cell transcriptomics enables characterization of high-resolution cell types in human cortex, which were used to revisit the idea of the canonical cortical microcircuit and to understand functional specialization of cortical areas. Deeply sampled single nucleus RNA-sequencing of eight regions spanning cortical structural variation showed a highly consistent cellular makeup for 24 cell subclasses and over 150 cell types. However, proportions of excitatory neuron subclasses varied dramatically, reflecting differences in intra- and extra-cortical connectivity across primary sensorimotor and association cortices. Astrocytes and oligodendrocytes also showed differences in laminar organization across areas that in part reflect functional requirements of long-range projection neurons. Primary visual cortex had a distinct cellular organization, including a more than two-fold increased ratio of excitatory to inhibitory neurons, novel types of layer 4 excitatory neurons and specialized inhibitory neurons. Finally, gene expression varied in matched neuron subclasses across areas and predicts differences in synaptic function, for example in serotonin signaling. Together these results provide a cellular and molecular definition of human cortical cytoarchitecture that reflects functional connectivity and predicts areal specializations.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.24

Topic: A.10. Development and Evolution

Support: NIA Grant U19AG060909

Title: A multimodal atlas of the molecular and cellular changes to cortex driven by Alzheimer's disease

Authors: *K. J. TRAVAGLINI[1], M. GABITTO[1], Y. DING[1], J. T. MAHONEY[1], J. ARIZA[2], T. CASPER[1], M. CLARK[1], P. K. CRANE[2], N. DEE[1], J. GLOE[1], J. GOLDY[1], T. GRABOWSKI[2], J. GUZMANN[1], M. J. HAWRYLYCZ[1], S. JAYADEV[2], E. S. KAPLAN[1], E. LARSON[3], C. LATIMER[2], E. MELIEF[2], E. MEYERDIERKS[1], S. MUKHERJEE[2], T. PHAM[1], V. M. RACHLEFF[2,1], A. TORKELSON[1], K. A. SMITH[1], C. KEENE[2], E. LEIN[1], B. LEVI[1], R. D. HODGE[1], J. A. MILLER[1];
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Abstract: Alzheimer's disease (AD) is the most common cause of dementia and to date there are no effective disease modifying drugs. Histopathology studies have long noted dramatic, progressive, and stereotyped changes across numerous brain regions, including protein aggregate formation and selective loss of molecularly defined neuronal populations. But the underlying molecular and cellular mechanisms that cause AD and facilitate its progression remain unknown or are only coarsely understood, hampering efforts to treat or cure the disease. To uncover these mechanisms, we characterized the transcriptomic and epigenetic landscapes of AD by applying single nucleus RNA and ATAC sequencing to ~8 million nuclei isolated from 3 cortical regions (medial entorhinal cortex, middle temporal gyrus, and prefrontal cortex) in 84 aged donors that span the histopathological and cognitive disease spectrums (including unaffected controls) as part of a broader Seattle AD Brain Cell Atlas (SEA-AD) effort. We identified ~130 highly resolved transcriptional cell types from the BRAIN initiatives' neurotypical references and leveraged recently developed machine learning approaches to integrate and hierarchically classify nuclei across donors. This enabled characterization of cell type abundance, gene expression, and chromatin accessibility differences that correlate with AD neuropathology, cognition functioning, and genetic background with unprecedented precision. Our comprehensive molecular atlas identified specific intratelencephalic excitatory neurons, inhibitory neurons, and microglia subsets that have altered abundances, gene expression, and/or chromatin accessibility as a function of both classical and quantitative neuropathology, suggesting these populations may be selectively vulnerable to disease processes or involved in disease etiology. Differentially expressed genes and accessible chromatin regions include some found by previous atlasing efforts and genome wide association studies as well as novel ones, providing new clues to the molecular pathways that underpin AD. The cell types and molecular pathways identified by our atlas provide new targets for the rational development of therapeutic interventions and as biomarkers for earlier detection of the disease.

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Poster

031. Comparative Cellular and Molecular Mechanisms

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.25

Topic: A.10. Development and Evolution

Support: NIH Grant 1U19AG060909-01

Title: Spatial mapping of human cortical cell types with Alzheimer's disease progression

Authors: *J. CLOSE[1], B. LONG[1], E. GELFAND[1], M. KUNST[1], Z. MALTZER[1], M. HUPP[1], D. MCMILLEN[1], J. CAMPOS[1], R. HODGE[1], J. MILLER[1], K. TRAVAGLINI[1], M. GABITTO[1], J. WATERS[1], C. KEENE[2], E. LEIN[1];
[1]Allen Inst. for Brain Sci., Seattle, WA; [2]Pathology, Univ. of Washington, Seattle, WA

Abstract: Alzheimer's disease (AD) follows a stereotyped progression with deposition of extracellular amyloid beta plaques and intracellular phosphorylated tau tangles accompanied by loss of neurons in affected brain regions. Recent single nucleus RNA sequencing (snRNAseq) characterization of cells in the human brain resulted in a detailed classification of transcriptomic types, and efforts are underway to describe these types in AD donor tissue. In parallel, we performed spatial mapping of these human cortical cell types in the same AD donor tissue in middle temporal gyrus (MTG), a cortical area affected in mid-late stages of AD progression. A 140-gene panel designed to identify MTG cell types was used to perform multiplexed error robust in situ hybridization (MERFISH) on MTG specimens from donors spanning AD neuropathic change (ADNC) stages 0-3 to confirm snRNAseq observations, and to determine cell type locations, proportions, and relationship to pathology. We have identified and mapped vulnerable cell populations as well as changes in proportions for key transcriptomic types during AD progression.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.26

Topic: A.10. Development and Evolution

Title: Inter-individual genomic and transcriptomic variation in human cortical cell type

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Abstract: Single cell transcriptomic analysis of human cortex has identified a diverse cellular makeup but is limited by diversity in postmortem samples leading to limited characterization of conserved features among donors. Here we assessed variation in cortical cellular abundance and gene expression across 75 adult individuals using single nucleus RNA-seq and whole genome sequencing from tissue that was acutely resected during epilepsy and tumor surgeries. Nearly all nuclei were confidently mapped to one of the 130 cell types identified in the middle temporal gyrus, indicating a highly consistent cellular architecture that could be compared across individuals. Cell type abundances varied from less than 1.5-fold to more than 4-fold across individuals and was not explained by demographic factors or disease state, except for a significant reduction of Parvalbumin-positive interneurons in epilepsy cases. Gene expression varied more between donors than within donors, particularly for excitatory neuron types including long-range projection neurons in superficial and deep layers that communicate with distant brain regions. Age, sex, ancestry, disease state and cell type explain greater than 1% of variation in expression for a median of 322 genes across these covariates. Variance in gene expression between donors was greater than across cell types for some genes including *LINC00486*, *ISG15*, and *CAMK2N1*, and these genes exhibited continuous or discrete variation across donors. Genomic variation was significantly associated with variable gene expression in all neuronal subclasses, including single nucleotide variants near *LRRC37A* and Alzheimer's disease-associated genes *APP* and *SORT1* that were specifically associated in excitatory neurons in layer 6b. Identifying genes whose expression divergence is explained by genetic variants enables us to determine genes with high fluctuation across donors whose variation is partially driven by cis-regulatory differences. Together, these analyses demonstrate a highly consistent cellular makeup across human individuals but with significant variation reflecting donor characteristics, disease condition, and genetic regulation.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.27

Topic: A.10. Development and Evolution

Support: NIA Grant U19AG060909

Title: Sea-ad: leveraging quantitative neuropathology, single cell omics, and spatial transcriptomics to create an open access atlas of Alzheimer's disease

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Abstract: Alzheimer’s disease (AD) is the most common form of dementia, representing ~70% of dementia cases, and affecting much of the aged population. AD is conventionally defined by characteristic deposition of specific pathological proteins; however, tools to probe the transcriptome, epigenome, and spatial organization of single cells in complex brain tissues allow a more refined look at how the neurotypical brain changes in AD. Linking these indicators of AD severity will be key for understanding disease mechanisms and can yield valuable insights into selective vulnerability or resistance to pathology. The Seattle Alzheimer’s Disease Brain Cell Atlas (SEA-AD) brings together experts in AD research and large-scale molecular/anatomical brain mapping to modernize AD tissue banking methods, and to combine traditional and quantitative neuropathology with emerging -omics technologies. The initial focus of SEA-AD is on middle temporal gyrus (MTG), where multimodal data was collected and integrated from 84 aged subjects of varying degrees of AD severity (including normal cognition) from two well-characterized cohorts—Adult Changes in Thought (ACT) and the University of Washington ADRC. SEA-AD anchors all results to a robust set of 127 cell types defined in young adult donors, with an additional 12 non-neuronal types identified in aged donors, including an APOE+ set of microglia corresponding to previously reported disease-associated microglia (DAM). More generally, transcriptomic and epigenetic data from aged donors can be assigned to cell types in this expanded reference with high confidence. Additional associations between cell types, molecular pathways, quantitative pathology, and traditional disease metrics are presented in companion posters; these results are consistent with and expand upon published results from other cohorts. The complete SEA-AD MTG atlas, which can be used to reproduce any presented results, is now freely available at portal.brain-map.org/explore/seattle-alzheimers-disease. It includes access to all data and associated donor metadata, an interactive pathology image viewer, tools for visualization and exploration of -omics data, and a tool for reference-based mapping of new RNA-seq experiments, along with extensive documentation and user support.

Disclosures: **J.A. Miller:** None. **M.J. Hawrylycz:** None. **J. Ariza:** None. **S. Ding:** None. **L.A. Esposito:** None. **R. Gala:** None. **E.S. Kaplan:** None. **E.J. Melief:** None. **A.L. Nolan:** None. **J. Nyhus:** None. **V.M. Rachleff:** None. **K.J. Travaglini:** None. **J.L. Close:** None. **P.K. Crane:** None. **M. Darvas:** None. **M.I. Gabitto:** None. **T.J. Grabowski:** None. **R.D. Hodge:** None. **S. Jayadev:** None. **C.S. Latimer:** None. **B.P. Levi:** None. **C.L. MacDonald:** None. **S. Mukherjee:** None. **K.A. Smith:** None. **M. Wang:** None. **E.B. Larson:** None. **C.D. Keene:** None. **E.S. Lein:** None.

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.28

Title: WITHDRAWN

Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 031.29

Topic: A.10. Development and Evolution

Support: AHA Postdoc Fellowship #915654
James S. McDonnell Foundation 220020467
NIMH Grant MH207672
Howard Hughes Medical Institute

Title: Human CLOCK enhances cognitive flexibility by altering cortical excitatory neuron function

Authors: *Y. LIU, M. R. FONTENOT, A. KULKARNI, N. KHANDELWAL, S.-H. PARK, C. DOUGLAS, M. HARPER, P. XU, N. GUPTA, J. R. GIBSON, J. S. TAKAHASHI, G. KONOPKA;
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Abstract: Alteration of expression rather than protein coding sequence of genes has been determined as a major mechanism for human brain evolution. Decades of comparative genomics between human and non-human primates have discovered lists of candidate genes. However, few of them have been functionally studied to understand how alterations in gene expression affected human brain evolution. *CLOCK* is a gene that has been repeatedly identified as having a human-specific brain expression pattern and is involved in brain function. Here, we generated a mouse

model that only expresses human *CLOCK* in a way that recapitulates expression of *CLOCK* in the human cortex. We tested the humanized (HU) mice in a set of behavioral experiments. HU mice outperformed wildtype mice in a set-shifting test for cognitive flexibility. We then did immunohistochemistry on frontal cortex and found that HU mice had increased neuron density. To understand the functional mechanisms of behavioral alterations, we examined the electrophysiological properties of excitatory neurons in the upper layers of frontal cortex and found that HU mice have greater frequency of excitatory postsynaptic currents, while the intrinsic excitability, resistance, and conductance of these neurons were unchanged. These results suggest that the excitatory neurons of HU mice might have more functional connections. Consistent with this prediction, we found that excitatory neurons in HU mice have increased complexity of dendritic branching and spine density. To understand the underlying molecular mechanisms, we did single-nuclei RNA-seq on frontal cortex. We found that mouse *Clock* and human *CLOCK* showed different spatiotemporal expression, and genes associated with dendrite growth and spine formation were upregulated in HU mice. To validate our findings in the mouse model and the downstream targets from single-nuclei RNA-Seq, we used CRISPR/Cas9 to generate *CLOCK* knockout (KO) human iPSC lines. We differentiated iPSCs to cortical-like excitatory neurons and rescued with overexpression of one of two CLOCK downstream genes (*TENM2* or *SORCS2*). We found that KO neurons have decreased dendritic complexity, and *TENM2* but not *SORCS2* overexpression partially rescued this phenotype. In summary, human *CLOCK* has an altered spatiotemporal expression pattern compared to mouse *Clock* and promotes dendrite growth and spine formation in excitatory neurons, resulting in more functional connections in frontal cortex and higher cognitive flexibility. Our results suggest that gain of functions in neurodevelopment through spatiotemporal alteration of gene expression might be an important molecular mechanism for human brain evolution.

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Poster

031. Comparative Cellular and Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 031.30

Topic: I.07. Data Analysis and Statistics

Support: O'Donnell Brain Institute NSTP Fellowship
NHGRI (HG011641)
SBE-131719
EF-2021635
T32DA007290
T32HL139438
MH103517

Title: Identifying epigenomic and transcriptomic alterations in human brain evolution at cellular resolution

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Abstract: Human evolution is marked by enlarged brain and increased cognitive abilities. Such traits must be genetically encoded which motivated decades of research to find human specific molecular traits linked to specific genetic modifications. Early approaches that compared humans to closely related species showed that most human specific molecular traits are likely caused by alterations in non-coding regions, leading to altered gene expression patterns. To understand both non-coding and gene expression patterns specific to humans at cellular resolution, we performed single-nuclei ATAC-seq (snATAC-seq) and single-nuclei RNA-seq (snRNA-seq) in human, chimpanzee and macaque adult cortex. This yielded ~73000 nuclei in snATAC-seq and ~150000 nuclei in snRNA-seq. We reproduced many previously identified neuronal subtypes and found certain neuronal subtypes to be more human specific than others indicating heterogeneity of human evolution at subtype resolution. Through motif analysis on human specifically accessible regions, we pinpointed human specific transcription factor activity for each cell type. We also found that human specific molecular features were more enriched in brain diseases compared to chimpanzees in matched cell types. Taken together, our results demonstrate the epigenomic and transcriptomic features of human brain evolution and uncover their potential link with brain diseases at the cell type level.

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Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.01

Topic: C.03. Parkinson's Disease

Support: 2020M3E5D9080661
2020R1A4A4079722

Title: Analysis of biological functions of synphilin-1 with integrated omics

Authors: J. HWANG[1], D. KWON[2], S. KIM[1], N. GEORGE[1], T. SHIN[1], *G. LEE[3];
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Abstract: One of the well-known causes of Parkinson's disease is the aggregation of α-synuclein that affects several intracellular functions including mitochondrial function and results in death of dopaminergic neurons. Among α-synuclein-interacting proteins, synphilin-1 has been reported to be found in Lewy body and have a neuroprotective effect. Although the relationship between α-synuclein and synphilin-1 is known, the function of synphilin-1 is not fully understood. Therefore, we focused on the effects of synphilin-1 on mitochondria to elucidate its neuroprotective effect. In this study, we used HEK293 cells which were stained with neurofilament (NF)-specific antibodies such as NF-M, NF-L, and NF-H and Synph-293 cells (HEK293 cells stably overexpressing synphilin-1). To discover the cellular changes due to synphilin-1, we performed *in vitro* experiments and analyzed transcriptome, proteome, and metabolome for *in silico* prediction. Synphilin-1 transfected HEK293 and Synph-293 cells showed a notable increase in ATP levels, which can be explained by transcriptome-proteome-metabolome-integrated network. Additionally, we investigated the uptake of nutrients to observe whether increment of ATP is induced by enhanced mitochondrial function rather than nutrients. Synphilin-1 transfected HEK293 and Synph-293 cells showed significant decrement of glucose. This observation is consistent with *in silico* prediction network, showing lower glucose uptake, higher concentration of ATP, and enhanced mitochondrial function. To confirm the improved mitochondrial function, we studied the mitochondrial stress resistance with TMRE assay. Synph-293 cells showed higher TMRE intensity than HEK293 cells in FCCP treated groups. This indicates that synphilin-1 may regulate mitochondrial function and help mitochondria to produce more ATP and resist against stress. Even though α-synuclein and synphilin-1 are not studied simultaneously, our findings suggest that synphilin-1 may have a neuroprotective role by regulating intracellular energy status and enhancing mitochondrial function in brain.

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Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.02

Topic: C.03. Parkinson's Disease

Support: NIH/NIA: R21AG067282

Title: The DIRAS proteins regulate neuronal autophagy

Authors: *A. A. PATIL[1], R. DIAZ ESCARCEGA[1], J. MORUNO MANCHON[1], Y. RUI[2], A. S. TSVETKOV[1];
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Abstract: Autophagy is a major degradation pathway for dysfunctional or abnormal cytoplasmic components. In neuronal cells, autophagy occurs at a basal level, and dysregulated autophagy is

associated with a wide variety of neurodegenerative disorders. Modulating neuronal autophagy is, therefore, a promising therapeutic strategy for ameliorating neurodegeneration. GTP-binding RAS-like proteins 1/2/3, also known as DIRAS 1/2/3, are involved in regulating autophagy in various cancer cells. DIRAS3 (ARHI) is a tumor suppressor, which is downregulated or absent in over 60% of primary ovarian cancers. Re-expression of DIRAS3 in these cancers upregulates autophagy and hinders cell growth. Intriguingly, over the years of evolution, mouse genome has lost the Diras3 gene and contains only Diras1 and Diras2; whereas human genome contains DIRAS1, DIRAS2 and DIRAS3, suggesting important differences between autophagic pathways in mouse and human cells. In our data, we show that DIRAS proteins are potent enhancers of neuronal autophagy. DIRAS proteins co-localize with beclin-1 and promote flux through autophagy in neurons. In neurons, DIRAS3 stimulates the degradation of mutant huntingtin, the protein that causes Huntington disease, and results in neuroprotection. Our study identifies the DIRAS proteins as regulators of neuronal autophagy and provides a novel target for blunting neurodegeneration.

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Poster

032. Cellular Studies of Dopamine Neurons

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.03

Topic: C.03. Parkinson’s Disease

Support: FAPESP, CAPES and CNPq (Brazil; A.F.F.F. was the recipient of a FAPESP fellowship, #2020/02109-3)

Title: Inhibition of transient receptor potential melastatin 2 channel limits the progression of 6-hydroxydopamine-induced hemiparkinsonism in mice

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Physiol. and Biophysics, Univ. of São Paulo, São Paulo, Brazil

Abstract: The transient receptor potential melastatin 2 (TRPM2) is a non-selective calcium channel involved in neurodegeneration in several models. However, a possible role of TRPM2 in Parkinson’s disease (PD) remains to be clarified. We have recently used the TRPM2 inhibitor AG490, a tyrphostin compound, and found a neuroprotective effect in a common mice model of PD when AG490 was administered half an hour before 6-hydroxydopamine (6-OHDA). We hypothesize that AG490 can be used as a treatment and not only as a prophylactic. To answer that, we induced the model in 3 month-old C57BL/6 male mice (Ethics approval: #8395080450). Ten µg of 6-OHDA in 1µl, or 1µl of saline were injected into the right striatum of mice. Three days after the surgery we performed motor behavior tests (n=12/group) to validate the model and initiated the treatment. Thirty mg/kg of AG490 or vehicle were intraperitoneally injected daily

for 4 days, starting on day 3 after 6-OHDA injections. In day 6, another set of behavior tests was performed. On day 7, brains were collected for immunofluorescence analyses of tyrosine hydroxylase (TH - dopaminergic neurons), TRPM2, GFAP (astrocytes), and Iba-1 (microglia) of the substantia nigra (n=6/group). Behavior data were analyzed by two-way ANOVA repeated measures and immunofluorescence data by two-way ANOVA. Bonferroni's posttest was performed on these data. On day 3, animals that received 6-OHDA showed impaired motor behavior in the apomorphine and rotarod tests when compared to animals that received saline. On day 6, the last day of AG490 treatment, 6-OHDA-injected, AG490-treated mice showed improvement in the apomorphine test, with a reduced number of asymmetric rotations when compared to 6-OHDA-injected, vehicle-treated mice ($p<0.01$). In the rotarod test, animals that received 6-OHDA and were treated with AG490 had a higher latency to fall than 6-OHDA-injected, vehicle-treated animals ($p<0.01$). Six-OHDA-injected, vehicle-treated mice had reduced TH (54±3, $p<0.01$), and increased TRPM2 (1.68±0.14, $p<0.01$), GFAP (2.79±0.37, $p<0.01$), and Iba-1 (2.48±0.19, $p<0.01$) staining when compared to control (saline-injected, vehicle-treated animals). Six-OHDA-injected, AG490-treated mice had higher TH (73±2, $p<0.01$), and less TRPM2 (1.06±0.03, $p<0.01$), GFAP (1.58±0.17, $p<0.01$), and Iba-1 (1.34±0.11, $p<0.01$) staining than 6-OHDA-injected, vehicle-treated mice. These results indicate that AG490 treatment reduced dopaminergic neuronal loss and neuroinflammation in the 6-OHDA animal model of PD during the progression of neurodegeneration. Thus, the TRPM2 channel may represent an important pharmacological target for the treatment of PD.

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Poster

032. Cellular Studies of Dopamine Neurons

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Program #/Poster #: 032.04

Topic: C.03. Parkinson's Disease

Support: NIH R21AA028800
NIH R21AG068607
NIH R21DA052419
NIH F31NS118811

Title: Transcriptomic analysis of vesicular glutamate transporter-expressing dopamine neurons reveals differential expression of genes mediating vulnerability to neurodegeneration

Authors: *S. A. BUCK[1], C. D. TREIBER[4], V. R. SUNDAR[1], Z. I. YANG[1], X. XUE[2], S. WADDELL[4], R. W. LOGAN[5], Z. FREYBERG[3];
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Abstract: Dopamine (DA) neurodegeneration is a key component of Parkinson's disease (PD), but some DA neurons are relatively protected from PD-induced neurodegeneration compared to others. Relative protection of DA neurons is associated with vesicular glutamate transporter 2 (VGLUT2) expression, and conditional knockout of VGLUT2 in DA neurons increases vulnerability while moderate upregulation of VGLUT2 confers increased protection in PD models. To date, however, the mechanism(s) behind this VGLUT2-associated neuroprotection remains unknown. We hypothesized that VGLUT2's protective properties stem from its ability to mobilize intracellular machinery to lower toxicity associated with DA metabolism. It is also possible that VGLUT2 may not directly confer protection, but rather it is either 1) a marker whose expression is correlated with expression of other protective proteins in these DA neurons, or 2) VGLUT2 may be one of several co-expressed genes that collectively boost resilience. To determine putative modulators of VGLUT2-mediated DA neuroprotection, we identified the genes that are differentially expressed (DE) in $VGLUT2^+$ DA neurons compared to $VGLUT2^-$ DA neurons across both *Drosophila* and mouse models. We performed single-cell RNAseq in *Drosophila* central brains to quantify differential gene expression in DA neurons expressing the fly ortholog of VGLUT2, *Drosophila* VGLUT (dVGLUT). In parallel, we performed bulk RNAseq from FACS-sorted mouse midbrain DA neurons to quantify differential gene expression in $VGLUT2^+$ DA neurons. Using these two RNAseq datasets, we compiled 180 DE candidate genes and 7 transcription factors identified to be upstream regulators of mouse DE transcripts. We obtained *Drosophila* UAS-RNAi lines for these 187 genes and crossed them with tyrosine hydroxylase (TH)-Gal4 flies to knock down gene expression specifically in DA neurons. We then screened the candidates for vulnerability to DA neurodegeneration by measuring changes in locomotion during exposure to paraquat, a pesticide model of PD. Of the 187 genes screened, over 50 were either significantly vulnerable to paraquat in both sexes or had a significant sex difference in vulnerability ($P<0.05$). These most vulnerable candidates were overrepresented in biological pathways including locomotion, development, oxidative phosphorylation and synaptic transmission. Future work will test these candidates for potential interaction with dVGLUT/VGLUT2 in DA neuroprotection.

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Poster

032. Cellular Studies of Dopamine Neurons

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.05

Topic: C.03. Parkinson's Disease

Support: NIH Grant 5R01AG048918

Title: Proteomics and phosphoproteomics reveal insights into metformin protection of selective dopamine neurons from methamphetamine-induced damage in nonhuman primates: implications for Parkinson's disease

Authors: *J. K. BLACKBURN[1], D. W. CURRY[1], B. STUTZ XAVIER[2], R. H. ROTH[1], J. D. ELSWORTH[1];
[1]Psychiatry, [2]Comparative Med., Yale Univ. Sch. of Med., New Haven, CT

Abstract: In Parkinson's disease and during normal aging there is a progressive loss of midbrain dopaminergic neurons. The loss of dopamine (DA) innervation to forebrain regions has been firmly linked with declines in motor and cognitive function in aging and PD. Despite knowing that mitochondrial alterations and oxidative stress play key roles in the loss of DA neuron function in aging and PD, there are currently no therapeutic treatments that can preserve the dwindling population of midbrain DA neurons. Rodent studies have indicated that the anti-diabetic drug, metformin, protects the brain against loss of DA neurons in models of PD. Metformin influences many cellular pathways, including AMP-activated kinase (AMPK) signaling, which plays a crucial role in maintaining cellular energetic homeostasis, and the Akt pathway, which regulates diverse cellular functions including cell growth, survival, proliferation and differentiation. However, the precise mechanisms by which metformin protects DA neurons are not yet known. To elucidate metformin's protective action in PD, we explored the changes in protein expression and activation in African green monkeys treated orally with metformin for 4 weeks (12.5 mg/kg for 1 week, then 25 mg/kg for 3 weeks). A subset of monkeys were also treated with the dopaminergic toxin, methamphetamine (METH) one week prior to terminus (0.5 mg/kg on day 22, 1.0 mg/kg on day 23). Metformin treatment reduced METH-induced dopaminergic toxicity in selective brain regions, including substantia nigra (SN) and dorsolateral prefrontal cortex (DFC) based on DA concentrations measured by HPLC. To investigate the basis of protection of DA neurons, we performed label-free quantification of proteome and Ti-O2 enriched phosphoproteome analysis of SN and detected 3102 proteins and 2570 phosphoproteins with a false discovery rate <1%. Pathway analysis of differentially regulated proteins reveals a biological categorization of these alterations and indicated that relevant metformin-induced changes depended on METH-exposure: AMPK and Akt pathways were only activated in monkeys treated with both metformin and METH, as were mTOR and Nrf2 signalling pathways. These results indicate that metformin preferentially promotes mitochondrial function, antioxidant functions, cell growth and survival under conditions of dopaminergic stress in primate brain. Our findings help evaluate the role and potential of AMPK activators as a neuroprotective treatment for PD and some sequelae of aging.

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Poster

032. Cellular Studies of Dopamine Neurons

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Program #/Poster #: 032.06

Topic: C.03. Parkinson's Disease

Support: P20NS123220
Parkinson's Foundation

Title: Single-cell transcriptomic atlas of the human substantia nigra in Parkinson’s disease

Authors: *M. LIANG;
Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY

Abstract: Marianna Liang, Qian Wang, Minghui Wang, Insup Choi, Lap Ho, Kurt Farrell, Kristin G. Beaumont, Robert Sebra, John F Crary, David A. Davis, Xiaoyan Sun, Lily Sarrafha, Joel Blanchard, Tim Ahfeldt, Bin Zhang, and Zhenyu Yue. Parkinson’s disease (PD) is the second leading neurodegenerative disorder characterized by degeneration of neuromelanin-containing dopaminergic (DA) neurons in the substantia nigra (SN). The cause of PD remains unclear however single-nucleus RNA sequencing (snRNAseq) has significantly advanced our understanding of neurodegenerative diseases but limited progress has been made in PD. We have generated by far the largest snRNAseq data from human SN including 9 healthy controls and 23 idiopathic PD cases across different Braak stages. A combination of immunostaining and validation against datasets from independent cohorts resulted in the identification of three molecularly distinct subtypes of DA-related neurons, including a RIT2-enriched population in aged human SN. RIT2 variants are linked to PD. All DA neuron subtypes degenerated in PD. Analysis of the DA neurons of the three subtypes from PD demonstrated alterations of common gene sets associated with neuroprotection. Validation in mouse, midbrain organoids, and human tissue identifies a RIT2 population that partially overlaps with TH in the ventral substantia nigra. Our result highlights the heterogeneity of DA neurons in the human SN and suggest a molecular basis for vulnerability and resilience of human DA neurons in PD.

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Poster

032. Cellular Studies of Dopamine Neurons

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Program #/Poster #: 032.07

Topic: C.03. Parkinson’s Disease

Support: Swiss National Foundation (SNF grant number 31003A_179527) to LT
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Spanish Ministry of Sciences and Innovation (PID2019-105795RB-I00) to TG-H

Title: Long-term uninterrupted supraphysiological GDNF intra-striatal administration is toxic and reverses neuroprotective effects.

Authors: *M. DUARTE AZEVEDO[1], N. PRINCE[1], M. HUMBERT-CLAUDE[1], V. MESA[2], K. DEMATOS[1], T. GONZÁLEZ-HERNÁNDEZ[2], L. TENENBAUM[1];

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Abstract: Glial cell line-derived neurotrophic factor (GDNF) protects nigro-striatal dopaminergic (DA) neurons and reduces motor symptoms when applied in the rodent striatum in toxin-induced Parkinson's disease (PD) models. Furthermore, GDNF reduces alpha-synuclein propagation induced by pre-formed fibrils[1]. However clinical trials based on intraputaminal GDNF delivery has so far failed to demonstrate significant clinical benefits[2]. Our hypothesis is that GDNF beneficial effects depend on the treatment regimen. We have previously described a doxycycline (Dox)-regulated AAV vector allowing to finely adjust GDNF dose and period of administration at clinically-acceptable Dox doses[4]. Using different Dox and vector doses, we have administered GDNF at concentrations ranging from 3-fold to 20-fold the endogenous tissue level (measured by ELISA), in the striatum of unilaterally 6-hydroxydopamine (6-OHDA)-lesioned female Wistar rats (n=108 in total). The rats were treated for 17 weeks either continuously or intermittently for 2 weeks with 2 weeks interruptions. Behavioral tests revealing motor impairments were: amphetamine-induced rotations and drug-free distance run and rotations in an openfield. Output measures were: GDNF localization (immunohistochemistry), DA neurons survival and cell size (VMAT2 immunostaining and western blot), Ret signaling (phospho-S6 ribosomal protein immunostaining), oxidative stress (8-oxo-2'-deoxyguanosine immunostaining) and neuroinflammation (IbaI immunostaining). Significant reduction of the motor impairments and restoration of striatal DA neurons innervation induced by the 6-OHDA lesion were observed at GDNF tissue concentrations ranging from 3- to 10-fold but not at 20-fold the endogenous level. In contrast, at the highest GDNF dose, motor deficits were not reversed and the striatal dopaminergic innervation was not increased relative to untreated rats. Strikingly, DA neurons harbored a higher level of DNA oxidation as compared to the lowest GDNF dose. However, when the treatment was applied intermittently, the highest GDNF dose also induced significant improvements, increased striatal re-innervation and did not induce increased levels of DNA oxidation. In future clinical trials, it will be important to control GDNF administration in order to avoid overdosage potentially reducing the clinical benefits.1.Chmielarz et al. doi.org/10.1101/75289 ; 2. Heiss, JD et al. 2019. DOI :10.1002/mds.27 ; 3. Tenenbaum, L. & Humbert-Claude, M. doi:10.3389/fnana.2017.00029; 4. Chtarto, A. *et al.* doi:10.1038/mtm.2016.27.

Disclosures: **M. Duarte Azevedo:** None. **N. Prince:** None. **M. Humbert-Claude:** None. **V. Mesa:** None. **K. DeMatos:** None. **T. González-Hernández:** None. **L. Tenenbaum:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.08

Topic: C.03. Parkinson's Disease

Title: Curcumin activates p62-mediated autophagy via regulating Nrf2-Keap1 pathway in rotenone-induced parkinsonian mouse model

Authors: *A. S. RATHORE[1], S. P. SINGH[2];
[1]Banaras Hindu Univ., Varanasi, India; [2]Banaras Hindu University/ Fac. of Sci., Varanasi, India

Abstract: Curcumin activates p62-mediated autophagy via regulating Nrf2-Keap1 pathway in rotenone-induced parkinsonian mouse model.Aaina Singh Rathore*, Surya Pratap Singh[1]Department of Biochemistry, Institute of Science, Banaras Hindu University, Varanasi – 221005, Uttar Pradesh, IndiaAbstract:Aggregation of misfolded alpha-synuclein (α-syn) protein in the form of Lewy bodies and Lewy neurites is a major characteristic of Parkinson's disease (PD). Autophagy plays a crucial role in degradation of abnormally aggregated proteins and organelles. p62 (an autophagy substrate) competes with Nrf2 (Nuclear factor erythroid 2 related factor 2) for binding with Keap1. Therefore, increased production of p62 causes activation of Nrf2 consequently transcriptional activation of its target genes. The same p62 binds with LC3-II present on the membrane of autophagosomes and marks the normal functioning of the autophagy pathway. The present study demonstrates the potential therapeutic properties of Curcumin in the management of PD by activation of autophagy via Nrf2-Keap1 pathway. The mice were pretreated for 7 days with oral administration of curcumin (80mg/kg body weight). Later, they were intoxicated with rotenone (2mg/kg body weight) for developing parkinsonism and, co-treated with curcumin for 35 days. The results indicated increased nuclear translocation of Nrf2 in the curcumin treated group as compared to the rotenone intoxicated group, which was confirmed by the increased levels of its downstream genes, NQO1 (NADPH: quinone oxidoreductase 1) and HO-1 (heme oxygenase-1). Tyrosine hydroxylase (TH) enzyme, dopaminergic neuron marker, also showed decreased expression in rotenone induced PD mice whereas curcumin treatment ameliorated the level of TH in the treatment group. Autophagy was evaluated by the change in expression of autophagic markers, p62 and LC3-II. Increased p62 expression and decreased LC3 expression in the rotenone mouse model of PD confirmed the compromised autophagy pathway, consequently increasing the aggregation of misfolded protein α-syn. Whereas, curcumin treatment showed significant decrease in expression of autophagy substrate p62 along with down-regulation of α-syn expression and increased level of LC3-II. Consequently, the findings reveal the neuroprotective role of curcumin in rotenone-intoxicated mice by activating p62-dependent Keap1-Nrf2 autophagy pathway.
Keywords: Parkinson's disease; Autophagy; Nrf2-Keap1 Pathway; Oxidative stress; Protein aggregation

Disclosures: **A.S. Rathore:** None. **S.P. Singh:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.09

Topic: C.03. Parkinson's Disease

Support: NRF-2022R1A2C1011996

Title: Histone Deacetylase 6 (HDAC6) inhibitor restores the cellular functions in Parkinson Disease (PD) model

Authors: *Y. PARK, S. SUN, H. SEO;
Hanyang Univ., Ansan, Korea, Republic of

Abstract: Histone deacetylase (HDAC) has critical roles to regulate gene expression and various cellular functions adjusting the acetylation level of histone or non-histone proteins. HDAC6, one of the Class IIb deacetylases, can catalyze both histone and non-histone proteins due to its structure. HDAC6 inhibition has been reported as a potential therapeutic target in several neurodegenerative diseases. Although the function of HDAC6 in PD is not clearly defined yet, HDAC6-specific inhibition has been reported as a therapeutic target of PD. In this study, we used HDAC6 specific inhibitor, tubacin, to find out the neuroprotective mechanisms of HDAC6 inhibition in PD. We determined that tubacin improved the motor coordination in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) PD mice, and increased the number of tyrosine hydroxylase (TH)-positive dopaminergic neurons. In the primary cortical neuron culture, tubacin increased the length and number of neurites. Tubacin increased the survival of microglia and neurons in PD cell model. These results suggest the potential therapeutic application of tubacin for PD.

Disclosures: Y. Park: None. **S. Sun:** None. **H. Seo:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.10

Topic: C.03. Parkinson’s Disease

Support: Barrow Neurological Foundation
Postdoctoral Fellowship for Basic Scientists

Title: Acmsd overexpression prevents neurodegeneration and gliosis in the aav-αsyn model of parkinson’s disease

Authors: *K. MEYERS[1], D. J. MARMION[2], I. M. SANDOVAL[2], E. QUANSAH[3], L. BRUNDIN[3], F. P. MANFREDSSON[2];
[2]Neurobio., [1]Barrow Neurolog. Inst., Phoenix, AZ; [3]Van Andel Inst., Grand Rapids, MI

Abstract: Alterations in the kynurenine pathway (KP) are linked to the onset and progression of Parkinson’s disease (PD). Neurotoxic metabolites of the KP, including 3-hydroxykynurenine (3-HK) and quinolinic acid (QA), are found in the plasma and cerebrospinal fluid of PD patients and correlate with symptom severity. Metabolites of the KP pathway include alpha-amino-beta-

carboxymuconate-epsilon-semialdehyde (ACMS), which is spontaneously converted into the NMDA receptor agonist, quinolinic acid (QA), an excitotoxic compound that promotes inflammation and neurodegeneration. 2-amino-3-carboxymuconate-6-semialdehyde decarboxylase (ACMSD) shifts the pathway away from QA production by enzymatically converting ACMS into picolinic acid (PA), a compound with neuroprotective effects. We therefore hypothesized that adeno-associated virus (AAV)-mediated overexpression of ACMSD in the context of the AAV-alpha-synuclein (αsyn) PD model, an animal model that leads to dose-dependent degeneration of dopaminergic neurons, would provide neuroprotective effects by shifting the KP towards enhanced production of PA/QA, reducing gliosis, inhibiting neurodegeneration and motor deficits. Unilateral stereotaxic injections were performed in rats with two doses, $1*10^{12}$ vector genomes (vg/ml) (low-dose) and $1*10^{13}$ vg/ml (high-dose) of AAV-αsyn together with either ACMSD or FLEX-GFP to the substantia nigra (SN), n = 10/group. Motor function was assessed with the cylinder and amphetamine-induced rotations test 4- and 8-weeks following vector delivery. 8-weeks following vector delivery, we dissected nigral and striatal tissue from a subset of animals in the low dose cohort (n=10) to quantify KP metabolites using ultra-high performance liquid chromatography (UPLC). Low and high dose treated animals were perfused for histological analyses of αsyn pathology including gliosis and neurodegeneration. Viral transduction was validated by immunohistochemical (IHC) GFP or ACMSD immunoreactivity (IR) in the SN. We performed stereological analyses of the dopaminergic and pan-neuronal markers tyrosine hydroxylase (TH) and HuC/D, respectively, to assess neuronal death. Densitometry and enumeration of Iba1 and GFAP IR cells was conducted, and morphological complexity of glial cells was assessed using a machine learning paradigm to assess gliosis. Our results show that ACMSD overexpression prevents gliosis and ameliorates neuronal loss due to αsyn overexpression with the concomitant restoration of motor function. Metabolite results are pending. These results suggest that ACMSD may serve as a novel therapeutic intervention for PD.

Disclosures: K. Meyers: None. **D.J. Marmion:** None. **I.M. Sandoval:** None. **E. Quansah:** None. **L. Brundin:** None. **F.P. Manfredsson:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.11

Topic: C.03. Parkinson's Disease

Support: NIH Grants 5 R01NS034239-25
NIH Grant R01 AG043540
Michael J Fox Foundation
California Institute of Biomedical Research
NIH Grant 2R01 NS034239
University of Nebraska Foundation Community Funds

Title: Immunomodulatory Potential of Granulocyte-Macrophage Colony-Stimulating Factor (GM-CSF) in Alpha-Synuclein Overexpressing Mice and Parkinson's Disease Patients.

Authors: *K. OLSON[1], Y. LU[1], K. NAMMINGA[1], A. WOODS[2], S. JOSEPH[2], R. MOSLEY[1], H. GENDELMAN[1];
[1]UNMC, Omaha, NE; [2]California Inst. of Biomed. Res., La Jolla, CA

Abstract: Aberrant innate and adaptive immune responses are linked to neuroinflammation and Parkinson's disease (PD) progression. Based on a large volume of previous work from our laboratory and others, we demonstrate that neurodestructive autoimmunity speeds neuronal injury while regulatory T cell (Treg) responses are protective against neural damage. Augmentation of peripheral immune cells to achieve neuroprotection is a novel therapeutic strategy for PD. Granulocyte-macrophage colony-stimulating factor (GM-CSF) has previously been successfully utilized as an immunomodulator in pre-clinical and clinical evaluations. However, its short half-life and limited bioavailability hinders its wide-spread clinical use. Therefore, a long-acting GM-CSF formulation (mPDM608) was created and assessed for its therapeutic potential in a preclinical alpha-synuclein overexpression model. Administration of mPDM608 to mice overexpressing alpha-synuclein within the substantia nigra resulted in increased levels of CD4+CD25+FoxP3+ regulatory T cells (Treg) and CD11b+Ly6G-Ly6C+ myeloid-derived suppressor cells (MDSC) within spleen and peripheral blood. Treatment also significantly increased nigrostriatal dopaminergic neuron survival and decreased reactive microglia. Therapeutic profiles were comparable to treatment with recombinant GM-CSF. Additionally, Phase 1 clinical evaluation of recombinant GM-CSF was assessed in PD patients and proved beneficial during a 30-month trial period. GM-CSF treatment resulted in improved Unified Parkinson's Disease Rating Scale (UPDRS) Part III scores that were coordinate with elevated Treg frequencies and stable immunosuppressive capacity. Together, these findings suggest that both formulations have the potential to provide clinical benefit for PD patients.

Disclosures: **K. Olson:** A. Employment/Salary (full or part-time):; University of Nebraska Medical Center. **Y. Lu:** A. Employment/Salary (full or part-time):; University of Nebraska Medical Center. **K. Namminga:** A. Employment/Salary (full or part-time):; University of Nebraska Medical Center. **A. Woods:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Calibr. **S. Joseph:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Calibr. **R. Mosley:** A. Employment/Salary (full or part-time):; University of Nebraska Medical Center. **H. Gendelman:** A. Employment/Salary (full or part-time):; University of Nebraska Medical Center.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.12

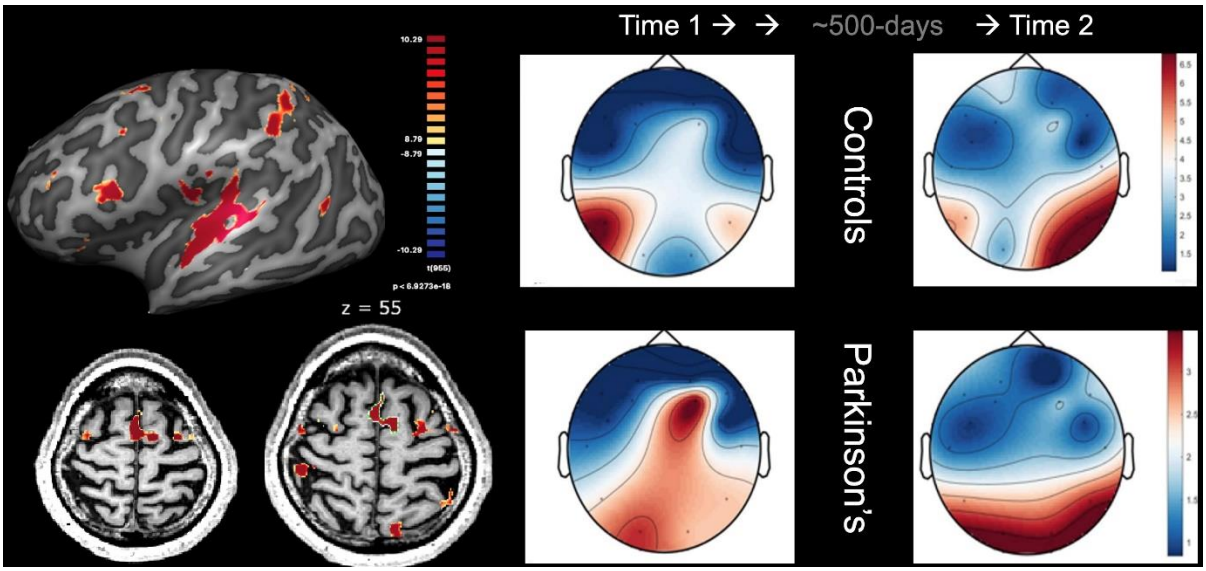
Topic: C.03. Parkinson's Disease

Support: Parkinson Society Canada
NSERC Discovery

Title: Cortical Modulation Resulting from Long-term Dance Training in Parkinson's disease: Evidence from fMRI and resting state EEG

Authors: J. R. SIMON[1], J. BEK[7], S. HOUSHMAND[2], K. A. BEARSS[1], R. E. BARNSTAPLE[3], R. COHAN[4], G. R. LEVKOV[5], P. M. DI NOTA[8], S. HOUSHANGI-TABRIZI[1], K. GHANAI[1], R. J. BAR[9], *J. F. X. DESOUZA[6];
[1]Psych, Neurosci. Grad. Diploma Program, [3]Dance, [4]Psych, [2]York Univ., Toronto, ON, Canada; [5]Biol., York Univ., North York, ON, Canada; [6]Psych, Neurosci. Grad. Diploma Program, Interdisciplinary Grad. Studies, York Univ., Toronto, ON, Canada; [7]Univ. of Toronto, Toronto, ON, Canada; [8]Psychology, Univ. of Toronto, Mississauga, Mississauga, ON, Canada; [9]Canada's Natl. Ballet Sch., Toronto, ON, Canada

Abstract: Dance is associated with positive outcomes in people with Parkinson's disease (PD), but little is known about the long-term effects and neural mechanisms of dance interventions for PD. Our longitudinal investigation utilises fMRI and resting-state EEG alongside behavioural data to examine the potential neuroplastic effects of dance. Results so far showed that regular dance participation may delay symptom progression (Bearss & DeSouza 2021). A single-case fMRI study examined neural plasticity modulations in a male with PD learning dance over 7 months of weekly classes. At 4 timepoints throughout training, fMRI was performed while the participant listened to music and imagined the dance movements from the classes for 1-minute blocks interspersed with 30s fixation blocks. A multi-study fixed effects (FFX GLM) analysis was conducted, comparing the activity of the imagery and rest blocks. A cluster threshold of 22 voxels was applied to the resulting statistical parametric map with Bonferroni correction of $p<.001$. Significantly activated clusters from this threshold were extracted as ROIs and average BOLD signals from the four timepoints were computed for each. Results showed high activation during the dance imagery in September in the supplementary motor area (SMA), right and left superior temporal gyrus (STG), and right and left insula (Fig; left). For SMA, right and left STG, and left insula, activation decreased in December (11 weeks), increased by January (18 weeks), and then decreased in April (29 weeks). The participant also was scanned multiple times over 3-years using 6-minute rsEEG before and after the dance class. Averaged alpha power shows changes across 16 dance participants with PD (bottom row) and 7 healthy control dance participants (top row). The people with PD showed a similar pattern to controls after dance training (right column) over an average period of 500 days (n=16). These multimodal neuroimaging findings provide new evidence on the potential neuroplastic effects of dance as a therapeutic activity for people living with PD using standard care.



Disclosures: **J.R. Simon:** None. **J. Bek:** None. **S. Houshmand:** None. **K.A. Bearss:** None. **R.E. Barnstaple:** None. **R. Cohan:** None. **G.R. Levkov:** None. **P.M. Di Nota:** None. **S. Houshangi-Tabrizi:** None. **K. Ghanai:** None. **R.J. Bar:** None. **J.F.X. DeSouza:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.13

Topic: C.03. Parkinson's Disease

Support: Cambridge Africa Alborada Research Fund
Company of Biologists travel grant

Title: Co-administration of Quercetin with Curcumin mitigate Neurodegeneration in Drosophila GSK 3 overexpression in Parkinson's disease

Authors: *J. A. OLANREWAJU, Esq.[1], S. RUSSELL[2];
[1]JOHN OLANREWAJU, babcock university, Ilishan Remo, Nigeria; [2]Dept. of Genet., Univ. of Cambridge, Cambridge, United Kingdom

Abstract: Aim: We explored the mechanistic interactions and potential therapeutic benefits of curcumin and quercetin co-administration with a specific focus on Glycogen synthase kinase 3 GSK-3 activity.Methods: We hypothesize that excess GSK-3 accumulation in the substantia nigra is driven by oxidative stress and aim to test the effects of these compounds on the localization and activity of GSK-3 in the well-established model organism Drosophila

melanogaster. We probed the dopaminergic neutrons characterization via Tyrosine Hydroxylase Confocal microscopy.Results: The antioxidant properties of curcumin with quercetin mediated an anti-inflammatory response, ameliorating oxidative stress in the brain. The co-administration of both compounds as well rescue the Dopaminergic neurons in the brain of the shaggy fly.Conclusion: The co-administration of curcumin and quercetin were able to reduce the loss of function of the nervous system typical of GSK 3 shaggy strain, by reducing the over expression of GSK 3 beta.Authors: John Olanrewaju, Department of Anatomy, Babcock University, Nigeria. Steve Russell, Department of Genetics, University of Cambridge, UK.There is no conflict of interest for any of the authors.Support: Cambridge Africa Alborada Fund.

Disclosures: J.A. Olanrewaju: None. **S. Russell:** None.

Poster

032. Cellular Studies of Dopamine Neurons

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Program #/Poster #: 032.14

Topic: C.03. Parkinson's Disease

Support: NIH F31NS118811
NIH R21AA028800
NIH R21AG068607
NIH R21DA052419

Title: Dynamic expression of Drosophila tyrosine hydroxylase and vesicular glutamate transporter in response to aging and paraquat exposure

Authors: *S. A. RUBIN[1], S. A. BUCK[1], R. R. MUSUKU[1], D. SHAH[1], Z. FREYBERG[2]; [1]Dept. of Psychiatry, [2]Dept. of Psychiatry and Cell Biol., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Parkinson's disease (PD) is characterized by neurodegeneration of dopamine (DA) neurons, particularly in the substantia nigra *pars compacta*. However, not all DA neurons are equally impacted since neighboring DA neurons within the ventral tegmental area are relatively protected from PD-induced degeneration. Yet, the mechanisms behind this selective vulnerability remain unknown. One hypothesis is that expression of the vesicular glutamate transporter 2 (VGLUT2) in DA neurons, which enables DA/glutamate co-release, also confers neuroprotection to these cells. Indeed, conditional VGLUT2 knockout in mouse DA neurons increases vulnerability in PD models. To further characterize the connection between VGLUT2 expression and DA neuron vulnerability, we used the genetically tractable *Drosophila* model to create luciferase reporters of: 1) tyrosine hydroxylase (TH), which is the rate-limiting enzyme of DA synthesis and is decreased in PD, and 2) *Drosophila* VGLUT (dVGLUT), the fly ortholog of VGLUT2. By combining GAL4-UAS and lexA-lexAop expression systems, we created intersectional genetic luciferase reporters of TH and dVGLUT expression specifically in adult TH^+/$dVGLUT^+$ neurons (i.e., dVGLUT-expressing DA neurons). We then performed luciferase

assays to measure TH and dVGLUT expression in DA/glutamate neurons in response to different cell insults including aging and exposure to paraquat, a pesticide PD model. In parallel, we compared our findings to a luciferase reporter of TH expression in all DA neurons (i.e., global TH reporter). Surprisingly, we found that TH expression increased in *Drosophila* with age, both in the intersectional TH reporter ($P<0.001$) and in the global TH reporter ($P<0.05$). This increase was also observed in the intersectional dVGLUT reporter ($P<0.01$). In response to 10mM paraquat, we observed higher relative TH expression in the intersectional reporter compared to the global TH reporter, particularly at 1 day post-exposure ($P<0.01$), but not at 3 or 5 days, suggesting that DA/glutamate neurons can acutely upregulate TH as part of a neuroprotective response. In conclusion, TH and dVGLUT expression are differentially expressed with age and paraquat, suggesting dynamic responses in DA neurons to these different stressors.

Disclosures: S.A. Rubin: None. **S.A. Buck:** None. **R.R. Musuku:** None. **D. Shah:** None. **Z. Freyberg:** None.

Poster

032. Cellular Studies of Dopamine Neurons

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.15

Topic: C.03. Parkinson's Disease

Support: NIH-R15 (1R15NS121784)
NIH-5P20GM103653
NIH-P20GM103446

Title: Senolytics treatment reduces senescent astrocytes and protects nigrostriatal neurons from alpha-synuclein preformed fibrils-induced damage in mice

Authors: *A. GHOSH[1], K. OFORI[1], D. VERMA[1], G. CABRERA[2], D. WHEELER[1], Y. KIM[1]; [1]Delaware State Univ. (Neuroscience), Dover, DE; [2]Delaware State Univ. (Biology), Dover, DE

Abstract: Parkinson's disease (PD) is well associated with protein aggregation and misfolding of a-synuclein, which results in progressive dopaminergic neuronal death. Although microglia and astrocytes are known to mediate neuronal death when the toxic milieu is not resolved within a reasonable timeframe, the impact of cellular senescence in damaged glial cells in PD pathology remains to be elucidated. Recently, the use of senolytics to eliminate toxic senescent cells in brains has revealed the potential application of senolytics for preventing neurodegenerative diseases. However, the clinical translation for senolytics application still has a major challenge due to the extent of non-specific cell death induction and off-target effects. This study aims to identify an optimal time window when senolytics application can be efficacious in rescuing damaged dopaminergic neurons in PFF-injected brains. Using 12 months old C57Bl/6 male and female mice (n=7-8/group), pre-formed fibrils (PFF) of mouse alpha-synuclein were stereotaxically injected into the dorsal striatum bilaterally. Then, the PFF-injected mice were

gavaged with a potent senolytics (ABT-263) with 50 mg/kg for 7 consecutive days at 1.5, 2.5- and 3.5-months post PFF-injection or only vehicle treatments: positive control (no PFF) and negative control (PFF only). At the end of 5 months post-PFF injection, senolytics-treated groups, especially 2.5 months post-PFF injection showed significant improvement from motor deficits in various behavioral analyses, such as rotarod, nesting, hindlimb clasping, grooming, and pole test. In the following immunohistochemical analyses, our results demonstrated that oral senolytic treatment prevented or rescued dopaminergic neuronal loss in the SNc and enhanced the intensity of TH+ staining in the striatum in a blinded analysis. Further staining for reactive astrocytes or senescent astrocytes showed a substantial decrease in the number of non-proliferative p21+ senescent astrocytes when the senolytic treatment started at 2.5 months post PFF injection. In conclusion, our ABT-263 treated *in vivo* results suggest that a senolytic induced the cell death of senescent astrocytes prematurely, which mitigates the 2nd damage by toxic glial cells for neuronal loss in PD pathology.

Disclosures: **A. ghosh:** None. **K. Ofori:** None. **D. Verma:** A. Employment/Salary (full or part-time):; Post doctoral fellow, Delaware State University. **G. Cabrera:** None. **D. Wheeler:** None. **Y. Kim:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Principal Investigator.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.16

Topic: C.03. Parkinson's Disease

Support: Barrow Neurological Foundation

Title: Longitudinal Assessment of Neurofilament Light Chain in the AAV-a-syn Model of Parkinson's Disease

Authors: *A. GRALEN[1], R. P. BOWSER[1], J. AN[1], I. M. SANDOVAL[2], K. MEYERS[3], F. P. MANFREDSSON[2];
[2]Neurobio., [1]Barrow Neurolog. Inst., Phoenix, AZ; [3]Res. Grants - Neurobio., Col. of Med., Phoenix, AZ

Abstract: Title: LONGITUDINAL ASSESSMENT OF NEUROFILAMENT LIGHT CHAIN IN THE AAV-α-SYN MODEL OF PARKINSON'S DISEASE
Addison Gralen*[1], Robert Bowser, PhD[1], Jiyan An[1], Ivette M. Sandoval[1], PhD[1], Kimberly Meyers, PhD[1], and Fredric P. Manfredsson, PhD[1]. [1]Department of Neurobiology, Barrow Neurological Institute, Phoenix, AZ, United States, 85013
Neurofilament light chain (NfL) is a cytoplasmic scaffolding protein that has been found to be increased in the cerebrospinal fluid (CSF) blood of human neurodegenerative disease and in

animal models. Increased NfL levels have been highly correlated to both disease onset, progression, and severity of disease and have also been detected prior to the onset of motor symptoms in murine models. Parkinson's Disease (PD) is a frequently occurring progressive neurodegenerative disease characterized primarily by the death of dopaminergic neurons on the substantia nigra (SN). Early detection and intervention are important in the treatment outcome of PD. There is no current standard for diagnosis prior to the development of motor symptoms. Further study in this area could give rise to potential avenues for disease monitoring and progression in murine models, therefore in this project we are aiming to measure and characterize plasma and CSF NfL content and how levels correlate with neuron death in the AAV-aSyn model of PD. Plasma was collected prior to vector delivery and monthly thereafter. Animals received $1x10^{12}$ vector genomes (vg/mL) of AAV-aSYn, or FLEX-GFP as control, after which we assessed motor performance monthly using amphetamine-induced rotations to provide a behavioral correlate of circulating Nfl levels. AAV-aSyn treated animals displayed ipsiversive turning in the amphetamine-induced rotations test which was absent in GFP control animals. 12 weeks following vector delivery, CSF was collected and animals were sacrificed and brains were processed for histology. Quantitative immunohistochemistry was used to assess the integrity of the nigrostriatal tract as well as indices of inflammation. AAV-αsyn overexpression resulted in a 30% loss of both tyrosine-hydroxylase (TH)-positive cells in the SN and TH-positive terminals in the striatum as compared to control. Quantification of NfL levels by Meso Scale Discovery assay is pending. We anticipate that NfL levels in plasma and CSF will mirror behavioral and histological measures, thus providing a longitudinal means of assessing neurodegeneration in this model.

Disclosures: **A. Gralen:** None. **R.P. Bowser:** None. **J. An:** None. **I.M. Sandoval:** None. **K. Meyers:** None. **F.P. Manfredsson:** None.

Poster

032. Cellular Studies of Dopamine Neurons

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Program #/Poster #: 032.17

Topic: C.03. Parkinson's Disease

Support: R21-DA047455
KIBM grant 2021-1751

Title: Gaba-to-dopamine switching in the substantia nigra affecting behavior

Authors: *D. DULCIS, J. LAI, A. PORCU, B. ROMOLI, S. POWELL;
Psychiatry, Univ. Of California San Diego Neurosciences Grad. Program, La Jolla, CA

Abstract: Previous work revealed an inverse correlation between smoking and Parkinson's disease (PD) that is associated with nicotine-induced neuroprotection of dopaminergic (DA) neurons against nigrostriatal damage in PD primates and rodent models. Nicotine, a neuroactive

component of tobacco, can directly alter the activity of midbrain DA neurons and induce non-DA neurons in the substantia nigra (SN) to acquire a DA phenotype. We investigated the recruitment mechanism of nigrostriatal GABAergic neurons to express DA phenotypes, such as transcription factor Nurr1 and DA-synthesizing enzyme tyrosine hydroxylase (TH), and the concomitant effects on motor function. Wild-type and α-syn-overexpressing (PD) mice treated with chronic nicotine were assessed by behavioral pattern monitor (BPM) and immunohistochemistry/*in-situ* hybridization to measure behavior and the translational/transcriptional regulation of neurotransmitter phenotype following selective Nurr1 overexpression or DREADD-mediated chemogenetic activation. Nicotine treatment led to a transcriptional TH and translational Nurr1 upregulation within a pool of SN GABAergic neurons in wild-type animals. In PD mice, nicotine increased Nurr1 expression, reduced the number of alfa-syn-expressing neurons, and simultaneously rescued motor deficits. Hyperactivation of GABA neurons alone was sufficient to elicit *de novo* translational upregulation of Nurr1 in non-DA neurons. In wild type mice, we discovered that concomitant overexpression of Nurr1 and DREADD-mediated activation of SN GABA neurons is sufficient to elicit neurotransmitter plasticity and acquisition of the TH phenotype within the activated neuronal pool. Retrograde labeling revealed that a fraction of these GABAergic neurons projects to the dorsal striatum, confirming that these neurons have the anatomical connectivity to potentially release the newly acquired dopamine in the striatum. Nicotine exposure initiates neuroprotective mechanisms counteracting the neurodegenerative effects of α-syn accumulation in DA neurons and contributing to Nurr1-mediated therapeutic effects. Revealing the mechanism of nicotine-induced DA plasticity protecting SN neurons against nigrostriatal damage could contribute to developing new strategies for neurotransmitter replacement in PD.

Disclosures: D. Dulcis: None. **J. Lai:** None. **A. Porcu:** None. **B. Romoli:** None. **S. Powell:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.18

Topic: C.03. Parkinson’s Disease

Title: Continuous, high-intensity exercise is more effective than spaced exercise in protecting against 6-OHDA toxicity.

Authors: *M. HANNA[1], P. ALFORD[2], W. NICHOLSON[2];
[1]Vanguard Univ., [2]Biol. Sci., Vanguard Univ., Costa Mesa, CA

Abstract: Parkinson's disease (PD) is a neurodegenerative movement disorder characterized by the degeneration of the dopaminergic neurons of the nigrostriatal pathway. Patients with PD typically experience motor symptoms, including tremors, rigidity, slowness of movement, and postural instability. Lewy Body pathology from misfolded α-synuclein aggregates is another

major hallmark of the disease. In animal models of PD, increased oxidative stress, reactive oxygen species, inflammatory cytokines, and microglial activation have been reported to play a significant role in mediating PD pathology. In this study, we tested if five weeks of high-intensity, spaced treadwheel exercise consisting of three 20 minute intervals separated by 30 minutes of rest would have a neuroprotective effect against the dopaminergic neurotoxin 6-hydroxydopamine in an animal model of Parkinson's disease. We also examined the differential effects of spaced exercise compared to 60 minutes of continuous exercise in 6-OHDA injected rats. After exercising for five weeks, rats were injected with 6-OHDA. Behavioral tests were assessed within one week by examining apomorphine-induced rotation, methamphetamine-induced rotation, and the paw reach test. Apomorphine and methamphetamine-induced rotation are used to assess the extent of impairment caused by 6-OHDA and directly correlate to the degree of dopaminergic loss. Results showed that rats in the continuous, high-intensity exercise group showed reduced apomorphine-induced contralateral rotation and methamphetamine-induced ipsilateral rotation compared to the spaced exercised group and the non-exercised 6-OHDA group. The data suggest that high-intensity, continuous exercise can act as a neuroprotectant factor by reducing the extent of dopaminergic neurodegeneration.

Disclosures: **M. Hanna:** None. **P. Alford:** None. **W. Nicholson:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.19

Topic: C.03. Parkinson's Disease

Support: NRF Grant 2021R1F1A1063591
NRF Grant 2022R1C1C1006166
HRF Grant HRF-202103-009

Title: The couple of netrin-1/α-synuclein regulates the survival of dopaminergic neurons via α-synuclein disaggregation

Authors: ***Y. LEE**[1], S. JANG[1], E. KANG[1], H. JEON[1], Y. JEONG[1], Y. KIM[1], D. HONG[1], S. LEE[1], X. LIU[2], S. KANG[2], E. AHN[1];
[1]Hallym Univ., Hallym Univ., Chuncheon-si, Korea, Republic of; [2]Dept. of Pathology and Lab. Med., Emory Univ. Sch. of Med., Atlanta, GA

Abstract: α-Synuclein has been proposed to play a key pathogen in Parkinson's disease (PD). Especially, the abnormal accumulation and aggregation of the misfolded α-Synuclein protein is the neuropathological hallmark of all α-Synucleinopathies, including Parkinson's disease. Netrins (netrin-1, netrin-3, and netrin-4) are secreted proteins, which are laminin-related and involved in axon guidance function and cell survival molecular pathway. Remarkably, only netrin-1 is highly expressed in healthy adult substantia nigra brain region and inversely correlates with the

expression of α-Synuclein, which led us to investigate the impact of the molecular interaction between α-Synuclein and netrin-1 in dopaminergic neuron fate. Triggering nigra dopaminergic neuronal death associated with netrin-1 reduction by DCC-4Fbn (peptide) treatment in the cell media. After that, we performed the TUNEL analysis, immunoblotting assay, qRT-PCR, cell toxicity test, intracellular ROS level analysis, and co-immunostaining. Moreover, direct molecular interaction between netrin-1 and α-Synuclein was examined by Th-T test, X-ray diffraction (PFFs number counting), Co-IP, and real time binding assay *in-vitro*. SNCA Tg and netrin-$1^{fl/fl}$ + Cre virus injected animal models were designed to verify the dopaminergic neuronal loss with escalation of hyperphosphorylated α-Synuclein S129, α-Synuclein aggregation, and motor defect with netrin-1 expression. Here we show that netrin-1 and α-Synuclein directly interact in pre-formed fibrils (PFFs) generation test, real time binding assay, and Co-IP with neurotoxin treated cell lysates. Of interest, we observed that the level of netrin-1 was significantly decreased upon human SNCA overexpressing transgenic mice brain (SN) region, which matched the analysis result from human PD patients database. In addition, we confirmed the mass dopaminergic neuronal death and motor dysfunctions in the netrin-$1^{fl/fl}$ + Cre virus injected animal models (Netrin-1 conditional KO). Our finding suggest that netrin-1 deprivation triggers dopaminergic neuronal cell death with α-Synuclein aggregation during aging process and netrin-1 can be the promising therapeutic or diagnostic molecule in α-Synucleinopathies.

Disclosures: **Y. Lee:** None. **S. Jang:** None. **E. Kang:** None. **H. Jeon:** None. **Y. Jeong:** None. **Y. Kim:** None. **D. Hong:** None. **S. Lee:** None. **X. Liu:** None. **S. Kang:** None. **E. Ahn:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.20

Topic: C.03. Parkinson's Disease

Support: NIH R25 grant, #5R25GM107754-08
NSF CREST grant, #HRD-2112556

Title: Investigating how the dopamine neural circuit activation response to the blue-light sensing circuit

Authors: *N. GAYLUAK[1], B. NELMS[2];
[2]Biol., [1]Fisk Univ., Nashville, TN

Abstract: Dopamine is a neurotransmitter important for motor control and cognitive function1 and is associated with feelings of pleasure, motivation, and sense of reward. Several diseases are associated with misregulation of dopamine levels, such as Parkinson's disease (PD), schizophrenia, and attention deficit hyperactivity disorder (ADHD). However, multiple neural circuits are likely to intersect and integrate with the dopaminergic pathway and may be able to

impact downstream signaling. The goal of this research project is to better understand the intersection of the dopaminergic circuit with a blue-light sensing circuit in the model organism Caenorhabditis elegans (C. elegans). In C. elegans, an accumulation of dopamine leads to the inhibition of motor activity and therefore paralysis. In a swimming-induced paralysis assay (SWIP), worms lacking the gene encoding the dopamine transporter (dat-1 mutants) paralyze rapidly due to accumulation of extra-synaptic dopamine. However, we have discovered that when paralyzed worms are exposed to blue light they are reanimated, suggesting that the blue light signaling pathway overrides the inhibition of motor neurons by extra-synaptic dopamine. Previous work by others has shown that worms possess neural circuitry for avoiding blue light. We postulate that the neural circuit activation specifically for evading blue light can override paralysis caused by extra-synaptic dopamine. In addition we will look at worms that lack a key gene (lite-1) for blue light avoidance. We are also testing if other wavelengths, such as green light, can have the same effect. Additionally, reanimation could be caused when the solution is simply warmed, therefore research will be done to eliminate the possibility that the response could be related to thermotaxis in response to the light heating the solution. Finally, we are examining if there is any correlation between how long it takes for C. elegans to paralyze and how long C. elegans takes to reanimate once exposed to blue light. The findings from this research may contribute further understanding of diseases that are associated with dysregulation of dopamine levels by examining the various neural circuits that intersect and integrate with the dopaminergic pathway.

Disclosures: **N. Gayluak:** None. **B. Nelms:** None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.21

Topic: C.03. Parkinson's Disease

Support: NIH R25 grant #5R25GM107754-08
CREST grant #HRD-2112556

Title: Neuroprotective Effects of ubh-1 and hsp-12.6 in DA neurons in C. elegans

Authors: *S. SHEHREEN;
Biol., Fisk Univ., Nashville, TN

Abstract: Parkinson's disease is a debilitating neurodegenerative disease affecting 1% of the population above 60. Understanding the specific molecules and mechanisms that protect dopaminergic neurons from degeneration and other hallmarks of the disease such as Lewy body formation, amyloid fibrils, and increases in neuromelanin in the substantia nigra, will inform better therapeutic approaches for treatment of Parkinson's patients. We are examining two specific genes that have homologs in the model organism *C. elegans*. Mutations in the *uch-l1*

gene lead to an autosomal dominant form of Parkinson's. UCH-L1 is a deubiquitinase and monoubiquitin stabilizer in the neuron. HSPB1 is a small heat shock protein found in the neuromelanin granules in the substantia nigra in Parkinson's patients and can prevent the formation of amyloid fibrils due to oxidized dopamine *in vitro*. Homologs of these genes, *ubh-1* and *hsp-12.*6 respectively, are upregulated in the dopaminergic neurons in *C. elegans*. Without the presence of UBH-1 and/or HSP-12.6, we expect accelerated neurodegeneration of the dopaminergic neurons because of increased oxidative stress. We will be using fluorescent microscopy to visualize dopaminergic neuronal integrity and oxidative stress over the lifespan of the worm, as well as lifespans assay to observe any differences in lifespan between wildtype and *ubh-1* and *hsp-12.6* mutants.

Disclosures: S. Shehreen: None.

Poster

032. Cellular Studies of Dopamine Neurons

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 032.22

Topic: C.03. Parkinson's Disease

Support: NIH R25 grant #5R25GM107754-0
NSF CREST grant #HRD-2112556

Title: Investigating the effects of comt-2 loss in c. elegans

Authors: *S. ASHSHAREEF[1], B. NELMS[2];
[2]Life and Physical Sci., [1]Fisk Univ., Nashville, TN

Abstract: Dopamine (DA) is a crucial neurotransmitter regulating nervous system function including cognition and motor control. Dysregulation of DA signaling is associated with diseases like Parkinson's and schizophrenia. DA levels can be regulated at multiple steps in the pathway such as biosynthesis, packaging, release, reuptake, and breakdown(metabolism). These DA disruptions are difficult to study in a gene-by-gene approach in humans, so we are utilizing a model organism, *C. elegans*, for experimental analysis. The aim of this project is to investigate one of the main enzymes that metabolize DA, which is Catechol-O-methyl Transferase (COMT). There are 5 homologs in C. elegans (COMT-1, COMT-2. COMT-3, COMT-4, COMT-5). This project aims to understand the impact of the loss of COMT-2 on DA metabolism. To determine this, we are using various behavioral assays that are influenced by DA levels such as Swimming Induced Paralysis, Basal Slowing, and Brood Size Assays. Due to a high degree of conservation between human and *C. elegans* DA signaling pathways, our results may give important insight into regulation of DA levels, DA metabolism, and its corresponding therapeutic implications.

Disclosures: S. AshShareef: None. **B. Nelms:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.01

Topic: B.04. Synaptic Transmission

Support: National Institute of Neurological Disorders and Stroke (NINDS) intramural fund

Title: Modular structures in the postsynaptic density imaged by Cryo-EM tomography

Authors: *J. JUNG, A. DOSEMECI, X. CHEN, T. S. REESE;
NINDS, Lab. of Neurobio., NIH, Bethesda, MD

Abstract: The postsynaptic density (PSD) is a dense protein complex present at the postsynaptic membrane in an excitatory synapse. Biochemical studies revealed various proteins constituting the PSD and their involvement in regulating synaptic functions. Electron microscopy studies have shown that the PSD forms an electron-dense disc-like structure at the postsynaptic membrane opposing the presynaptic active zone in the mammalian brain. Moreover, in recent advanced light microscopy studies, the PSD was found to contain dynamically movable clusters of receptors and scaffolding proteins aligned with presynaptic active zone proteins. Here we employed Cryo-EM tomography to visualize structural details of isolated PSDs quickly frozen without any heavy metal staining at a few nanometer resolution in 3D that may reveal any dynamic elements in the PSD consistent with the moving clusters imaged by light microscopy. We found that isolated PSDs were generally round and flat, while exhibiting irregular, lumpy structures intricately connected to each other. PSDs often had gaps or holes through the complex scaffolding structure. Such structural features were observed regardless of sonication designed to loosen or break up weakly bound structural components or modules in the PSD. To delineate the PSD and its modular structures efficiently, we used an automatic segmentation approach developed in our laboratory. This approach helped expedite PSD segmentation and obtain, without time-consuming manual segmentation, its individual modules that likely correspond to discrete protein complexes. Most modules were observed randomly located and connected to each other. Also, we found some modules large enough to span the entire thickness of the PSD, probably containing many PSD proteins linked to each other that reside on the cytoplasmic or extracellular surface of the PSD. Our findings demonstrate that modular structures exist in the isolated PSD with or without sonication. This suggests that the modular structures, at least in part, may be a different structural representation of dynamic clusters containing key PSD proteins and complexes.

Disclosures: J. Jung: None. **A. Dosemeci:** None. **X. Chen:** None. **T.S. Reese:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.02

Topic: B.04. Synaptic Transmission

Support: NIH Grant DA022727
NIH Grant NS106906
NIH Grant NS111976
NIH Grant NS110385

Title: Tau STED super resolution microscopy enables the study of the nanoscale organization of glutamate receptors in living cells and brain tissue

Authors: *R. E. CAIN, M. B. DALVA;
Thomas Jefferson Univ., Philadelphia, PA

Abstract: Synapses are the site of neuronal communication and are essential for brain function, yet studying how synapses are organized has been challenging due to their small size. A variety of super-resolution imaging techniques, including Stimulated Emission Depletion (STED) microscopy, have been used to break this barrier. Super-resolution imaging reveals that pre- and postsynaptic proteins including PSD95, and amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) and N-methyl-D-aspartate (NMDA) glutamate receptors, form discrete nanoclusters whose number scales linearly with spine size. Live-cell STED imaging of over-expressed and fluorescently tagged pre and postsynaptic proteins reveals that these proteins move dynamically after induction of structural plasticity. However, phototoxicity from live-cell STED imaging and the need to express exogenously tagged synaptic proteins provide challenges to this approach. Similarly, studying synaptic proteins in brain tissue has been limited by the resolution that can be achieved in dense tissue. To overcome these problems we are developing two new approaches, Tau (τ) STED imaging and CRISPR knock in of fluorescence tags into endogenous glutamate receptors. τSTED is a recent development that collects the fluorescent lifetimes of individual photons during scanning and uses this information to identify super-resolved photons. τSTED can be used to achieve super resolution with up to a third of the STED depletion laser power, minimizing photobleaching, phototoxicity, and background fluorescence. We describe approaches to use a Leica SP8 Tau STED system to image glutamate receptors in tissue and living cultured neurons at high spatial and temporal resolution. Recently an Open Resource for the Application of Neuronal Genome Editing (ORANGE) has been developed based on Homology independent targeted insertion technology to tag endogenous proteins with fluorescent tags. We describe endogenously tagging AMPARs and NMDARs, which can be imaged with live-cell τSTED at a high temporal resolution to visualize the dynamics of glutamate receptors in living neurons on the nanoscale. Studying the nano-organization of synaptic proteins within brain tissue has been challenging due to the lower resolution that can be achieved in dense tissue. Here, using τSTED imaging in brain sections, we show the capability to study the nanoscale organization of endogenous glutamate receptors in spines in brain tissue. Super resolution imaging has pushed our understanding of synaptic structure, and τSTED can push our understanding even further, through improving resolution in live-cell and tissue nanoscale imaging.

Disclosures: **R.E. Cain:** None. **M.B. Dalva:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.03

Topic: B.04. Synaptic Transmission

Support: HHMI Gilliam Fellowship
R35NS111562

Title: Shank3 Phosphorylation Bidirectionally Regulates Synaptic Scaling

Authors: *A. GUERRERO, C.-H. WU, V. TATAVARTY, G. TURRIGIANO;
Brandeis Univ., Waltham, MA

Abstract: Shank3 is a postsynaptic scaffold protein important for the formation of dendritic spines and synapses and its mutations have been associated with neurodevelopmental disorders including intellectual disability and autism spectrum disorders (ASD). Shank3 is necessary for the expression of synaptic scaling, a form of homeostatic plasticity that bidirectionally modulates post-synaptic strength in response to activity perturbations, stabilizing neuronal activity. The molecular mechanisms by which Shank3 regulates synaptic scaling are not yet known. In cultured cortical neurons, our lab recently identified two sites on Shank3 that show a persistent hypo-phosphorylation during activity blockade and transient hyper-phosphorylation in response to an increase in activity. Using electrophysiology and immunocytochemistry, we demonstrated that introducing Shank3 phosphorylation (phosphomimetic mutant) blocks scaling up while preventing Shank3 phosphorylation (phosphodeficient mutant) abolished scaling down. Our data thus suggest that the phosphorylation state of these two sites seem to act as a bidirectional switch permissive to scale in a particular direction as a response to activity. Here I aim to further investigate (1) the upstream mechanisms that may regulate activity-dependent changes in Shank3 phosphorylation, and (2) the molecular machinery downstream of Shank3 phosphorylation that allows for synaptic scaling. Our results show that protein phosphatase 2A (PP2A) activity maintains hypo-phosphorylation during scaling up, while multiple activity-dependent kinases are required for the hyper-phosphorylation during scaling down. In terms of pathways downstream of Shank3 phosphorylation state, I found that enhancing metabotropic glutamate receptor 5 (mGluR5) by using a positive allosteric modulator rescued scaling up in neurons expressing the Shank3 phosphodeficient mutant. Notably, mGluR5 has been shown to associate with Shank3 through Homer1, a postsynaptic protein also crucial for scaling, and it is likely that mGluR5 pathways could be the candidate mechanism mediated by Shank3 phosphorylation to regulate synaptic scaling. Future work will focus on validating this model *in vivo*.

Disclosures: **A. Guerrero:** None. **C. Wu:** None. **V. Tatavarty:** None. **G. Turrigiano:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.04

Topic: B.04. Synaptic Transmission

Support: NIDA RO1

Title: Quantal Synaptic Events in Single Dendritic Spines

Authors: *H. JAFRI[1], M. B. DALVA[2];
[1]Farber Inst. of Neurosci. Thomas Jefferson Univ., Philadelphia, PA; [2]Thomas Jefferson Univ., Philadelphia, PA

Abstract: Dendritic spines are the primary sites of glutamatergic transmission in the brain and the sites of synaptic and structural plasticity, which are thought to be neural correlates of learning and memory. Synapses contain biomolecules such as PSD95, glutamate receptors, and calcium sensors (synaptotagmins) to facilitate fast and efficient neurotransmission. Super-resolution imaging of spines reveals that the nano-organization of synaptic proteins is modular. Key pre- and postsynaptic proteins, such as Synpatophysin1, VGlut1, Bassoon, and PSD95, form nanopuncta that scale in number, but not size, with respect to spine area. Following chemically-induced Long Term Potentiation (cLTP), potentiated spines both grow in size and increase in the number of nanomodules they contain. This suggests that nanomodules may represent functional components of synaptic signaling domains. Neurotransmission at the spine level is not well studied. Electrophysiology recordings are limited by signal decay over space. Recordings from soma or dendrites may not be identical to the activity in individual spines. Therefore, we developed a technique to visualize mini-synaptic NMDAR dependent-calcium transients (mSCTs), or spontaneous calcium events, in spines allowing us to investigate how neurotransmission occurs at single spine resolution. We combined this with the super resolution technique, Stimulated Emission Depletion (STED) nanoscopy, to determine the functional impact of nanomodular synaptic organization. To visualize mSCTs and relate them to synaptic nanomodules, cortical neurons were transfected with FingR PSD95-mTurquoise2 to label endogenous PSD95 in spines, and GCaMP8f to visualize calcium signaling. mSCTs were isolated by inhibiting neuronal activity, AMPARs, L-type calcium channels, and intracellular calcium stores. Spontaneous calcium events that were initiated in and restricted to the heads of spines were analyzed. Following the blockade of the NMDAR with APV, GCaMP fluorescence was held at baseline levels, indicating that transients were NMDAR-dependent. We found that the amplitudes of mSCTs varied widely within and between spines and form a skewed Gaussian distribution. In addition, the number of mSCTs per minute (frequency) varied widely, up to 20-fold. Consistent with the hypothesis that nanomodules reflect a functional synaptic unit, the cumulative distribution of mSCTs in two PSD nanomodule spines is significantly skewed towards larger amplitude events. Taken together, these findings suggest that there are functional differences between spines which may be correlated to nano-organization.

Disclosures: H. Jafri: None. **M.B. Dalva:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.05

Topic: B.04. Synaptic Transmission

Support: NIH grant NS089578
DOD/USAMRAA grant W81XWH1810388
NIH grant 1F31NS122477

Title: Polarity protein Par3 regulates dendritic spine morphogenesis and cognition in vivo

Authors: *M. M. VOGLEWEDE, E. N. OZSEN, M. SUN, H. ZHANG;
Robert Wood Johnson Med. School, Rutgers Univ., Piscataway, NJ

Abstract: Dendritic spines are small, highly polarized protrusions on excitatory neurons serving as sites of postsynaptic input. Plasticity of dendritic spines is necessary for learning, while stable dendritic spines are thought to encode long-term memories. The polarized nature of dendritic spines suggests their plasticity and stability may be mediated by polarity proteins. The polarity protein Partitioning defective 3 (Par3) regulates mature dendritic spine formation in vitro, and several single nucleotide polymorphisms (SNPs) and copy number variation (CNV) of *Pard3*, which encodes Par3, are associated with intelligence, schizophrenia, and autism spectrum disorder (ASD). Together, these data implicate Par3 in mature dendritic spine stabilization, which may play a role in cognition and social interaction. However, the mechanisms of Par3 in dendritic spine plasticity and cognition *in vivo* remains completely unknown. We established a novel Par3 mouse model to conditionally knockout Par3 in postnatal forebrain pyramidal neurons. We found that loss of Par3 *in vivo* increases immature dendritic spines and increases overall dendritic spine density in the CA1 region of the hippocampus. In addition, loss of Par3 *in vivo* alters hippocampal-dependent spatial learning, phosphorylation of cytoskeletal regulating proteins, and levels of synaptic proteins. Together, our data suggest that Par3 plays a role in regulation of dendritic spine stability and cognitive functions.

Disclosures: M.M. Voglewede: None. **E.N. Ozsen:** None. **M. Sun:** None. **H. Zhang:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.06

Topic: B.04. Synaptic Transmission

Support: Boehringer Ingelheim Fonds Foundation
NIH RO1NS106031
Vallee Foundation Scholar
McKnight Scholar

Title: Conserved synaptic AMPA-to-NMDA ratio in human and mouse neocortex

Authors: *D. VARDALAKI[1], T. L. PHAM[1], S. S. CASH[2], M. T. HARNETT[1];
[1]MIT, Cambridge, MA; [2]Dept Neurol, Mass Genl Hosp, Boston, MA

Abstract: The integrative properties and plasticity of glutamatergic synapses are controlled by the relative expression of AMPA and NMDA receptors. Studies in rodents show that development, learning, addiction, and stress can change the ratio of these receptors and alter neuronal output. However, far less is known about the expression and function of these receptors in human neurons. Here, we measured AMPA:NMDA at individual spiny synapses in human and mouse cortical neurons using current-clamp electrophysiology and super-resolution immunohistochemistry. We found that AMPA:NMDA is conserved among human and mouse synapses, despite substantial interspecies differences in the morphology of spines, dendrites, and somas. Our findings indicate that neurons in the mammalian cortex actively regulate glutamatergic receptor content of synapses. This process, as well as other conserved physiological operations, may be specifically organized to coordinate with the increased size and complexity of human neuron dendritic trees to give rise to new or enhanced computational functions.

Disclosures: D. Vardalaki: None. **T.L. Pham:** None. **S.S. Cash:** None. **M.T. Harnett:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.07

Topic: B.04. Synaptic Transmission

Title: Unique versus shared functions of MDGAs in regulating synapse properties

Authors: *S. KIM, G. JANG, H. KIM, D. LIM, J. EUN, J. UM, J. KO;
DGIST, DGIST, Daegu, Korea, Republic of

Abstract: MDGAs (MAM domain-containing glycosylphosphatidylinositol anchors) are immunoglobulin superfamily of adhesion molecules composed of six immunoglobulin domains, a fibronectin III repeat, a MAM domain, and a GPI anchor. MDGA1 and MDGA2 were reported as suppressive factors for inhibitory and excitatory synapse development, respectively. However, various genetic manipulation studies have raised fundamental questions about the overall physiological role of MDGAs. Here, we have systematically analyzed the effects of conditional genetic deletions of MDGA paralogs, either individually or both, in cultured mouse hippocampal

cultured neurons. We found that conditional genetic deletions of MDGA1 increased inhibitory synapse density without altering excitatory synapse density, whereas conditional genetic deletion of MDGA2 specifically increased excitatory synapse density. Strikingly, no synaptic alterations were found upon simultaneous deletion of both MDGA1 and MDGA2. These observations were consistently found in cultured neurons deleted sparsely and globally with individual MDGA paralog, suggesting cell-autonomous MDGA loss-of-function effects. These anatomical phenotypes were further corroborated by whole cell patch clamp recordings, revealing the specific effect of each MDGA deletion on miniature postsynaptic currents. Furthermore, MDGA gain-of-function experiments consistently supported specific action of each MDGA paralog on specific synapse types. Lastly, Sholl analysis showed that each MDGA paralog is required for dendritic branching and neuronal development. We are currently doing extensive structure-function analyses using various MDGA variants to elucidate the mechanistic basis underlying MDGA-regulated synapse and neuron development.

Disclosures: S. Kim: None. **G. Jang:** None. **H. Kim:** None. **D. Lim:** None. **J. Eun:** None. **J. Um:** None. **J. Ko:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.08

Topic: B.04. Synaptic Transmission

Support: K99MH124920
R01NS036715
RF1MH120119

Title: Btbd11 supports cell-type-specific synaptic function

Authors: *A. BYGRAVE[1,2], A. SENGUPTA[3], E. JACKERT[2], M. AHMED[2], B. ADENUGA[2], H. L. GOLDSCHMIDT[2], R. C. JOHNSON[2], H. ZHONG[4], F. L. YEH[5], M. SHENG[6], R. L. HUGANIR[7];
[1]Tufts Univ., Boston, MA; [2]Johns Hopkins Univ., Baltimore, MD; [3]NIDA, NIH, Baltimore, MD; [4]Vollum Institute, OHSU, Portland, OR; [5]Genentech, South San Francisco, CA; [6]Stanley Ctr. for Psychiatric Res., Cambridge, MA; [7]Dept. Neurosci., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Glutamatergic synapses exhibit cell-type-specific differences in basal synaptic transmission and plasticity. We assessed cell-type-specific specializations in the composition of glutamatergic synapses in mouse forebrain. We identified Btbd11 as an inhibitory interneuron-specific and synapse-enriched protein in hippocampal and cortical neurons. Presently, the function of Btbd11 remains completely unexplored. With biochemical experiments we found that Btbd11 directly binds with core postsynaptic proteins including the important scaffold protein

Psd-95. Intriguingly, we observed that Btbd11 shows hallmarks of liquid-liquid phase separation (LLPS) when co-expressed with Psd-95, supporting the idea that the glutamatergic post synaptic density on dendritic shafts in interneurons exists, at least in part, in a phase separated state. LLPS is postulated to promote the organization of intracellular space, through formation of membraneless organelles. We evaluated Btbd11 function in inhibitory neurons by generating conditional knockout mice. We observed that knockout of Btbd11 from inhibitory interneurons decreased glutamatergic signaling onto parvalbumin-positive interneurons. Further, both *in vitro* and *in vivo*, we identified that Btbd11 knockout disrupted network activity with Ca^{2+} imaging and electrophysiology, respectively. At the behavioral level, Btbd11 deletion from interneurons increased exploratory behaviors and reduced measures of anxiety. Finally, Btbd11 knockout sensitized mice to pharmacologically induced hyperactivity following NMDA receptor antagonist challenge. In conclusion, our findings revealed a cell-type-specific mechanism that supports glutamatergic synapse function in inhibitory interneurons, regulating circuit function and animal behavior.

Disclosures: **A. Bygrave:** None. **A. Sengupta:** None. **E. Jackert:** None. **M. Ahmed:** None. **B. Adenuga:** None. **H.L. Goldschmidt:** None. **R.C. Johnson:** None. **H. Zhong:** None. **F.L. Yeh:** None. **M. Sheng:** None. **R.L. Huganir:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.09

Topic: B.04. Synaptic Transmission

Support: NIH Grant DA041876
Department of Pharmacology and Toxicology and Indiana University School of Medicine Start-up

Title: Spinophilin cell type-specifically regulates striatal plasticity and striatal-dependent behaviors

Authors: C. W. MORRIS[1], D. S. WATKINS[2], ***A. J. BAUCUM II**[3];
[1]Indiana University-Purdue Univ. Indianapolis, Indianpolis, IN; [3]Pharmacol. and Toxicology, [2]Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Spinophilin is a major synaptic protein phosphatase 1 (PP1) interacting protein that interacts with both metabotropic glutamate receptor 5 (mGluR5) and dopamine D2 receptors (D2Rs). Herein, we leverage our recently generated conditional spinophilin KO mice to delineate the cell type-specific role of spinophilin on mediating striatal behaviors associated with mGluR5 and D2R function. Specifically, we find that loss of spinophilin globally impacts motor learning on a rotarod device as well as locomotor sensitization to the psychostimulant, amphetamine. Moreover, we have found that loss of spinophilin in dopamine D2R-containing indirect pathway

medium spiny neurons (iMSNs), but not dopamine D1R-containing direct pathway medium spiny neurons (dMSNs), attenuates motor learning on a rotarod device. Conversely, loss of spinophilin in neither iMSNs nor dMSNs impacted amphetamine-dependent locomotor sensitization. Finally, we show that loss of spinophilin in either dMSNs or iMSNs impacts a repetitive motor output, grooming, associated with excessive mGluR5 function. Together, these data implicate spinophilin within specific striatal MSNs on changes associated with motor learning and enhanced motor output. Detailing specific mechanisms by which spinophilin impacts these behaviors is important as it will lead to an understanding of biochemical and signaling changes that mediate normal motor learning and excessive motor output associated with striatal dysfunction.

Disclosures: C.W. Morris: None. **D.S. Watkins:** None. **A.J. Baucum II:** None.

Poster

033. Postsynaptic Organization and Structure

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Program #/Poster #: 033.10

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH126017

Title: N-linked Glycosylation Sites Critically Modulate the Synaptic Abundance of Neuroligins

Authors: *T. CAST, O. BENNER, L. S. MINIMIDE, Z. LENNINGER, J. R. BAMBURG, S. CHANDA;
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Abstract: Neuroligins (NLGNs) are a class of synaptic cell-adhesion molecules (CAMs) that play instructive roles in synapse organization. Transcriptional and translational regulations of NLGNs, as well as their binding affinity or specificity for other synaptic proteins have been shown to determine NLGNs' synaptic availability and functional contribution at various neural circuits. However, it remains unclear whether posttranslational modifications (PTMs), especially N-linked glycosylation of NLGNs, can also influence their cellular properties. This is especially critical, as autism-associated mutations in the NLGN family have been shown to disrupt protein maturation and glycosylation. First, we found that different NLGN variants are differentially glycosylated across several brain regions in an isoform-dependent but sex-independent manner. In cortex and hippocampus of adult mice (C57BL/6; >P30; 4 male, 4 female) the ratio of maturely glycosylated NLGN1 is significantly higher than in cerebellum or hindbrain. However, maturation of NLGN2 is identical throughout male and female brain regions, indicating NLGNs may be functionally regulated through glycosylation *in vivo*. Investigating the direct role of N-linked glycosylation, we found that removal of individual N-glycosylation residues though alanine-substitution significantly enhanced NLGNs' retention at endoplasmic reticulum (ER), and considerably reduced their cell-surface transport. Other molecular properties of NLGNs,

including folding stability and neurexin (NRXN) interaction were unaffected. As a consequence of ER retention, glycosylation-deficient NLGNs exhibited severe impairment in dendritic distribution, and limited association with both glutamatergic and GABAergic synapses. Our results suggest that N-linked glycosylation is an essential PTM that facilitates NLGNs' proper trafficking through secretory pathway, and promotes their efficient localization at synapses.

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Poster

033. Postsynaptic Organization and Structure

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Program #/Poster #: 033.11

Topic: B.04. Synaptic Transmission

Support: NSF RUI grant IOS1754986 (MLL) and IOS1755019 (MMF)

Title: Investigating roles of the extracellular matrix in synapse formation

Authors: *H. MCKILLOP[1], S. O'KEEFE[1], L. MACQUARRIE[1], M. M. FRANCIS[2], M. L. LEMONS[1,2];
[1]Biol. and Physical Sci., Assumption Univ., Worcester, MA; [2]Neurobio., Univ. of Massachusetts Med. Sch., Worcester, MA

Abstract: Proper synapse development depends on complex molecular interactions between pre- and postsynaptic scaffolds as well as the extracellular matrix (ECM). While significant progress has been made in understanding roles for neuronal scaffolds at synapses, our understanding of how the ECM contributes to the development and maintenance of synaptic connections remains more limited. Here, we investigate potential roles for ECM interactions in synapse patterning using a powerful genetic model, the nematode *Caenorhabditis elegans*. To date, our studies have primarily focused on the outgrowth of recently characterized *C. elegans* GABAergic dendritic spines. We first asked if the ECM molecule, *cle-1*/collagen XVIII, is present near sites of dendritic spine outgrowth. We found that endogenously GFP-tagged CLE-1/collagen XVIII was localized adjacent to and surrounding dendritic spines. In addition, we found that the number of dendritic spines was significantly decreased in *cle-1(cg120)* mutant animals, suggesting the NC1 region, which is largely deleted in the *cg120* animals, might be important for proper dendritic formation.
To pursue how *cle-1*/collagen XVIII may be influencing dendritic spine development, we chose to test if integrins, a family of receptors that bind to many ECM molecules including collagen, are required for proper spine formation. Integrins are heterodimeric receptors, containing one α and one β subunit, and at least 24 different integrin combinations have been documented in vertebrates. Fortunately, *C. elegans* has only two integrins, and both require the sole β subunit, *pat-3*. *pat-3* null alleles are lethal. Therefore, we employed 3 CRISPR engineered *pat-3*/β

integrin alleles[1] to test if integrin signaling plays a role in dendritic spine formation. Interestingly, the number of dendritic spines was significantly decreased in only one *pat-3*/β integrin mutant allele (*kq8042*) that has a Y>A mutation in a NPxY phosphotyrosine motif. This mutation is predicted to impair *pat-3/*β integrin cytoplasmic tail binding to intracellular partners such as talin. Surprisingly, we found ~20% of dendritic spines in *pat-3(kq8042)* mutants were dorsally directed, while wild type animals have ventrally directed spines. We are currently investigating the basis for this change in directionality, in particular the hypothesis that positioning of presynaptic cholinergic release sites is altered in instances where we observed dorsally directed spines. In summary, our studies suggest the ECM molecule *cle-1*/collagen XVIII and an ECM binding partner, integrins, are required for synapse development.
Citation: Hanna et al., 2020. MicroPubl Biol. 10.17912.

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Poster

033. Postsynaptic Organization and Structure

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Program #/Poster #: 033.12

Topic: B.04. Synaptic Transmission

Support: NIH R01 Brain441406-ZL-29425
HHMI

Title: Subcellular localization of neurotransmitter receptors in the mushroom body cell types

Authors: *A. BHUKEL[1], P. SANFILIPPO[2], A. KIM[2], J. YOO[2], H. BEVIR[2], A. YUEN[2], P. MIRSHAHIDI[2], L. ZIPURSKY[2], Y. ASO[1];
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Abstract: Due: June 17th 2PM EDT
Title: Subcellular localization of neurotransmitter receptors in the mushroom body cell types.
A. Bhukel, P. Sanfilippo, *A. Kim, J. Yoo, H. Bevir, A. Yuen, P. Mirshahidi,* S.L. Zipursky, Y. Aso .
Recent Electron Microscopy (EM) based reconstruction of neuronal circuits produced extensive connectivity maps of the *Drosophila* brain, including dense maps of the mushroom body (MB), the learning and memory center. In the FlyEM's hemibrain connectome data, there are approximately 1.8 million predicted synapses inside a mushroom body. These synapses were annotated primarily by predicting locations of presynaptic active zones. Postsynaptic densities (PSDs) are presumed sites of neurotransmitter receptor clustering but were difficult to observe in Drosophila brains, especially in the MB. Absence of PSDs in the EM data leaves the possibility that some of the predicted connections use volume transmission instead of synaptic transmission. Additionally, EM connectome predicted many axo-axonal connections in the MB lobes.

Therefore, knowledge about precise localization of neurotransmitters and composition of neurotransmitters at PSDs is prerequisite to validate and correctly interpret EM connectome. To address this issue, we developed a method to generate constitutive and conditional alleles of neurotransmitter receptors at endogenous genomic loci to visualize receptors in whole brain and in single cells, respectively. In this work, we used generated knock-in lines for excitatory nicotinic acetylcholine (nAChR) receptors and inhibitory glutamate GluClalpha and GABA Rdl receptors to better understand the nature of synaptic transmission in the neural circuits of the MB. Our confocal screen for these receptors in population of MB-neurons revealed striking specificity of receptor localization in select brain areas. Moreover, we noticed specific dendritic and/or axonal localization in single neurons. nAChRs preferentially localized to dendrites while GluClalpha was uniformly distributed throughout neurons. Rdl localization on the other hand appeared to be highly cell-type specific. The diversity of neurotransmitter receptor localization suggests complex regulation of the localization which goes beyond individual neurotransmitter identity. This set of reagents combined with the knowledge of subcellular localization of neurotransmitter receptor subunits will be useful for fine perturbation of circuits, understanding plasticity mechanisms and understanding logic of specific circuits.

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Poster

033. Postsynaptic Organization and Structure

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Program #/Poster #: 033.13

Topic: B.04. Synaptic Transmission

Support: NIMH Grant MH066198
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Title: Nano-organization of spontaneous GABAergic transmission directs its autonomous function in neuronal signaling

Authors: *N. J. GUZIKOWSKI[1], E. T. KAVALALI[2];
[1]Vanderbilt Univ. Sch. of Med., [2]Vanderbilt Univ., Vanderbilt Univ., Nashville, TN

Abstract: Earlier studies delineated the precise arrangement of proteins that drive neurotransmitter release and postsynaptic signaling at excitatory synapses. However, spatial organization of neurotransmission at inhibitory synapses remains unclear. Here, we took advantage of the molecularly specific interaction of antimalarial Artemisinins, and an inhibitory synapse scaffold protein, gephyrin, to probe the functional organization of gamma-aminobutyric acid A receptor ($GABA_AR$) mediated neurotransmission in central synapses. Short-term application of Artemisinins severely contracted the size and density of gephyrin and $GABA_AR$ $\gamma2$ subunit clusters. This size contraction elicited a neuronal activity-independent increase in

Bdnf expression due to a specific reduction in GABAergic spontaneous, but not evoked, neurotransmission. The same functional effect could be mimicked by disruption of microtubules that link gephyrin to the neuronal cytoskeleton. These results suggest the GABAergic postsynaptic apparatus possesses a concentric center-surround organization, where the periphery of gephyrin clusters selectively maintain spontaneous GABAergic neurotransmission facilitating its autonomous function regulating *Bdnf* expression.

Disclosures: **N.J. Guzikowski:** None. **E.T. Kavalali:** None.

Poster

033. Postsynaptic Organization and Structure

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Topic: B.04. Synaptic Transmission

Support: NIH Grant R37MH080046
NIH Grant F32MH119687
NIH Grant F31MH117920
NIH Grant F31MH124283

Title: Nanoscale organization of synaptic scaffold proteins across development

Authors: *S. R. METZBOWER, P. A. DHARMASRI, A. D. LEVY, M. ANDERSON, T. A. BLANPIED;
Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Through their numerous protein-protein interaction domains, the MAGUK family of synaptic scaffold proteins provides a framework to retain and position receptors, signaling molecules, and other key synaptic components. While SAP102 and PSD-95, two of the most abundant and critical MAGUKs at excitatory glutamatergic synapses, have similar protein structures, they also have key divergent properties. SAP102 expression peaks earlier in development and is required for synaptogenesis, while PSD-95 peaks later and is associated with synapse maturation. In addition, SAP102 functionally associates with GluN2B-containing NMDARs, while PSD-95 is more strongly associated with GluN2A-containing NMDARs. PSD-95, along with presynaptic release machinery and AMPARs, forms distinct subsynaptic nanoclusters (NCs) that play a role in synaptic function. However, the subsynaptic distribution of SAP102, its relation to PSD-95 NCs, and how either MAGUK's nano-organization changes throughout development, is unknown. Because of the importance of SAP102 and PSD-95 in establishing and maintaining synaptic function across development, we set out to determine their nanoscale organization with respect to one another in cultured hippocampal neurons at one, two, and three weeks in vitro. Using 2-color DNA-PAINT super-resolution imaging we found that SAP102, like PSD-95, has a non-random distribution within the synapse, and both proteins are organized into subsynaptic NCs with high local protein density. Interestingly, SAP102 NCs

tended to be both smaller and denser than PSD-95 NCs, suggesting they might play a distinct structural role. In synapses that contained both SAP102 and PSD-95, their nanoscale distributions showed a high degree of cross-correlation, indicating their NCs are likely to overlap within subsynaptic nanodomains that may serve to bring together critical synaptic elements. However, for synapses with only one MAGUK present, either PSD-95 or SAP102 showed enhanced subsynaptic clustering compared to synapses where both proteins were present, highlighting how overall synapse protein composition may determine synaptic nanostructure. Across development, SAP102 NCs were relatively stable both in size and density, while PSD-95 NCs were larger in older synapses, suggesting that as synapses mature and PSD-95 emerges as the primary scaffold, there is a nanostructural rearrangement of PSD-95 that may underly functional maturation. The nano-organization of these two MAGUK family members could be a powerful tool that shapes synaptic signaling through positioning of receptors and other critical signaling molecules.

Disclosures: S.R. Metzbower: None. **P.A. Dharmasri:** None. **A.D. Levy:** None. **M. Anderson:** None. **T.A. Blanpied:** None.

Poster

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Topic: B.04. Synaptic Transmission

Support: NIH/NIMH F31 MH124283
NIH/NIMH R37 MH080046
NIH/NIA R01 AG065594
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Title: Direct visualization of triheteromeric NMDA receptor trafficking and organization

Authors: M. C. ANDERSON[1], A. NIGAM[3], J. W. JOHNSON[3], ***T. A. BLANPIED**[2]; [1]Program in Neurosci., [2]Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD; [3]Neurosci., Univ. of Pittburgh, Pittsburgh, PA

Abstract: Glutamatergic signaling via NMDA receptors (NMDARs) is critical in synaptic plasticity, brain development, excitotoxicity, and numerous degenerative and cognitive disorders. Each NMDAR comprises four subunits: two obligatory GluN1 subunits and two GluN2A-D or GluN3 subunits. Importantly, NMDARs with different GluN2 compositions display strikingly different biophysical characteristics, and unique protein interactions and signaling. Thus, identifying how neurons control synaptic abundance of specific NMDAR subtypes has been a longstanding goal in neuroscience. Unfortunately, our understanding of these mechanisms has been restricted by inability to visualize one of the key classes of NMDARs in neurons, triheteromeric receptors that contain GluN1 and two different GluN2 subunits. Indeed, the

subcellular distribution of triheteromeric NMDARs remains mysterious and the mechanisms controlling their trafficking to and from synapses remain almost totally unknown. This is a crucial shortcoming, because in many parts of the brain including the hippocampus, triheteromeric receptors containing both GluN2A and GluN2B are among the most common subtypes of NMDARs. Here, we introduce a new method for direct visualization of triheteromeric NMDARs in neurons using bimolecular complementation. We tagged the N-termini of GluN2A and GluN2B subunits with two components of split fluorescent proteins or of a split HaloTag enzyme that is catalytically active only when the two parts are brought within close proximity. Thus, after transfection of these tagged subunits, standard fluorescent imaging can be used to detect specifically NMDARs containing both GluN2A and GluN2B subunits in a single receptor complex. Whole-cell recordings demonstrate that activation of split-tagged NMDARs by glutamate, as well as inhibition by ifenprodil are unaltered by the presence of the tags. Further, the split-tagged subunits do not undergo detectable interreceptor complementation, and neurons expressing the complementing receptor subunits displayed no morphological abnormalities. Using this tool, we find that triheteromeric receptors traffic to synapses and display unique subcellular trafficking characteristics. Moreover, we can utilize the unique permeability properties of HaloTag ligands to directly test surface trafficking and compartment-specific organization of triheteromeric NMDARs. These probes will fill longstanding gaps in our knowledge of NMDARs and lay necessary groundwork for investigation of other aspects of triheteromeric NMDAR trafficking in healthy neurons and disease models.

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Poster

033. Postsynaptic Organization and Structure

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Title: Subtype-specific positioning of endogenous NMDARs near key pre- and postsynaptic nanodomains

Authors: *M. C. ANDERSON[1], A. D. LEVY[2], P. A. DHARMASRI[1], S. R. METZBOWER[2], T. A. BLANPIED[2];
[1]Program In Neurosci., [2]Physiol., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: NMDAR subunit composition dictates receptor channel kinetics, protein interactions, and subcellular trafficking. Indeed, the position of NMDAR subtypes within and outside a synapse can influence their specific roles in downstream processes such as cell death, plasticity, and gene expression. Further, the fine subsynaptic position of NMDARs may impact their activation by glutamate release, as it does for AMPARs. Scaffold proteins (e.g. PSD-95), release machinery (e.g. Munc13), and glutamate receptors concentrate in densely packed subsynaptic nanoclusters (NCs). These NCs are all aligned across the synapse, which serves to position glutamate release directly over concentrations of AMPARs and facilitate their activation. Previous work has shown that GluN2A- and GluN2B-containing NMDARs also form NCs whose reorganization is involved in basal synaptic transmission as well as plasticity. However, little is known about the organization of endogenous NMDAR subtypes relative to their postsynaptic scaffolds or sites of vesicular release. We used CRISPR to label endogenous GluN2B with EGFP and a GluN2A-specific antibody to label endogenous GluN2A in cultured primary hippocampal neurons. We then performed four-color Exchange-PAINT to super-resolve surface GluN2A and GluN2B relative to their postsynaptic scaffolds PSD-95 or SAP102, or to the presynaptic release site marker Munc13. Consistent with previous data, both GluN2A and GluN2B form small, dense NCs within and outside the synapse. Quantitative PAINT analysis of GluN2B NCs suggests the smallest contain 1 to 2 labeled subunits, potentially representing tri- and diheteromeric receptors, respectively, and that multiple receptors may group together to form larger NCs. We presumed that NMDARs are anchored to PSD-95 and their protein densities would be well overlapped. Surprisingly, nanometer-scale renderings of GluN2 and PSD-95 distributions showed a lack of visible colocalization of high-density regions, and each subunit showed *anti*-enrichment at short distances from PSD-95 NCs. Nevertheless, PSD-95 density with respect to GluN2A or GluN2B NCs is enhanced within 150 nm, suggesting the receptors may be adjacent to, yet not overlapping with, the MAGUK NCs. To test this, we measured a density-based colocalization index of receptor to MAGUK, which confirmed the tendency for PSD-95 colocalization to occur on receptor NC edges. Further analysis of NMDAR subtype positioning with respect to key constituents of synaptic functional architecture will provide clarity on diverse mechanisms controlling the role of NMDARs in neurons.

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Poster

033. Postsynaptic Organization and Structure

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Topic: B.04. Synaptic Transmission

Support: NIH Grant NS108087
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NIH Grant NS128543

Title: Spinal cord Magi-2 (S-SCAM) is an obligate scaffold for the NMDA receptor influencing the development of inflammatory pain in mice

Authors: *G. SHEEHAN[1], A. ROSZCZYK[1], A. BHATTACHARJEE[2];
[1]SUNY at Buffalo, Buffalo, NY; [2]Pharmacol. and Toxicology, SUNY-Buffalo, Buffalo, NY

Abstract: Chronic pain has become a global health issue affecting 1 in 5 individuals. Additionally, in the United States, the opioid epidemic was responsible for over 100,000 deaths in 2021 alone. This has been fueled in part by the need for the prescription of addictive opioid analgesics and has highlighted the importance of the search for novel targets for treating chronic pain. As the first sight of central integration of nociceptive neural signals, the spinal cord dorsal horn (SCDH) represents a compelling target for such novel analgesics. We found that the synaptic WW-domain containing scaffolding protein Magi-2(S-SCAM) was differentially expressed in superficial spinal cord dorsal horn neurons indicating a role in mediating excitatory synaptic transmission from high threshold nociceptive fibers. Utilizing a Magi-2 transgenic mouse line where we found Magi-2 protein levels were reduced by ~50% compared to wildtype littermates, we observed that Magi-2 deficiency attenuated the second phase of the formalin model of inflammatory pain. These mice showed a baseline reduction in the NR1 subunit of the NMDA receptor in SCDH tissue suggesting that diminished glutamatergic synaptic transmission was responsible for the formalin behavioral phenotype. Spinal cord slice electrophysiology experiments were performed to test this assertion. To further confirm the role of Magi-2 in the dorsal horn during inflammatory pain, we turned to shRNA-mediated knockdown of Magi-2. AAV9 carrying shRNA targeting Magi-2 was intrathecally injected into 8wk old wildtype C57Bl/6 mice two weeks before unilateral hind paw injection of Complete Freund's Adjuvant (CFA). Magi-2 shRNA resulted in a 50% reduction of Magi-2 protein in the SCDH and attenuated the thermal hyperalgesia associated with the CFA model of chronic inflammatory pain. Current experiments are aimed at characterizing the synaptic protein changes that occur as a result of Magi-2 knockdown. *In-vitro* experiments revealed that heterologous co-expression of Magi-2 and NR1/NR2D was able to increase NR1 protein levels 2-fold suggesting that Magi-2 increases NMDA receptor stability. Future experiments will further characterize this interaction.

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Poster

033. Postsynaptic Organization and Structure

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Program #/Poster #: 033.18

Topic: B.04. Synaptic Transmission

Title: Presenilin and APP regulate synaptic kainate receptors

Authors: *G. BARTHET[1], A. MOREIRA DE SA[2], P. ZHANG[3], J. M. CASTANHEIRA[4], A. GORLEWICZ[5], C. MULLE[6];

[1]Wyss Ctr. for Bio and NeuroEngineering, Geneva, Switzerland; [2]Bordeaux Univ., Bordeaux, France; [3]CNRS, Bordeaux, France; [4]Univ. Bordeaux 2, Vila Real, Portugal; [5]Nencki Inst. of Exptl. Biol., Warsaw, Poland; [6]Interdisciplinary Inst. For Neurosci., Bordeaux, France

Abstract: Kainate receptors (KARs) form a family of ionotropic glutamate receptors which regulate the activity of neuronal networks by both pre- and post-synaptic mechanisms. Their implication in pathologies is, until now, mostly documented for epilepsy. The higher prevalence of epileptic symptoms in Alzheimer disease (AD) patients questions the role of KARs in AD. Here we investigated whether the synaptic expression and function of KARs was impaired in mouse models of AD. We addressed this question by immunostaining and electrophysiology at synapses between mossy fibers and CA3 pyramidal cells (Mf-CA3 synapses), in which KARs are abundant and play a prominent physiological role. We observed a decrease of the immunostaining for GluK2 in the stratum lucidum in CA3, and of the amplitude of synaptic currents mediated by GluK2-containing KARs in an amyloid mouse model (APP/PS1) of AD. Interestingly, a similar phenotype was observed in CA3 pyramidal cells with a genetic deletion of either presenilin or APP/APLP2 as well as in organotypic cultures treated with γ-secretase inhibitors that prevent Aβ production. Finally, the GluK2 protein interacts with full-length and C-terminal fragments of APP. Overall, our data suggest that APP stabilizes KARs at synapses possibly through a trans-synaptic mechanism, and that this interaction is under the control the γ-secretase proteolytic activity of presenilin.

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Poster

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Topic: B.04. Synaptic Transmission

Support: Max Planck Society

Title: Local mitochondrial mechanisms fueling synaptic plasticity

Authors: *R. FAN, V. RANGARAJU;
Max Planck Florida Inst. for Neurosci., Max Planck Florida Inst. for Neurosci., Jupiter, FL

Abstract: Cognitive functions are highly energy-consuming processes. Mainly, synapses that are sites of neurotransmission and plasticity require instant and localized energy supply. Consistent with this notion, our previous work revealed that locally stable mitochondrial compartments fuel synaptic protein synthesis during plasticity. However, the molecular signal that drives local mitochondrial energy production in response to spine-specific energy demands is unclear. Given that mitochondrial calcium can activate enzymes of the Kreb's cycle to produce energy, we hypothesize that calcium could be the local molecular signal that drives mitochondrial energy

production during synaptic plasticity. To address this question, we specifically targeted the genetically-encoded calcium indicator, GCaMP6f, to the mitochondrial matrix and measured mitochondrial calcium dynamics in response to single-spine stimulation by two-photon glutamate uncaging. Our results show that, upon spine-stimulation, mitochondrial calcium displays a synchronous increase with that of spine calcium influx. Furthermore, this mitochondrial calcium influx is confined within 30-μm spatial compartments at the base of the stimulated spine. Contrary to recent studies, the mitochondrial calcium uniporter facilitates the mitochondrial calcium influx independent of the endoplasmic reticulum. Using a highly sensitive luciferase-based ATP reporter targeted to the mitochondrial matrix, we observe local mitochondrial ATP increases upon single-spine stimulation. We are currently investigating if this local increase in ATP synthesis is mitochondrial calcium-dependent and is required to fuel synaptic plasticity within spatially-confined dendritic compartments.

Disclosures: R. Fan: None. **V. Rangaraju:** None.

Poster

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Swedish Research Council 2016–01936
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Title: Human Dopamine D1 Organization Contributes to Functional Brain Architecture

Authors: *R. PEDERSEN, J. JOHANSSON, A. SALAMI;
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Abstract: Background Individual differences in dopamine D1 availability are related to functional brain network dynamics. Moreover, inter-regional co-variations in dopamine D1 receptor (D1DR) availability reflect distinct functional subsystems. However, little is known about the spatial topology of D1DRs and whether it is of relevance for functional network interaction. In this work, we set out to investigate whether (1) differences in region-to-region D1DR co-variation is associated with functional network connectivity, and (2) functional connectivity differences correspond to distinct D1DR systems. Finally, (3) the co-variance structure of inter-regional D1DR associations differ across the life span. **Methods** We used baseline data from the DyNAMiC study, a large-scale life-span study including PET ([11C]-SCH23390) and fMRI/MRI imaging (N = 165, 50% female. age range: 20-79 years. We applied between-subject inter-regional correlation analysis and PCA decomposition to investigate whether the functional organization of dopamine D1DR availability map onto the functional architecture of the brain. **Results** We found distinct functional subsystems of cortical and

subcortical D1DR availability, largely corresponding to functional network topology. Moreover, the degree of D1DR co-variability was related to the strength of functional connections within functionally integrated regions. These results suggest that D1DR profiles are systematically aligned with patterns of functional connectivity, specifically within the intrinsic functional systems, above and beyond spatial proximity, in concordance with the notion that receptor profiles inform inter-regional signaling.

Disclosures: **R. Pedersen:** None. **J. Johansson:** None. **A. Salami:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.21

Topic: B.04. Synaptic Transmission

Support: NIH Grant MH118425
Brain and Behavior Research Foundation Grant #30264
Summer Undergraduate Research Program Code=97270s1

Title: Exacerbated novelty-induced hyperlocomotion and increased neuronal activity in the dentate gyrus upon GluA1 carboxyl-terminal domain truncation

Authors: *A. V. KOLLI, G. SANDOVAL, K. V. SOLORIO, V. A. VERA, M. SANDOVAL, J. DÍAZ-ALONSO;
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Abstract: GluA1 is one of four subunits that constitute α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors, or AMPARs. GluA1 containing AMPARs are highly enriched in the hippocampus, a region of the brain widely recognized to be critical for learning and memory formation. The GluA1 subunit is essential for long-term potentiation (LTP) of excitatory synapses, an important mechanism for learning. Much of the research into the mechanisms controlling activity-dependent GluA1 synaptic trafficking have focused on the cytoplasmic carboxy-terminal domain (CTD). However, the function of this domain of GluA1 in synaptic transmission and LTP is still debated. Using multiple complementary genetic approaches combined with electrophysiology, we determined that LTP of the Schaffer collateral->CA1 synapse can occur in the absence of the GluA1 CTD. Surprisingly, we recently found that mice expressing a CTD-truncated GluA1 subunit (ΔCTD GluA1 KIs) display certain behavioral abnormalities including excessive novelty-induced hyperlocomotion. We also found that, in the hippocampus, the absence of the GluA1 CTD leads to a mild, yet significant subcellular redistribution of AMPARs. The goal of this study is to determine the circuit-specific role of the GluA1 CTD in synaptic transmission and plasticity, and its specific impact in novelty detection in mice. 4-5 month old male and female WT and ΔCTD GluA1 mice were exposed to a novel environment in the open field apparatus. Subsequently, c-Fos expression was analyzed to

evaluate the impact of the GluA1 CTD in neuronal activity in different brain regions. We found significantly exacerbated neuronal activation in different brain regions, including hippocampal dentate gyrus and CA3, in ΔCTD GluA1 mice compared with WT counterparts upon exposure to a novel environment. This increased neuronal activity in the dentate gyrus and CA3 suggests that synaptic transmission in these hippocampal regions, crucial for novelty detection among many other functions, is modulated by mechanisms targeting the GluA1 CTD. Specifically, these results suggest a surprising AMPAR gain of function upon the loss of the GluA1 CTD. Ongoing whole-cell patch-clamp experiments are aimed at determining the circuit specific impact of the GluA1 CTD deletion on synaptic transmission and plasticity. Our combined findings suggest that the GluA1 CTD plays an important modulatory role in some brain circuits important for novelty detection and expand our understanding of the region-specific molecular rules controlling AMPAR trafficking and function.

Disclosures: **A.V. Kolli:** None. **G. Sandoval:** None. **K.V. Solorio:** None. **V.A. Vera:** None. **M. Sandoval:** None. **J. Díaz-Alonso:** None.

Poster

033. Postsynaptic Organization and Structure

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 033.22

Topic: B.04. Synaptic Transmission

Support: NIH/NINDS Intramural Research Program

Title: A common hub for general anesthetic-induced unconsciousness and analgesia in the central amygdala

Authors: *W. HAN[1], W. LU[2];
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[2]NINDS/NIH, NINDS/NIH, Bethesda, MD

Abstract: General anesthesia (GA), a reversible state of unconsciousness with the absence of pain sensation, maximally eliminates painful stimulation and arousal during the surgery and invasive diagnostic procedure. However, inappropriate GA can lead to prolonged recovery or intraoperative awareness and an increased risk of postoperative complications and posttraumatic stress disorder. Thus, identifying molecular mechanisms within specific cell types responsible for regulating GA will help improve clinical GA monitoring. We have recently identified that Shisa7, a postsynaptic transmembrane protein, is critical to the trafficking and kinetic properties of $GABA_AR$ at inhibitory synapses in mice. Shisa7 also potently enhances the pharmacological action of diazepam, an induction agent for GA, on $GABA_ARs$. Here we report that Shisa7 also regulates the pharmacology of other $GABA_AR$-related GAs (i.e. propofol, etomidate, and isoflurane). Specifically, Shisa7 KO diminished GA-induced potentiation of $GABA_AR$-mediated whole-cell currents in cultured hippocampal neurons and blunted GA-induced loss of righting

reflex (LORR) in mice. In addition, the *Von Frey* test demonstrated that a hypnotic dose of GA completely abolished the pain-induced reflexive to the mechanical stimulus in control mice. In contrast, the same mechanical stimulus induced a strong pain-elicited reflexive in Shisa7 KO mice. Together, these data indicate that Shisa7 is critical to GA-induced LORR and analgesia. We, therefore, hypothesized that GA potentially induces LORR in part by activating analgesic circuits that depend on the Shisa7 function. Histochemical experiments further revealed that GAs strongly triggered cFos activation in the mouse central amygdala (CeA). Significantly, GA-induced cFos expression was reduced in the CeA of Shisa7 KO mice. Moreover, GAs activated a common subpopulation of neurons expressing somatostatin (SOM) in CeA. Notably, chemogenetic inhibition of these SOM+ neurons in CeA blunted GA-induced LORR and enhanced pain-elicited reflexive in mice. Given a high expression of Shisa7 in the CeA and an essential role of Shisa7 in regulating GA, these results allow us to study how Shisa7 functions in GA-related physiology and behavior. Together, our data identify Shisa7, an essential modulator of inhibitory synaptic transmission, as a potential common target for regulating neural circuit function in consciousness and pain and improving the clinical application of GA.

Disclosures: **W. Han:** None. **W. Lu:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 034.01

Topic: B.04. Synaptic Transmission

Support: Novo Nordisk Foundation NNF20OC0065309
Independent Research Fund Denmark 1030-00452B

Title: D1-medium spiny neurons exhibit a temporally regulated differential dopamine sensitivity.

Authors: *A. KONOMI PILKATI[1], T. F. ANDREASEN[3], A. T. SÖRENSEN[2], K. L. MADSEN[4], U. GETHER[5];
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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 input types, such as glutamatergic, 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 by transducing striatal primary cultures derived from D1-Cre mice pups or wild-type E19 rat embryos with the genetically encoded protein kinase A (PKA) sensors ExRai-AKAR2 or AKAR4. By using live imaging 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. Thus, the D1R-MSNs appear to have a DA-response spectrum. The response profile of a given neuron is largely preserved after 1h and often even after 24h. However, a fraction of the neurons shifts their sensitivity, becoming either more or less sensitive towards DA. Moreover, we observe that overexpression of D1R in MSNs increases the fraction of hyperresponsive neurons, suggesting that differential receptor expression levels at least in part may contribute to the observed phenotype. 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.

Disclosures: A. Konomi Pilkati: None. **T.F. Andreasen:** None. **A.T. Sörensen:** None. **K.L. Madsen:** None. **U. Gether:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.02

Topic: B.04. Synaptic Transmission

Support: NIH Grant F30MH122146

Title: Neurexins in serotonergic neurons regulate serotonin transmission and complex mouse behaviors

Authors: *A. CHEUNG, K. FUTAI;
Neurobio., Univ. of Massachusetts Chan Med. Sch. Grad. Program in Neurosci., Worcester, MA

Abstract: Extensive serotonin (5-HT) innervation throughout the brain corroborates 5-HT's modulatory role in numerous cognitive activities. Volume transmission is the major mode for 5-HT transmission but mechanisms underlying 5-HT signaling are still largely unknown. Abnormal brain 5-HT levels and function have been implicated in autism spectrum disorder (ASD). Neurexin (Nrxn) genes encode presynaptic cell adhesion molecules important for the

regulation of synaptic neurotransmitter release, notably glutamatergic and GABAergic transmission. Mutations in Nrxn genes are associated with neurodevelopmental disorders including ASD. However, the role of Nrxn genes in the 5-HT system is poorly understood. We generated a mouse model with all three Nrxn genes disrupted specifically in 5-HT neurons to study how Nrxns affect 5-HT transmission. Loss of Nrxns in 5-HT neurons impaired 5-HT release in the dorsal raphe nucleus and dorsal hippocampus and decreased serotonin transporter distribution in specific brain areas. Furthermore, 5-HT neuron-specific Nrxn knockout reduced sociability and increased depressive-like behavior. Our results highlight functional roles for Nrxns in 5-HT neurotransmission and the execution of complex behaviors.

Disclosures: A. Cheung: None. **K. Futai:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

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Topic: B.04. Synaptic Transmission

Support: FONDECYT Grant Nº 120–0474
DIUV-CI Grant N°01/2006
Puente Grant UVA20993
ANID Grant n°21180628

Title: Modulation of synaptic transmission in Nucleus Accumbens core by the activation of 5-HT_{2A}receptor in male mice

Authors: *C. ESTAY-OLMOS[1,2], R. SOTOMAYOR-ZARATE[1], M. FUENZALIDA[1];
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Abstract: Serotonin (5-HT) is a neurotransmitter that plays an important role in brain functions and social behavior and their dysfunction is implicated in many mental disorders. 5-HT can modulate synaptic function, excitability of neocortical neurons and their discharge rate through the activation of several receptor subtypes, classified into seven families from 5-HT_1 to 5-HT_7 (including 14 different receptor subtypes). The widespread localization of 5-HT receptors and the high density of serotonergic axons arising from dorsal and median Raphe nuclei onto prefrontal cortex (PFC), ventral tegmental area (VTA), and nucleus accumbens (NAc) suggest an important role for 5-HT in synaptic function and a powerful modulator of excitatory/inhibitory (E/I) balance within mesocorticolimbic related areas. The 5-HT_{2A} ($G_{q/11}$-protein coupled receptor) activation reduces the synaptic transmission in PFC, through retrogradely endocannabinoids (eCBs) release and cannabinoid receptor type 1 (CB_1) activation. Currently, the role of serotonergic transmission in NAc has been little studied despite the fact that at least 4

subtypes of 5-HT receptors ($5HT_{1B, 2C, 2A}$ and $_7$) are expressed. GABAergic medium spiny neurons (MSNs) comprise more than 95% of the NAc neural population, but how 5-HT modulate the excitability and inhibitory synaptic transmission in MSNs has been poorly studied. To assess this question, we hypothesized that 5-HT modulates GABAergic transmission in NAc core mainly through $5\text{-}HT_{2A}$ activation in male mice. Using electrophysiological techniques such as whole cell patch clamp in current and voltage clamp mode, we evaluated whether $5\text{-}HT_{2A}$ activation modulates the evoked, spontaneous and miniature inhibitory transmission. We observed that $5\text{-}HT_{2A}$ activation (with specific agonists) produces a long-term depression of inhibitory synaptic transmission (iLTD), that depends on intracellular calcium rise, stimulating the synthesis and mobilization of eCBs (mainly 2-arachidonoylglycerol (2-AG)) by MSNs. 2-AG retrogradely activate CB_1 on presynaptic neurons decreasing NAc GABA release. We also observed that activation of $5\text{-}HT_{2A}$ through the eCBs release and CB_1 activation can depress the glutamatergic transmission in MSNs of NAc core. These results provide new insights into the cellular mechanisms underlying maintenance of optimal E/I balance in the NAc, a necessary information to understand how 5-HT regulates the NAc circuitry and determine its role in several neuropsychiatric disorders such as addiction, schizophrenia and depression**.**

Disclosures: C. Estay-Olmos: None. **R. Sotomayor-Zarate:** None. **M. Fuenzalida:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.04

Topic: B.04. Synaptic Transmission

Support: NIH R00 MH106757
Pilot Discovery Award from CTSI-CN
Young Investigator Award from the Brain and Behavior Research Foundation

Title: Early life adversity alters the maturation of synapses onto dorsal raphe serotonergic neurons

Authors: *A. KISNER, S. WOODS, A. POLTER;
The George Washington Univ., Washington, DC

Abstract: Environmental inputs during early life period play an important role in shaping the maturation of the brain. Adverse experiences during this time can have long-lasting effects on brain function and increase susceptibility to stress-related neuropsychiatric disorders later in life. These events can alter neuronal connectivity and are themselves linked to dysregulation of neuromodulatory systems. In particular, serotonergic neurons of the dorsal raphe nucleus (DRN) exert widespread and complex neuromodulatory effects that are necessary for fine-tuning neural circuit formation in the forebrain and the expression of affective and social behaviors. While serotonin regulates a wide range of brain functions and behavior, few studies have focused on the

maturation of DRN serotonergic neurons and how maturation of serotonergic circuits is altered by early-life experience. Here, we show that exposure to early life stress shifts the developmental trajectory of excitatory and inhibitory synapses onto DRN serotonergic neurons. To better understand the impact of early life experience on maturation of synapses onto DRN serotonin neurons, we implemented a limited bedding model to induce early life stress (ELS) from PND 4 to PND 11. We then used acute slice electrophysiology to assess the function of DRN synaptic inputs at key developmental timepoints. Our results demonstrated that ELS decreases the activity of both excitatory and inhibitory synaptic inputs onto juvenile (PND 15) serotonergic neurons and these changes persisted throughout adulthood. ELS also induced an increase in the ratio of AMPA to NMDA receptor-mediated excitatory transmission (mean AMPAR/NMDAR ratio ELS 17.1 ± 3.4, n = 12 cells vs control 7.3 ± 1.3, n = 8 cells) onto DR serotonergic neurons specifically in adolescent mice. Moreover, serotonergic neurons from juvenile and adult mice subjected to ELS have a reduced excitability (PND15: mean #APs ELS 9.3 ± 2.3 n = 9 cells vs control 14.5 ± 2.3 n = 11 cells and PND80: mean #APs ELS 13.5 ± 1.5 n = 8 cells vs control 21 ± 3.1 n = 10 cells at 300 pA somatic current injection). Intriguingly, this deficit in excitability is transiently reversed in adolescent mice. Given the potential involvement of aberrant serotonin signaling in stress-related disorders, our findings may serve as an important point for understanding neural mechanisms underlying dysregulations in synaptic connections onto DRN serotonergic neurons and potential targets for novel treatments to promote stress resilience following early life adversity.

Disclosures: **A. Kisner:** None. **S. Woods:** None. **A. Polter:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 034.05

Topic: B.04. Synaptic Transmission

Support: NIH 1R01NS112365-01A1
NSF 1750199

Title: Measuring Spontaneous Neurotransmission at Individual Hippocampal Boutons

Authors: *A. J. RALOWICZ, M. B. HOPPA;
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Abstract: Neurotransmitter release from synaptic vesicle fusion is the underlying fundamental process of neuronal communication at the majority of synapses. While evoked release following an action potential has been well characterized, spontaneous neurotransmission has been much less studied. In the hippocampus the probability of vesicle fusion during evoked transmission can vary by an order of magnitude between synapses along a single axon. However, the rates and control of spontaneous release have been much less studied. This is a difficult problem to study

as pyramidal neuron axons in the hippocampus have thousands of presynaptic boutons where vesicle release is independently regulated. The classic electrophysiology recordings to measure spontaneous release cannot provide spatial information about where spontaneous release events are occurring at the individual synapse level. We have taken advantage of a new generation of fluorescent glutamate sensors (iGluSnFR3.v857 developed by Podgorski Lab Janelia/Allan Brain Institute) to make repeated measurements of evoked and spontaneous fusion from cultured hippocampal neurons. We measured spontaneous release to occur at an average rate of 0.1 Hz, but with rates that varied by more than 10 fold across time measured between individual presynaptic terminals and almost 60% of boutons competent for evoked release never engaging in spontaneous release at all (n = 13 cells and 577 synapses). Currently, it is unknown if and how the occurrence of spontaneous release contributes to the frequency or magnitude of evoked vesicle fusion events. Recent work has shown that spontaneous release may utilize distinct release sites as well as discrete sets of postsynaptic receptors. Using GluSnFR3 we also measured evoked release probability and amplitude along with spontaneous release frequency from individual synapses in the same group of cells. We found that boutons with higher spontaneous neurotransmission frequency also had significantly higher evoked release probability and evoked release magnitude. We then measured the frequency of spontaneous release when increasing the relative size of the readily releasable pool (RRP) by inhibiting CDK5 using Roscovatine. Spontaneous release frequency increased across majority of boutons and again we saw an increases in evoked release regardless of initial evoked release probability and magnitude. Taken together, these findings suggest that spontaneous release is a regulated process dependent on vesicle availability in the RRP. This may suggest that spontaneous vesicle release is communicating a critical aspect of presynaptic strength and function to the postsynaptic target.

Disclosures: A.J. Ralowicz: None. **M.B. Hoppa:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.06

Topic: B.04. Synaptic Transmission

Support: NIMH Grant R01MH124934

Title: Acetylcholine enhances excitatory synaptic drive preferentially onto pyramidal tract neurons in the mouse prefrontal cortex

Authors: *A. T. GULLEDGE;
Mol. and Systems Biol., Geisel Sch. of Med. At Dartmouth Col., Hanover, NH

Abstract: Acetylcholine (ACh) acts postsynaptically in the mouse medial prefrontal cortex (mPFC) to selectively enhance the excitability of pyramidal tract (PT) neurons, but has little impact on the excitability of intratelencephalic (IT) projection neurons. ACh can also act

presynaptically in the cortex to suppress excitatory synaptic transmission onto both PT and IT neurons. For instance, ACh induces a dose-dependent reduction of glutamate release from commissural afferents arriving from the contralateral cortex (by 87 ± 2% at 20 µM ACh, n = 17; $p < 0.001$). To better understand how ACh regulates the relative excitatory drive of PT and IT neurons, long epochs (30 min) of spontaneous excitatory synaptic potentials (sEPSPs) were recorded in pairs of PT and IT projection neurons in brain slices of mPFC from female and male mice. Bath-application of 20 µM ACh (with 10 µM eserine) reversibly enhanced sEPSP amplitudes (by 39 ± 4%; $p = 0.003$), frequencies (by 60 ± 4%; $p < 0.001$), and half-widths (by 18 ± 1%; $p < 0.001$) preferentially in PT neurons (n = 16 pairs of IT and PT neurons). In contrast, in IT neurons only sEPSP amplitudes were enhanced (modestly, by 14 ± 2%; $p = 0.04$). IT sEPSP frequencies (+2 ± 2% change; $p = 0.62$) and half-widths (+4 ± 1%; $p = 0.14$) were not modulated by ACh. The effects of ACh on sEPSPs in PT neurons were blocked by the co-application of either atropine (1 µM; n = 10) or tetrodotoxin (TTX; 1 µM; n = 12), demonstrating that ACh acts at muscarinic ACh receptors to enhance action-potential-dependent excitatory drive preferentially in PT neurons. In both PT and IT neurons, ACh reversibly reduced the coefficient of variation (CV) of sEPSP amplitudes by about 20% (-20 ± 5% in PT neurons and -19 ± 6% in IT neurons; $p < 0.01$ for each relative to baseline CV), an effect that was similarly blocked by atropine (+4 ± 7%; $p = 0.11$) or TTX (+9 ± 3%; $p = 0.13$). Finally, we found that coincident inputs (i.e., those occurring within 1 ms) were very rare in both PT-IT (0.4 ± 0.2% of all sEPSPs) and PT-PT (0.5 ± 0.1% of all sEPSPs; n = 14) pairs, but were reversibly increased by ACh selectively in PT-PT pairs (to 1.6 ± 0.6% of all sEPSPs; $p = 0.03$ vs IT-PT pairs that remained at 0.4 ± 0.1%). These data suggest that, even as ACh suppresses glutamate release at many cortical synapses, including those from commissural afferents, it simultaneously activates an excitatory network in the prefrontal cortex that preferentially excites PT projection neurons to drive corticofugal output.

Disclosures: A.T. Gulledge: None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.07

Topic: B.04. Synaptic Transmission

Support: NIH/NIDA grant, 0255-6803

Title: Nicotine modulates septal projections to medial habenula

Authors: M. ISHIKAWA, G. VOREN, V. MATHIS, M. SHIN, P. J. KENNY;
Neurosci., Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Nicotine-induced activation of nicotinic acetylcholine receptors (nAChRs) located in the medial habenula (MHb) to the interpeduncular nucleus (IPN) precipitates an aversive

behavioral state that promotes nicotine avoidance behaviors. MHb neurons receive afferents almost exclusively excitatory and inhibitory neurons in the septum via the stria medullaris. Little is currently known about the actions of nicotine on synaptic inputs from septal nuclei to MHb neurons, especially. Using chemogenic and optogenetic manipulations coupled to slice electrophysiology techniques, we demonstrated that MHb neurons receive functional synaptic inputs from the triangular septal nucleus (TNS), located in posterior septum, that are modulated by nicotine treatment. Chemogenetic stimulation of the TNS decreased intravenous nicotine self-administration behavior in rodents. Using in vivo electrophysiology recordings in freely moving rats, we show that experimental-delivered and self-administered nicotine infusions bi-directionally modulate the activity of TNS neurons and the strength of their oscillations in theta power band. Optical stimulation of TNS terminals using parameters that recapitulates TNS neuron activity when in theta oscillation induced maximal increases in post-synaptic excitatory transmission in MHb neurons. Consumption of quantities of nicotine sufficient to precipitate craving-like nicotine seeking during periods of enforced abstinence induced striking deficits in the rate of spontaneous firing of TNS neurons. Nicotine abstinence was also associated with markedly decreased strength of functional connectivity in the TNS-MHb circuit, reflected by deficits in presynaptic release probability from TNS terminals in the MHb. Taken together, these results suggest that excitatory inputs from the TNS to the MHb play an important role in controlling nicotine intake and craving for the drug during periods of abstinence. Thus, interventions that reverse the actions of nicotine on this septum-habenula circuit may provide new approaches for the treatment of tobacco use disorder.

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Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.08

Topic: B.04. Synaptic Transmission

Support: R01 MH116003
R01 NS118731

Title: Anatomical characterization of glutamate delta receptor 1 in the lateral habenula

Authors: *D. CHOI[1], Y. SMITH[2], S. DRAVID[3], J.-F. PARE[1];
[1]Emory Univ., Atlanta, GA; [2]Yerkes Res. Ctr., Udall Ctr. Excel. For Parkinson's Dis. and Dept. of Neurol., Atlanta, GA; [3]Creighton Univ., Omaha, NE

Abstract: Glutamate delta receptors (GluDs) are synaptogenic molecules that form trans-synaptic complexes with the presynaptic protein neurexin and the intermediary protein cerebellin to regulate the formation and maintenance of synapses. Although the importance of GluD2 in the

synaptic development of the cerebellar cortex is well established, much less is known about GluD1. However, recent studies have suggested synaptogenic functions of GluD1 in the striatum, hippocampus, and central amygdala. Despite its strong expression, nothing is known about the cellular localization and function of GluD1 in the lateral habenula (LHb), a subcortical structure that processes reward-related behaviors and regulates monoaminergic systems. LHb dysregulation has been linked to psychiatric disorders such as major depressive disorder and schizophrenia, both of which have genetic association with *GRID1*, the gene that encodes for GluD1. It then stands to reason that disruption of GluD1 synaptic signaling in the LHb may contribute to the pathobiology of these disorders. A deeper understanding of GluD1 function in LHb necessitates a detailed map of its localization at the cellular, subcellular and subsynaptic levels. In this study, we used light and electron microscopy immunogold and immunoperoxidase methods to directly address this issue in mice. At the light microscopic level, the full extent of LHb displays strong cellular and neuropil GluD1 immunoreactivity that distinguishes it from the neighboring medial habenula, devoid of GluD1 immunolabeling. In light with these observations, preliminary electron microscopy data from immunoperoxidase-stained LHb tissue of three wild-type mice showed that GluD1 is mainly expressed in dendritic profiles, with lower expression in neuronal somata, spines, and glial processes. In some dendrites, the peroxidase deposit was aggregated at specific synapses, while in others it was diffusely expressed along the plasma membrane and the intracellular compartment. Data obtained so far from the immunogold-stained LHb tissue confirmed a strong expression of GluD1 at a subset of axo-dendritic and some axo-somatic symmetric synapses. Extra-synaptic and intracellular labeling was also found in many dendritic profiles. Studies are in progress to determine the source(s) of LHb afferents that express GluD1. This study provides the first description of the subcellular and subsynaptic localization of GluD1 in the LHb. Our results lay the foundation to determine how GluD1 regulates synaptic transmission in the LHb and how GluD1 disruption may contribute to psychiatric disorders associated with LHb dysfunction.

Disclosures: **D. Choi:** None. **Y. Smith:** None. **S. Dravid:** None. **J. Pare:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.09

Topic: B.04. Synaptic Transmission

Support: NIH Grant NS115947

Title: Investigating mechanisms to regulate LAR-RPTP function at excitatory synapses and the consequences for long distance transport

Authors: *Z. LENNINGER, D. PIERCE, F. HOERNDLI;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Neuronal mechanisms of excitatory synaptic transmission require a tight control of the number and function of ionotropic glutamate receptors (iGluR) at synapses. One of the key regulatory factors affecting synaptic transmission is the activity dependent long-distance transport of iGluRs. However, the molecular mechanisms that coordinate transport, synaptic delivery, and removal of iGluRs by this long-distance transport is poorly understood. We have recently found a new role for the receptor tyrosine phosphatase isoform PTP-3A, a homologue of the vertebrate leukocyte common antigen-related receptor-type protein tyrosine phosphatase (LAR-RPTP), in coordinating transport and synaptic retention of the *C. elegans* AMPA receptor, GLR-1. Our previous data show a differential role for the N- and C-terminal domains of PTP-3A, the former modulating GLR-1 transport events while the latter regulates GLR-1 retention at the synapse. These findings suggest a model in which synaptic activity could lead to the cleavage of PTP-3A. Here, we report evidence that PTP-3A is cleaved into separate C- and N-terminal portions. Furthermore, we show selective synaptic co-localization of PTP-3A and GLR-1 based on cell specific expression of fluorescently tagged PTP-3A and GLR-1. Finally, we investigate different conditions that may lead to the modulation of PTP-3A cleavage *in vivo* in *C. elegans*. In addition to cleavage, it was previously proposed that the synaptic scaffold and kinesin adaptor liprin-α may regulate LAR-RPTP synaptic localization and function. The *C. elegans* homologue of liprin-α, SYD-2, has been shown to interact with the kinesin UNC-104 and found at presynaptic sites, but its role in modulating the postsynaptic function of PTP-3A is currently unknown. Here, we show that GLR-1 transport and synaptic localization in *syd-2* loss of function mutants is similar to the loss of *ptp-3a*. Finally, we will use genetic epistasis, GLR-1/PTP-3A co-localization, and cleavage of PTP-3A by immunohistochemistry analyses to determine the mechanisms through which SYD-2 regulates PTP-3A function and localization at GLR-1 synapses.

Disclosures: Z. Lenninger: None. **D. Pierce:** None. **F. Hoerndli:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.10

Topic: B.04. Synaptic Transmission

Support: CIHR FDN-143238

Title: Postsynaptic NMDAR signaling promotes spontaneous and evoked neurotransmitter release in the developing retinotectal system

Authors: *M. R. VAN HORN[1], L. TIMMINS[1], S. GLASGOW[2], P. M. KESNER[1], V. HIGENELL[1], F. COOKSON[1], E. S. RUTHAZER[1];
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Abstract: The NMDA receptor plays an important role in activity-dependent refinement of sensory connections, which occurs during normal neural circuit development. In addition to their traditional postsynaptic localization, recent studies have provided evidence that NMDARs are also expressed at the presynaptic terminal in some systems, where they have been shown to modulate neurotransmission and synaptic plasticity. Here we tested for the presence of presynaptic NMDARs in the retinotectal system of the developing Xenopus laevis tadpole. Bath application of NMDAR antagonists, specifically targeting GluN2B-containing receptors, decreased miniature excitatory postsynaptic current (mEPSC) frequency. Furthermore, bath application of the NMDAR antagonist APV resulted in a significant increase in paired-pulse ratio (PPR). Interestingly, rearing the tadpoles in MK801 to block Ca^{2+} influx through the ion channel of the NMDARs did not prevent the APV-induced decrease in mEPSC frequency or the increase in PPR, suggesting that NMDARs can regulate both spontaneous and evoked synaptic release independent of Ca^{2+} influx through the ion channel. To test for the functional presence of NMDARs in RGC axons, we performed two-photon imaging of RGC axon terminals in isolated whole-brain preparations from animals in which RGCs were expressing the genetically-encoded calcium indicator GCaMP6s. Using this preparation, we found wash-on of NMDA produced robust Ca2+ transients in RGC axon terminals. However, we found that the NMDA-mediated increase in axonal Ca2+ was still observed when NMDARs in RGCs were knocked-down using antisense morpholino oligonucleotides against GluN1. Conversely, NMDA mediated Ca2+ increases were significantly reduced when NDMARs were knocked-down in the postsynaptic neurons. These findings suggest that postsynaptic NMDAR activation leads to an increase in presynaptic Ca2+. To determine if postsynaptic NMDARs mediate the change in presynaptic release properties observed during NMDAR blockage, we repeated the electrophysiology experiments in animals that had either pre- or postsynaptic NMDARs knocked-down and found that APV was ineffective in decreasing mEPSC frequency in animals where postsynaptic NMDARs were knocked-down. Taken together, these results suggest that activation of postsynaptic NMDARs can modulate evoked and spontaneous neurotransmission, likely through both ionotropic and non-ionotropic mechanisms. Additional studies are required to determine the downstream signaling pathways mediating these changes.

Disclosures: M.R. Van Horn: None. **L. Timmins:** None. **S. Glasgow:** None. **P.M. Kesner:** None. **V. Higenell:** None. **F. Cookson:** None. **E.S. Ruthazer:** None.

Poster

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Program #/Poster #: 034.11

Topic: B.04. Synaptic Transmission

Support: European Research Council (ERC)
Marie Skłodowska-Curie Actions (grant agreement 754411)
Fond zur Förderung der Wissenschaftlichen Forschung (Z 312-B27, Wittgenstein award)

Title: Developmental changes in synaptic transmission and connectivity shape information storage in the CA3 cell network

Authors: *V. M. VARGAS BARROSO, J. F. WATSON, P. M. JONAS;
ISTA, Klosterneuburg, Austria

Abstract: *Developmental changes in synaptic transmission and connectivity shape information storage in the CA3 cell network.*
AUTHORS: V. Vargas-Barroso, Jake F. Watson, P. JonasInst. of Sci. and Technol. Austria (ISTA), Klosterneuburg, Austria
CA3–CA3 recurrent synapses are crucial for learning and memory. Previous studies showed that synaptic connectivity (SC) between CA3 pyramidal neurons (PNs) is sparse, but endowed with nonrandom connectivity motifs (Guzman et al., 2016, Science 353, 1117–1123). Whether these rules are conserved across species, and how they emerge during development has not been determined. We recorded octuples of CA3 PNs (3487 tested connections total); unitary synaptic responses were measured through current and voltage-clamp. Under identical conditions, SC was higher in mice than in rats (~postnatal day P21). In rats, connection probability was 0.99% for CA3–CA3 and 0.75% for CA3–CA1 synapses, whereas in mice, it was 2.7% for CA3–CA3 and 1.23% for CA3–CA1 synapses ($P < 0.004$ and 0.52, respectively). As the number of cells is higher in rats than in mice (~330,000 versus ~100,000; Boss et al., 1987, Brain Res 406, 280–287), our results suggest an inverse relation between cell number and SC. Next, we tested SC among CA3 PNs in mice at three different developmental stages, P7–8, P18–22, and P45–50. Average connectivity was developmentally down-regulated, with connection probability values of 3.31%, 2.7%, and 1.01%, suggesting pruning of SC in CA3. Also, connectivity motifs increased during development, from 1 to 1.5 and 3.2 above the chance level ($P < 0.23$, 0.058, and 0.01, respectively). Notably, reciprocal and divergence motifs were enhanced. Our results indicate that nonrandom connectivity motifs emerge during network development. To explore how these connectivity rules affect network function, we implemented different connectivity values and cell numbers into a biologically inspired full-scale model of pattern completion (Hopfield, 1982, PNAS 79, 2554–2558; Guzman et al., 2016, Science 353, 1117–1123). Storage capacity increased linearly with cell number and connectivity values in the low parameter limit, but saturated with higher cell number and connectivity. Thus, sparse connectivity may optimize the storage of information in the hippocampal CA3 network.
This project received funding from the European Research Council (ERC) and Marie Skłodowska-Curie Actions (grant agreement 754411) under the European Union's Horizon 2020 research and innovation program (grant agreement 692692), and the Fond zur Förderung der Wissenschaftlichen Forschung (Z 312-B27, Wittgenstein award). The authors have no conflicts of interest to declare.

Disclosures: V.M. Vargas Barroso: None. **J.F. Watson:** None. **P.M. Jonas:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Centre National de la Recherche Scientifique
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Fondo di Ateneo per la Ricerca

Title: Excitatory drive of cortical fast-spiking GABAergic interneurons is set by D-serine acting on NMDA receptors.

Authors: *I. N. DE OLIVEIRA SOUZA[1,2], P. LECOUFLET[1], S. MALDERA[1], B. POTIER[1], L. POLLEGIONI[3], J.-P. MOTHET[1];
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Abstract: N-methyl-D-aspartate receptors (NMDARs) populate GABAergic interneurons, where they play a critical role in shaping circuit motifs and memory. However, we are largely ignoring whether and how NMDARs at GABAergic interneurons are gated by signals released in their surrounding microenvironment. Here we explore the dynamics of the co-agonist site occupancy by D-serine and glycine at glutamatergic synapses onto parvalbumin positive GABAergic interneurons in the adolescent prefrontal cortex, an area central to complex cognitive operation. By combining cellular electrophysiology with the use of unique pharmacological interventions and genetic manipulations, we report that the firing activity of layer 5 FS-PV+ interneurons and their excitatory synaptic coupling with principal neurons is under the control of NMDA receptors which are gated by D-serine but not glycine and that the identity of the co-agonist is not determined by the synaptic regime of the excitatory input. We further show that D-serine-deficient mice, a model of NMDAR hypofunction that exhibits schizophrenia-like phenotypes display attenuated firing pattern of the interneurons and no long-term potentiation. Our study extends the physiological implications of D-serine in brain physiopathology by uncovering its control of inhibitory synaptic networks through NMDARs.

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Poster

034. Physiological Synaptic Transmission and Modulation

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Topic: B.04. Synaptic Transmission

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European Commission NEUROTWIN grant (857562) to DAR
Wellcome Trust Collaborative Award (UNS120639) to LLL and DAR

Title: Imaging GABA release at individual synapses using genetically encoded optical sensor variants

Authors: *O. KOPACH[1], T. P. JENSEN[1], L. L. LOOGER[2], J. S. MARVIN[2], I. KOLB[2], J. P. HASSEMAN[3], D. A. RUSAKOV[1];
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Abstract: The genetically encoded, fluorescence intensity-based optical sensor of the inhibitory neurotransmitter GABA, iGABASnFR, has enabled monitoring of GABA release from multiple axons evoked by either electric stimulation or by epileptiform discharges (Marvin et al., 2019). Here we report experiments with the recently updated sensor variant, iGABASnFR2, that allowed us to detect GABA release from individual presynaptic axonal boutons activated by firing of a single interneuron, in acute brain slices. We used in vivo AAV9-based transduction of iGABASnFR2 variants, employing either a neuronal or astrocytic promoter, combined with high-resolution multiplexed two-photon excitation imaging. The sensor transduction protocol provided a sufficient level of iGABASnFR2 expression after three- to four- weeks post-injection to enable monitoring of GABA release events from individual axonal boutons of CA1 interneurons held in whole-cell mode. In conditions close to physiological, iGABASnFR2 expressed by either neurons or astrocytes showed the potential to detect GABA release events at a single synapse.

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Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.14

Topic: B.04. Synaptic Transmission

Support: NIH intramural awards to CJM and KZ
CIHR Operating grant to KT

Title: Of mice and men: evolutionary conservation of detonator synapse properties

Authors: *K. A. PELKEY[1], G. VARGISH[1], L. PELLEGRINI[3], D. CALVIGIONI[1], J. CHAPETON[2], X. YUAN[1], S. HUNT[1], R. CHITTAJALLU[1], K. TOTH[3], K. ZAGHLOUL[2], C. J. MCBAIN[1];

[1]NICHD, [2]NINDS, NIH, Bethesda, MD; [3]Cell. and Mol. Med., Univ. of Ottawa, Ottawa, ON, Canada

Abstract: As fundamental units of nervous system information transfer, synapses are foundational building blocks necessary for survival, consciousness, and behavior of multicellular organisms. Indeed, normal synaptic communication underpins most basic nervous system functions from control of internal body homeostasis to complex cognitive processes. Conversely, synaptopathy yields disorders such as autism. Though investigated to exquisite detail in model organisms, synapses have undergone minimal functional interrogation in the human brain. This is particularly true for specialized synapses such as the hippocampal mossy fiber (MF) "detonator synapse" which is considered essential for contextual memories. The ability of MF-CA3 synapses to support spatial memory encoding is theorized to stem from various peculiar synaptic features. Indeed, a rich literature describes several unique properties of MF synapses including sparse innervation by large multi-release site terminals supporting a remarkable frequency-dependent dynamic range of transmission onto the most proximal dendrites of principal cell targets, dramatic susceptibility to presynaptic modulation, rapid AMPAR dominated kinetics with minimal NMDAR contribution, "detonator" capabilities, and presynaptic NMDAR-independent long-term plasticity. Ultimately, the goal of examining synapses in rodents to such granular detail is for insight into human brain function. Thus, translation and relevance of rodent MF findings to human hippocampal function demands validation that human MFs display similar unique properties. In patient tissue resected for treatment of epilepsy we confirmed that human MF transmission exhibits all unique hallmark features described in rodents, including AMPAR dominated synapses with small contributions from NMDARs and KARs, large dynamic range with strong frequency-facilitation, NMDAR-independent presynaptic long-term potentiation, and strong cAMP sensitivity of release modulated by group II mGluRs. Moreover, serial array tomography EM confirmed evolutionary conservation of MF synapse ultrastructure. While data interpretation could be confounded by the diseased nature of resected tissue the astonishing congruence of core features shared between rodent and human MF synapses argues that basic properties of MFs dissected in rodents are also critical to human MF function. However, of interest from the disease perspective, we observed a dramatic selective deficit in GABAergic inhibitory tone onto human MF postsynaptic targets, suggesting that unrestrained detonator excitatory drive contributes to circuit hyperexcitability in human epilepsy.

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Poster

034. Physiological Synaptic Transmission and Modulation

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Topic: B.04. Synaptic Transmission

Support: ERC
SFB 1089

Title: Primary thalamus recruits and spares an equally high heterogeneous population of inhibitory neurons in the deep layers of the Barrel cortex.

Authors: *L. MESSORE[1], F. YÁÑEZ[2], J. M. GUEST[3], M. OBERLAENDER[4];
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Abstract: Synaptic inhibition can be described as being mediated by mainly two circuit configurations -feedforward (FF) and feedback inhibition (FB). However, which neurons takes part in each configuration and how informative their cellular properties are to their embedding in the circuit configuration, remains a topic of debate. Here, we analyze the electrophysiological response of L4 and L5 inhibitory and excitatory neurons, both to a sensory evoked multi-whisker stimulus and to a direct optogenetic stimulation of the thalamocortical synapses. Consistent with the FF and FB circuits described in the literature, we find a clear distinction between a subset of inhibitory neurons whose response precedes and those who succeeds the excitatory response. This distinction is maintained after an optogenetic activation of the thalamocortical synapses, which could indicate a difference in the thalamic input these populations receive. We confirm this by anatomically assessing the thalamocortical input by injecting a trans-synaptic virus tracer in the primary thalamus and barrel cortex. We then label the recorded neurons for a post hoc morphological reconstruction, and analyze how the classical cellular properties such as their molecular identity, axon and dendrite morphologies, and spiking activity reflect their place and function in the cortical circuitry. Our data shows that the aforementioned cellular properties are equally heterogeneous in both circuit configurations. We conclude that consistent with previous studies in the field, inhibitory neurons either precede or succeed the excitatory activation, as described for the FF and FB circuit configurations. Nevertheless, we do not see a clear distinction between these populations in term of their classical cellular properties, and it seems that primary thalamus targets and spares an equally heterogeneous population of inhibitory neurons in L4 and L5 of the barrel cortex.

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Poster

034. Physiological Synaptic Transmission and Modulation

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Topic: B.04. Synaptic Transmission

Support: NIH NINDS 1RO1NS112365-01A1
NSF CAREER IOS 1750199

Title: Vamp-associated protein (vap) regulates presynaptic organelle calcium handling and synaptic transmission

Authors: *C. D. PATON, L. C. PANZERA, M. B. HOPPA;
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Abstract: The endoplasmic reticulum (ER) is known to contact organelles throughout the cell to regulate calcium homeostasis, as well as protein and lipid synthesis. Membrane contact sites (MCS) are known to form between the ER and a multitude of organelles including mitochondria, peroxisomes, endosomes, as well as the plasma membrane. MCSs are typically spaced by 10-20nm and maintained by proteins from both organelle membranes, or a protein lipid interaction. Elucidating the molecular identity and function of various MCSs is challenging experimentally due to limited experimental techniques and redundancy in interorganellar tethering machineries. Recent experiments form our group has demonstrated that the voltage-gated potassium (Kv) channel 2.1 has both a non-conducting role in the axon, where it forms a stable junction between the ER and PM via ER VAMP-associated protein (VAP). Loss of Kv2.1 does not alter the axonal action potential, but greatly impairs both neurotransmitter release and ER Ca^{2+} uptake. Restoration of this function requires Kv2.1's VAP binding domain. We have developed CRISPR based knockdown approach to acutely deplete both isoforms of VAP (VAPA and VAPB) to better understand its role in synaptic transmission and calcium handling. We combined knockdown with noninvasive optical indicators of exocytosis and calcium in cultured rat hippocampal neurons. We demonstrate that loss of VAPA/B decreases axonal synaptic vesicle release probability and calcium handling between various organelles during electrical stimulation. Through a more complete understanding of how VAP contributes to calcium homeostasis, we can gain insight into its molecular role in the axon and potential role in diseases of the axon.

Disclosures: **C.D. Paton:** None. **L.C. Panzera:** None. **M.B. Hoppa:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Topic: B.04. Synaptic Transmission

Support: NIH Grant R01NS115508

Title: Regulation of hippocampal synaptic transmission by STIM proteins

Authors: *K. S. KORSHUNOV, M. E. MARTIN, M. PRAKRIYA;
Pharmacol., Northwestern Univ. Feinberg Sch. of Med., Chicago, IL

Abstract: Store-operated calcium entry (SOCE) is a ubiquitous Ca^{2+} influx mechanism initiated by Ca^{2+} release from the endoplasmic reticulum (ER), which triggers the opening of the Ca^{2+}-

release activated Ca^{2+} (CRAC) channels formed by the Orai proteins. Previous work indicates that CRAC channels formed by Orai1 are important for synaptic plasticity and cognitive functions involving learning and memory. However, the mechanism(s) by which Orai1 channels are activated in response to store depletion in hippocampal neurons is not well-understood. In the canonical SOCE pathway, Orai1 is activated by the ER Ca^{2+} sensors, STIM1 and STIM2, which sense the depletion of Ca^{2+} stores and physically interact with Orai1 channels to gate them open. In this study, we addressed the roles of STIM1 for synaptic transmission using conditional STIM1 knockout mice in hippocampal slice recordings. Previous work in neuronal cultures has indicated that STIM1 and STIM2 regulate several aspects of hippocampal neuronal function including synaptic release probability and amplitude of excitatory postsynaptic currents. Our current studies in slices indicate that deletion of the STIM1 proteins evokes significant alterations in several aspects of hippocampal synaptic transmission and changes in membrane excitability. We anticipate that these studies will expand on the growing knowledge of the role of SOCE proteins in regulating synaptic plasticity and excitability in the brain.

Disclosures: K.S. Korshunov: None. **M.E. Martin:** None. **M. Prakriya:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.18

Topic: B.04. Synaptic Transmission

Title: Docosahexaenoic acid and arachidonic acid promote synchronized neuronal activity

Authors: *S. MORITA[1,2], T. KONDO[1,3,4], H. TOKUDA[2], Y. KANEDA[2], T. ROGI[2], T. IZUMO[2], M. NAKAI[2], H. INOUE[1,3,4];
[1]iPSC-based Drug Discovery and Develop. Team, RIKEN BioResource Res. Ctr. (BRC), Kyoto, Japan; [2]Inst. for Hlth. Care Sci., Suntory Wellness Ltd., Kyoto, Japan; [3]Ctr. for iPS Cell Res. and Application (CiRA), Kyoto Univ., Kyoto, Japan; [4]Medical-risk Avoidance based on iPS Cells Team, RIKEN Ctr. for Advanced Intelligence Project (AIP), Kyoto, Japan

Abstract: Docosahexaenoic acid (DHA) and arachidonic acid (ARA) are essential fatty acids found in fish, meat, and eggs. They are also the major polyunsaturated fatty acids (PUFA) present in the brain. Some studies have indicated that supplementation with DHA and ARA has positive effects on human brain function, whereas a deficiency of these PUFAs may increase the risk of disease. However, information on how DHA and ARA function in the brain is lacking. In neurons, these lipids play critical roles in the formation, maintenance, and function of synapses. In particular, DHA has been reported to promote synaptogenesis, synaptic connectivity, and neural network maturation. In contrast, similar data on ARA are lacking. In the present study, we examined the effects of DHA and ARA on neural networks in long-term cultures of neurons differentiated from human induced pluripotent stem cells (iPSCs). Human iPSCs derived from healthy individuals were differentiated into neurons and then mixed with astrocytes to establish a

coculture system. The free forms of DHA and ARA were dissolved in dimethyl sulfoxide (DMSO) and mixed with the culture medium. After 16 weeks of culture with DHA and ARA, the cells were analyzed for changes in lipids, morphology, and neuronal activity. The fatty acids constituting the phospholipids were changed by the addition of DHA and ARA in the culture medium. The cells showed an increased ratio of DHA and ARA, and immunostaining revealed that the neurites of the neurons were longer and more branched, and thereby formed a more complex morphology. In addition, the sizes of the presynaptic and postsynaptic sites were both increased. Similar effects on neurite length, branching, and complex morphology were observed with treatments of either DHA or ARA alone or DHA and ARA combined. The effects of either DHA or ARA alone on synaptic size were similar, but stronger effects were observed when they were combined. Because the observed changes in neuronal morphology predicted changes in neuronal activity, we used a multi electrode array (MEA) system to record and analyze temporal changes in neuronal activity. Increased synchronized neuronal activity was observed when DHA and ARA were combined. Collectively, these results indicate that DHA and ARA increase synchronized neuronal activity through changes in neurite outgrowth and synaptic formation; thus, both lipids are important for the maturation of neural networks.

Disclosures: **S. Morita:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **T. Kondo:** None. **H. Tokuda:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **Y. Kaneda:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **T. Rogi:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **T. Izumo:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **M. Nakai:** A. Employment/Salary (full or part-time):; Suntory Wellness Ltd. **H. Inoue:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Topic: B.04. Synaptic Transmission

Support: Roy J. Carver Charitable Trust/Iowa Neuroscience Institute
IO1BX004440 (VA Merit Review Award)

Title: Contributions of Acid Sensing Ion Channels to synaptic transmission and plasticity in cerebellar Purkinje cells

Authors: J. HARDIE, J. A. WEMMIE;
Univ. of Iowa, Iowa City, IA

Abstract: The cerebellum is critical for motor learning and cognition, and is increasingly recognized as an important site of dysfunction in a number of neurological illnesses. Accumulating evidence suggests that pH dynamics may play a critical role in the cerebellum. Purkinje cells (PCs) throughout the cerebellar cortex express abundant levels of acid-sensing ion

channels (ASICs). These channels are permeable to Na^+ and Ca^{2+} and are activated when extracellular pH becomes acidic. Although their function in PCs is not clear, the abundant ASIC expression suggests that they likely play important roles in the cerebellar cortex. Previous studies from our laboratory found that whole animal *ASIC1a*$^{-/-}$ mice were deficient in eye-blink conditioning, a form of associative learning that is dependent on the cerebellum. Here, we probed the role of ASIC1A in PC physiology, synaptic transmission, and plasticity using whole cell patch-clamp recordings in acute cerebellar slices. We found that delivering extracellular acid to the soma and dendrites of PCs results in depolarizing inward current in wild-type mice that is not present in ASIC1a$^{-/-}$ mice. Additionally, we identified a component of the excitatory postsynaptic current (EPSC) in PCs upon parallel fiber stimulation that was sensitive to the ASIC inhibitor amiloride, and absent in *Asic1a*$^{-/-}$ mice. Moreover, loss of this EPSC was accompanied by deficits in climbing fiber (CF)-PF long-term depression (LTD). With fluorescent calcium imaging, we found a marked reduction in intracellular calcium entry in *Asic1a*$^{-/-}$ compared to *Asic1a*$^{+/+}$ mice during parallel fiber stimulation. Thus, ASIC1A is present in cerebellar PCs, contributes to parallel fiber synaptic transmission, and is required for CF-PF plasticity, potentially by increasing Ca^{2+} influx during PF synaptic transmission. These findings suggest important roles of pH-dependent signaling in the cerebellum, which may contribute to physiological underpinnings of motor learning and cognition.

Disclosures: **J. Hardie:** None. **J.A. Wemmie:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.20

Topic: B.04. Synaptic Transmission

Title: Activity-dependent changes in ph at the mouse neuromuscular junction

Authors: *R. DURBIN[1], D. HEREDIA[2], T. W. GOULD[2], R. B. RENDEN[2];
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Abstract: Changes in pH at the synapse during stimulation impact neurotransmission, and altered pH regulation may contribute to various disease states. During activity at sensory ribbon synapses, the synaptic cleft acidifies due to protons released from vesicles. At more conventional glutamatergic synapses, the cleft alkalinizes due to Ca^{2+} extrusion and H^+ antiport via the Plasma Membrane Calcium ATPase (PMCA). Here, we tested whether neurotransmission at the vertebrate neuromuscular junction (NMJ) affects synaptic cleft pH. Using fluorescence imaging of cytosolic Ca^{2+} and cleft pH transients at the mouse levator auris longus (LAL) neuromuscular junction, *ex-vivo* LAL preparations with intact motor nerves from 3-6 months old C57Bl/6 mice were used. Stimuli were driven via electrical field stimulation of the facial nerve innervating the LAL. To facilitate imaging, we blocked muscle contraction with either 3-(N-butylethanimidoyl)-

4-hydroxy-2H-chromen-2-one (BHC), a myosin inhibitor that allows for intracellular release of Ca^{2+}; or μ-conotoxin, a Nav1.4 channel blocker that inhibits the release of intracellular Ca^{2+} stores during transmission. Cytosolic Ca^{2+} in muscle was measured in transgenic mice expressing GCaMP3 in selectively muscle. We found significantly smaller Ca^{2+} transients when preparations were incubated with μ-conotoxin instead of BHC, confirming the release of Ca^{2+} from intracellular stores when BHC is applied. Synaptic cleft pH transients were measured following adeno-associated virus transduction with pHusion-ex, a pH-sensitive fluorescent probe. We did not find any significant pH transients for brief high frequency stimulation trains (50Hz, 250ms); however, when postsynaptic intracellular Ca^{2+} release was intact, we observed significant alkalization followed by prolonged acidification during sustained high frequency stimulation (50 Hz, 20 sec). When stimulation duration was reduced (5 sec, 50 Hz), we saw alkalization but no cleft acidification. In the presence of μ-conotoxin, no pH transients were observed, indicating intracellular Ca2+ release is required to alkalinize cleft pH. Antibody staining identified a unique organization of PMCA adjacent to synaptic terminals and postsynaptic receptors, surrounding the motor endplate. Despite identifying the pH changes due to stimulation and the presence of PMCA, the mechanisms underpinning the alkaline and acidic pH changes remain unclear. Using PMCA and monocarboxylate transporters as targets, future experiments directly elucidating these mechanisms are underway.

Disclosures: **R. Durbin:** None. **D. Heredia:** None. **T.W. Gould:** None. **R.B. Renden:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.21

Topic: B.04. Synaptic Transmission

Support: ZRM supported by Higher Committee for Education Development (HCED) scholarship in Iraq.
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W.P.P. was supported by Brazilian National Council for Scientific and Technological Development (CNPq) under grant number 202970/2014-0

Title: Alterations in synaptic transmission by acute changes in pH: extracellular and cytoplasmic

Authors: *R. VASCASSENNO[1], W. PIEDADE[2], Z. R. MAJEED[3], S. L. E. BLUMICH[4], E. BRAILOIU[5], R. L. COOPER[6];

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Abstract: The presynaptic terminals package transmitter into vesicles based on a proton gradient. We address issues related to altering this gradient in influencing synaptic responses. We are addressing two issues: (1) the influence of pHi on vesicular packaging of neurotransmitter; (2) response of glutamate receptors on postsynaptic targets with altering extracellular and intracellular pH. These investigations are being addressed at the model crayfish and Drosophila neuromuscular junctions (NMJs). These two projects are interrelated as transmission at glutamatergic synapses is retarded in the presence of CO2 which cannot be fully accounted for by a reduced pHi within the presynaptic nerve terminal or within the postsynaptic muscle fiber since the EPSPs increase in amplitude with rebound acidification after a pulse of NH4Cl. High (40 mM) proprionic acid acidifies both the pre- and post-synaptic cells. When used the frequency and amplitude of mini's increases despite a slight membrane depolarization. However, evoked transmission is blocked. Examining low pH on mammalian glutamatergic neurons with Fura 2 (Ca2+ indicator) in culture indicated Ca2+ release from ER as a potential mechanism to explain some of the observations for the increase in frequency of minis. The use of high [CO2] containing saline blocks evoked and mini's as well as the sensitivity of glutamate receptors. These NMJs are glutamatergic and the evoked (non-spiking) synaptic potentials and spontaneous (quantal) events are readily measured. Addressing the mechanisms underlying these observed phenomena may help in understanding synaptic depression after high frequency stimulation and feedback process in synaptic transmission. These studies tackle fundamental principles which are likely present in glutamatergic neurons for all animals.

Disclosures: R. Vascassenno: None. **W. Piedade:** None. **Z.R. Majeed:** None. **S.L.E. Blumich:** None. **E. Brailoiu:** None. **R.L. Cooper:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 034.22

Topic: B.04. Synaptic Transmission

Support: NIH Grant 5R01NS111749-04

Title: Snap25 differentially contributes to gαi receptor function in the nucleus accumbens

Authors: *J. C. ZEPEDA[1], K. M. MANZ[2], Z. ZURAWSKI[1], H. E. HAMM[1], B. A. GRUETER[2];
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Abstract: The nucleus accumbens (NAc) is a brain structure which guides vital motivated-behaviors such as food-seeking and reproduction through activation of complex circuitry. NAc medium spiny projection neurons (MSNs) require glutamatergic drive from cortical, thalamic, and limbic brain inputs in order to generate action potentials and in turn MSNs project long-range GABAergic axons to other brains regions. Neuromodulatory systems within the NAc are able to dampen glutamatergic neurotransmission through activation of Gi/o G-protein-coupled receptors (GPCRs). Previous work has shown that Gβγ subunits directly bind to the t-SNARE protein SNAP-25, but it remains unknown what contribution SNAP-25 may have on Gi/o GPCR signaling within the NAc. We hypothesized that SNAP-25 may differentially couple to the Gβγ complexes of different Gi/o GPCRs located in the NAc. In order to assess this possibility, we used a mouse line which contains a C-terminal three amino acid deletion of Snap25 that impairs its ability to associate with the Gβγ complex (Snap25Δ3) to survey how SNAP-25 may be contributing to Gi/o GPCR signaling at excitatory synapses within the NAc. Using a combination of whole-cell voltage-clamp electrophysiology and pharmacology we demonstrate that the Snap25Δ3 mice have altered basal excitatory neurotransmission properties, and that SNAP-25 contributes to the depression of glutamatergic signaling via GABAB, 5HT1 B/D, and Mu-opioid receptors, but not Kappa-opioid, CB1, group II mGluRs, and H3 receptors in the NAc core. The NAc is conserved from mice to humans and has been strongly implicated within various human brain disorders such as generalized anxiety disorder, eating disorders, major depressive disorder, and substance use disorders. These studies advance our understanding of key molecular mechanisms that guide neuromodulation and excitatory neurotransmission within the NAc.

Disclosures: J.C. Zepeda: None. **K.M. Manz:** None. **Z. Zurawski:** None. **H.E. Hamm:** None. **B.A. Grueter:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.23

Topic: B.04. Synaptic Transmission

Support: Holman Research Grant

Title: Pre- and post-synaptic loss of bone morphogenic protein family expression in subsets of neurons differentially modulates motor behavior in Drosophila melanogaster

Authors: *K. M. LEMBKE[1], M. PRYOR[2], S. TRAVIS[2];
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Abstract: Bone morphogenic proteins (BMPs) are a highly conserved family of essential signaling molecules that regulate a variety of cellular processes, including cell differentiation, proliferation, and tissue patterning. In the central nervous system, BMP signaling influences not only regulates pattern formation, but also synaptic connectivity and synaptic homeostasis, though

the exact mechanisms of these actions remain obscure. At the Drosophila melanogaster neuromuscular junction (NMJ), the BMP ligand Decapentaplegic (Dpp) is necessary for normal synaptic morphology and intact synaptic homeostasis. Canonically, Dpp binds its receptors Thickveins (Tkv), Saxophone (Sax), and Punt (Put), some of which are equally necessary for normal synaptic morphology. It is also possible that, non-canonically, Dpp wields its homeostatic function at the NMJ via an interaction with the type 2 voltage-gated calcium channel (Cav2) Cacophony, which is required for both neurotransmission, synaptic homeostasis, normal motor neuron burst rhythmicity, and normal motor behavior. And while Dpp is not essential for neurotransmission, the necessity of pre- and post-synaptic BMP expression for normal motor physiology is not well understood. The goal of this work is to understand how loss of BMP signaling affects motor behavior and physiology in third instar Drosophila larvae. We used the Gal4/UAS system to knock down the expression of a variety of BMPs in subtypes of neurons and glia. We characterized the effect of these loss of functions on larval behaviotypes, including crawling. Our results show that loss of BMP expression differentially modulates motor behavior in a cell-specific manner.

Disclosures: **K.M. Lembke:** None. **M. Pryor:** None. **S. Travis:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

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Program #/Poster #: 034.24

Topic: B.04. Synaptic Transmission

Support: ANR-18-CE37-0020-01

Title: How the hypothalamic supramammillary-hippocampal network is altered in a mouse model of the 22q11.2 deletion syndrome

Authors: *E. LEPICARD, V. CHEVALEYRE, R. PISKOROWSKI;
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Abstract: Hippocampal area CA2 plays a central role in the encoding of social information. This small region is targeted by long-range glutamatergic axons from the hypothalamic supramammillary nucleus (SuM) that enable the detection of social novelty. In area CA2, these glutamatergic inputs form synapses with parvalbumin-expressing (PV+) interneurons (INs) and pyramidal neurons (PNs). Using a mouse model of the 22q11.2 deletion syndrome (DS), we have previously described a reduction in PV+ IN density in area CA2 that is similar to observations made in postmortem studies from schizophrenic and bi-polar patients. Furthermore, social memory of these mice is strongly impaired. In this study, we aim to examine the local circuitry between the SuM and area CA2. Specifically, how excitatory and feedforward inhibitory (FFI) transmission from the SuM to CA2 PNs are changed in a 22q11.2 DS model. To answer this question, we have crossed SuM-cre mice with 22q11.2 DS mice (LgDel) and used viral vectors

to selectively express channelrhodopsin-2 (ChR2) in SuM neurons. We found that FFI from SuM axons onto CA2 PNs is decreased in both female and male LgDel mice while the direct excitation to PNs is unchanged. Furthermore, we have found a decrease PV+ IN density in the deep pyramidal layer of CA2, where SuM axons are localized. Thus, the ability of SuM inputs to convey social novelty information to the hippocampus is likely compromised by the change in PV+ IN function, leading to compromised social memory.

Disclosures: E. lepicard: None. **V. Chevaleyre:** None. **R. Piskorowski:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.25

Topic: B.04. Synaptic Transmission

Title: Deletion of the primate-specific immunoglobulin BTN3A2 reduces the number of neurites and impacts synchronous activity of human cortical excitatory neurons

Authors: D. CABEZAS[1], M. ALSAQATI[1], J. HADDON[1], Y. ZHU[1], S. WAINWRIGHT[1], P. MAYCOX[2], *M. PAPAKOSTA[2], R. HODGSON[2], J. SCHACHTER[2], M. LI[1], L. GRAY[1], A. HARWOOD[1], J. HALL[1], L. WILKINSON[1];
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Abstract: Deletion of the primate-specific immunoglobulin BTN3A2 reduces the number of neurites and impacts synchronous activity of human cortical excitatory neurons
Authors (*presenting author, **sponsoring author): *D.F.D. Cabezas[1],M. Alsaqati[1], J. Haddon[1], Y. Zhu[1], S. Wainwright[1], P. Maycox[2], **M. Papakosta[2], R. Hodgson[2], J. Schachter[2], M. Li[1], L. Gray[1], A.J. Harwood[1], J. Hall[1], L.S. Wilkinson[1]
[1]Cardiff University, Neuroscience and Mental Health Innovation Institute, Cardiff, UK[2] Takeda Development Center Americas, Inc. (TDCA)
Abstract: The BTN3A subfamily of immunoglobulins is widely known for their activity in the immune system. However, very little is known of the activity of these proteins in the nervous system. Recent studies have pinpointed BTN3A2 as a potential candidate risk gene for schizophrenia, a neurodevelopmental disorder which impacts the normal neuronal activity leading to a complex mix of sensory, emotional and cognitive symptoms. We decided to investigate the role of BTN3A2 in neural development and how reduced expression of the gene could impact co-ordinated neuronal function. Using CRISPR/Cas9, we generated three hESC lines (BTN3A2KO). Deletion of BTN3A2 did not impede general features of neuronal differentiation. Similar to the control parental line, BTN3A2KO cells expressed markers of dorsal neural progenitors (PAX6, FOXG1, NES), and were able to generate rosettes (labelled by NCADH). At later stages of development/differentiation, cells expressed deep cortical layer markers (TBR1 and CTIP2). Neuronal activity of BTN3A2KO neurons was studied using microelectrode array (MEA) plates from day 50 to 100 of *in vitro* differentiation. Compared to

the control cells, neurons carrying the BTN3A2KO exhibited reduced synchronicity and firing rate. Using a sparse labelling approach by transfecting a GFP coding plasmid, we identified that BTN3A2KO cells showed a reduced number of neurites at days 70 and 80 of *in vitro* differentiation. These results reveal a previously uncharacterised role for BTN3A2 during cortical development, where a reduced dosage of this protein impairs neuronal connectivity leading to electrophysiological changes, in particular a disruption to the ability of neurons to develop co-ordinated synchronous activity. The data may be of relevance to synaptic abnormalities increasingly thought to be key contributors to the pathogenesis of neurodevelopmental disorders including schizophrenia.

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Poster

034. Physiological Synaptic Transmission and Modulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 034.26

Topic: B.04. Synaptic Transmission

Support: MH66198
GM007347
MH081060
MH070727

Title: The effect of brain derived neurotrophic factor on presynaptic function

Authors: *C. WANG[1], E. T. KAVALALI[2], L. M. MONTEGGIA[3];
[2]Vanderbilt Brain Inst., [1]Vanderbilt Univ., Nashville, TN; [3]Vanderbilt Brain Inst., Nashville, TN

Abstract: Brain-derived neurotrophic factor (BDNF) plays a critical role in modulating synaptic physiology and contributes to the fundamental pathophysiology of neuropsychiatric disorders. Numerous studies have delineated the critical role of BDNF in the treatment of major depression. Despite its importance in the pathophysiology of major depression, its function at the presynaptic terminal, a fundamental unit of neurotransmission, remains poorly elucidated. To investigate how BDNF affects the dynamics of a single synapse, we applied exogenous BDNF in primary hippocampal cultures and measured presynaptic parameters with optical imaging. We further corroborated our findings by via genetic deletion of BDNF's high affinity TrkB receptor. We found that there appears to be a differential effect on different modes of neurotransmitter release, and that these effects occur through a calcium-dependent mechanism. Understanding the mechanism of BDNF at the presynaptic level is a critical question that remains largely

unanswered. Investigating this question at the level of single synapses can provide important and novel information about BDNF's effect on synaptic physiology and signaling.

Disclosures: C. Wang: None. **E.T. Kavalali:** None. **L.M. Monteggia:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.27

Topic: B.04. Synaptic Transmission

Support: A&S Summer Research Fellowship (MW)
SCoBIRC Endowed Chair #3 (P.G.S., H.J.V.)
Department of Biology, University of Kentucky
Chellgren Fellowship (RLC)

Title: Examining the effect of iron (ferric) on physiological processes in invertebrate models

Authors: *M. WAGERS[1], H. J. VEKARIA[2], P. G. SULLIVAN[2], R. L. COOPER[3];
[1]Biol., [2]Neurosci., [3]Univ. of Kentucky Dept. of Biol., Univ. of Kentucky, Lexington, KY

Abstract: Iron (Fe3+) is an essential element for maintaining life in plants and animals and is found in soil, fresh waters and marine waters. Iron is used within mitochondrial electron transport complexes as well as a co factor in numerous proteins, thus its importance in cellular function for various organisms. However, overexposure results in toxicity and iron accumulation in the mammalian central nervous system is associated with various neurological disorders. Although current literature has investigated long term effects of iron overload on organisms, studies of the acute effects are lacking. Thus, the present study seeks to ascertain the acute effects of iron overload on development, behavior, survival, cardiac function, and glutamatergic synaptic transmission in the Drosophila melanogaster. Additionally, the effects of iron overload on proprioception and mitochondrial function were investigated using blue crab (Callinectes sapidus). Acute exposure depresses development, behavior, survival, and cardiac function. Synaptic transmission is reduced but ionotropic glutamatergic receptors remain functional, suggesting a blockage of pre synaptic voltage gated Ca2+ channels. Fe3+ also reduces excitability and function in proprioceptive neurons. Thus, Fe3+ likely blocks stretch activated channels and voltage gated Na+ channels. The effects are partly reversible with acute exposure, indicating the cells are not rapidly damaged. Mitochondrial function remains present in nerve bundles after 10 min exposure to Fe3+ suggesting Fe3+ does not rapidly permeate the cells. This study is relevant in demonstrating the effects of acute Fe3+ exposure on various physiological functions and holds future applications in both clinical and environmental studies.

Disclosures: M. Wagers: None. **H.J. Vekaria:** None. **P.G. Sullivan:** None. **R.L. Cooper:** None.

Poster

034. Physiological Synaptic Transmission and Modulation

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Program #/Poster #: 034.28

Topic: B.04. Synaptic Transmission

Support: R35GM128823

Title: Neuronal activity-driven O-GlcNAcylation promotes mitochondrial bioenergetics

Authors: S. B. YU[1], R. G. SANCHEZ[1], Z. D. PAPICH[1], T. C. WHISENANT[2], ***G. PEKKURNAZ**[1];
[1]Neurobio., [2]Ctr. for Computat. Biol. and Bioinformatics, Univ. of California San Diego, La Jolla, CA

Abstract: Neuronal activity is an energy-intensive process that is largely sustained by activity-dependent mitochondrial ATP synthesis. Here, we describe a novel signaling pathway involving the enzyme O-GlcNAc transferase (OGT) that is capable of regulating mitochondrial bioenergetics via O-GlcNAcylation. Here, we demonstrate that neuronal activity upregulates O-GlcNAcylation in neuronal processes, and identify mitochondria as the loci of this post-translational modification. This pathway allows neuronal mitochondria to sense glucose availability and promotes mitochondrial bioenergetics to compensate for high energy expenditure. Mapping of the neuronal mitochondrial O-GlcNAcome identified proteins that participate in oxidative phosphorylation and ATP production as the primary targets of OGT. Finally, by measuring activity-dependent ATP synthesis, we demonstrate that neurons fail to replenish ATP after neuronal stimulation in the absence of O-GlcNAc cycling. Our findings suggests that O-GlcNAc cycling mediates glucose-dependent feedback control in neurons to optimize mitochondrial performance based on neuronal activity and energy demand. This mechanism thereby couples glucose metabolism to mitochondrial bioenergetics and plays a key role in sustaining neuronal energy homeostasis.

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Poster

035. Synaptic Plasticity

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 035.01

Topic: B.04. Synaptic Transmission

Support: CIHR, FDN-147473
Canada Research Chair Tier 1 (950-232211

Title: Insulin induces long-term depression of glutamatergic projections to ventral tegmental area dopamine neurons from the pedunculopontine tegmental nucleus

Authors: *D. NEYENS, S. BORGLAND;
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Abstract: Dopamine neurons of the ventral tegmental area (VTA) confer salience, reward, and motivational properties to external and internal cues as they relate to appetitive and motivated behavior. Importantly, these neurons are sensitive to peptides that signal metabolic status, such as insulin. VTA insulin signaling decreases dopamine release and the contextual associations of palatable food and psychomotor responses to cocaine. Previous work from our lab has shown that insulin induces an endocannabinoid mediated long-term depression (LTD) in glutamatergic projections to VTA dopamine neurons; however, the identity of these glutamatergic inputs was unknown. Here, we used optogenetics and slice electrophysiology to selectively stimulate glutamatergic projections to VTA dopamine neurons from the pedunculopontine tegmental nucleus (PPTg), a region that encodes sensorimotor and reward-related stimuli and sends dense projections to the VTA. Eight-week-old male and female mice received bilateral injections into the PPTg of a viral construct containing ChR2 (AAV2 CamKII-hCHR2(H134R)-EYFP). Virus expression within the PPTg and projections to the VTA were histologically confirmed at 4-6 weeks, and all electrophysiology was performed within this window. Acute activation of ChR2 with blue light (5 mW, 0.1 ms, 473 nm) in the presence of picrotoxin produced robust optically-evoked excitatory postsynaptic currents (oEPSCs) in nearly all dopamine neurons recorded from horizontal VTA slices. These oEPSCs were compound events containing both monosynaptic and polysynaptic input, as determined by treatment with 500 nM TTX and 100 µM 4-AP. Bath application of insulin induced LTD in the majority of compound oEPSCs, which were afterwards confirmed to contain monosynaptic oEPSCs. Slices were then pretreated with TTX/4-AP to measure the effect of insulin on monosynaptic inputs from the PPTg. Interestingly, we observed a more modest effect of insulin to induce LTD on isolated monosynaptic inputs compared to compound oEPSCs, suggesting that insulin-induced LTD is present in polysynaptic circuits. Future experiments will address the mechanism of the polysynaptic effect, if these effects are mediated by insulin receptor signaling, and the possible involvement of endocannabinoids. Taken together, these findings suggest that insulin signaling in VTA dopamine neurons depresses glutamatergic input from the PPTg.

Disclosures: D. Neyens: None. **S. Borgland:** None.

Poster

035. Synaptic Plasticity

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 035.02

Topic: B.04. Synaptic Transmission

Title: MDGA1 negatively regulates amyloid precursor protein-mediated synapse inhibition in the hippocampus

Authors: *J. KIM[1], S. KIM[1], H. KIM[1], I.-W. HWANG[2], S. BAE[1], S. KARKI[3], D. KIM[1], R. OGELMAN[2], G. BANG[4], J. KIM[4], T. KAJANDER[3], J. UM[1], W. OH[2], J. KO[1];
[1]Dept. of Brain Sci., Daegu Gyeongbuk Inst. of Sci. and Technol., Daegu, Korea, Republic of; [2]Univ. of Colorado Sch. of Med., Max Planck Florida Inst. for Neurosci., Aurora, CO; [3]Univ. of Helsinki,, Helsinki, Finland; [4]Korea Basic Sci. Inst., Ochang, Korea, Republic of

Abstract: Balanced synaptic inhibition, controlled by multiple synaptic adhesion proteins, is critical for proper brain function. MDGA1 (meprin, A-5 protein, and receptor protein-tyrosine phosphatase mu [MAM] domain-containing glycosylphosphatidylinositol anchor protein 1) suppresses synaptic inhibition in mammalian neurons, yet the molecular mechanisms underlying MDGA1-mediated negative regulation of GABAergic synapses remain unresolved. Here, we show that the MDGA1 MAM domain directly interacts with the extension domain of amyloid precursor protein (APP). Strikingly, MDGA1-mediated synaptic disinhibition requires the MDGA1 MAM domain and is prominent at distal dendrites of hippocampal CA1 pyramidal neurons. Down-regulation of APP in presynaptic GABAergic interneurons specifically suppressed GABAergic, but not glutamatergic, synaptic transmission strength and inputs onto both the somatic and dendritic compartments of hippocampal CA1 pyramidal neurons. Moreover, APP deletion manifested differential effects in somatostatin- and parvalbumin-positive interneurons in the hippocampal CA1, resulting in distinct alterations in inhibitory synapse numbers, transmission, and excitability. The infusion of MDGA1 MAM protein mimicked postsynaptic MDGA1 gain-of function phenotypes that involve the presence of presynaptic APP. The overexpression of MDGA1 wild type or MAM, but not MAM deleted MDGA1, in the hippocampal CA1 impaired novel object recognition memory in mice. Thus, our results establish unique roles of APP-MDGA1 complexes in hippocampal neural circuits, providing unprecedented insight into trans-synaptic mechanisms underlying differential tuning of neuronal compartment-specific synaptic inhibition.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.03

Topic: B.04. Synaptic Transmission

Support: FONDECYT Regular # 1201848
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Millennium Science Initiative Program ACE210014

Title: Serotonin via 5-HT2a receptors induces an input specific form of inhibitory synaptic plasticity in the prefrontal cortex

Authors: *R. C. MEZA[1], C. ANCATÉN-GONZÁLEZ[1], N. SANGUINETTI[1], M. FUENZALIDA[2], P. R. MOYA[1], C. Q. CHIU[1], A. E. CHÁVEZ[1];
[1]Ctr. Interdisciplinario de Neurociencias (CINV), Univ. de Valparaiso, Valparaiso, Chile; [2]Inst. de Fisiología, Univ. De Valparaiso, Valparaiso, Chile

Abstract: Serotonergic (5-HT) fibers from the raphe nuclei are known to regulate neuronal excitability and glutamatergic synaptic function in the prefrontal cortex (PFC) by activating different 5-HT receptor subtypes (5-HTRs). However, little is known about the mechanisms by which 5-HT tune inhibitory synaptic strength in the PFC. In whole-cell patch recordings, we find that brief pharmacological activation of 5-HT2aRs induces a long-term depression of inhibitory postsynaptic currents (IPSC-LTD) onto layer II/III pyramidal neurons elicited by local electrical stimulation of synaptic inputs. 5-HT2aR-mediated IPSC-LTD is expressed presynaptically and requires the activation of type 1 cannabinoid receptors (CB1Rs). We hypothesize that 5HT2aR activation may trigger endocannabinoid production to recruit presynaptic CB1Rs to subsequently suppress GABA release. Indeed, application of CB1R agonist WIN55,212-2 also reduces IPSCs presynaptically and this CB1R-mediated depression is occluded by a previous induction of 5-HT2aR-mediated IPSC-LTD, indicating a common mechanism in plasticity expression. Notably, repetitive optogenetic activation of 5-HT fibers alone is sufficient to trigger IPSC-LTD onto layer II/III pyramidal neurons that is frequency dependent and requires both 5-HT2aRs and CB1Rs. Interestingly, 5-HT2aR- and CB1R-mediated IPSC-LTD is input specific, occurring at inhibitory synapses from somatostatin- but not parvalbumin-positive GABAergic interneurons. Thus, our findings reveal a novel form of 5-HT-mediated regulation of GABAergic synaptic strength that is input-specific and strongly support a crosstalk between 5-HT2aRs and CB1Rs to modulate GABAergic inhibition in the PFC.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.04

Topic: B.04. Synaptic Transmission

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Title: Functional hemispheric asymmetry of medial habenula synapse associated with distinct modulation of emotion-related behaviors in mice

Authors: *C. ÖNAL, P. KOPPENSTEINER, R. SHIGEMOTO;
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Abstract: The habenula is a phylogenetically conserved bilateral brain structure known to modulate negative emotions. Hemispheric asymmetry in cortical areas plays a key role in behavior and cognition in human and experimental animals. Although habenular asymmetry is prominent in several vertebrate species, there is no evidence for left-right asymmetry in the mammalian habenula. To investigate the asymmetry in synaptic transmission in the medial habenula (MHb) to the interpeduncular nucleus (IPN) pathway in mice, we performed targeted electrical stimulation of left or right MHb-derived axons in acute slices. Using paired-pulse stimulation with 50 m.s. inter-stimulus interval (n=53 cells) and 50 Hz high-frequency stimulation (n=11 cells), we discovered that the probability of neurotransmitter release from left MHb terminals was significantly lower than that of right MHb terminal. Furthermore, activation of presynaptic $GABA_B$ receptors (GBR) using GBR agonist baclofen (1 μM) potentiated the release from left MHb terminals significantly stronger than that from right MHb terminals resulting in similar synaptic strength between the left and right (n=30 cells, 952.9±153.6, 543.4±68.49 % increase respectively). Finally, we selectively suppressed left or right cholinergic MHb neurons using stereotaxic injection of floxed inhibitory DREADDs-expressing AAV into 8-12 weeks old male $ChAT^{Cre}$ mice and performed cued fear conditioning. Chemogenetic inhibition of left but not right MHb significantly decreased the expression of auditory cue-conditioned fear memory (n=8 mice, mean 43.04±1.3, 51.97±2.68 respectively). Our study provides the first evidence for a functional asymmetry of the MHb-IPN pathway in mammals and its potential involvement in emotion-related behavior. Physiologically, the right MHb dominates neurotransmission at rest, whereas activation of GBR equalizes the left and right MHb-derived synaptic inputs in the IPN. The side-specific inhibitory effect on the fear expression may reflect the laterality in the $GABA_B$-mediated regulation of this pathway.

Disclosures: C. Önal: None. **P. Koppensteiner:** None. **R. Shigemoto:** None.

Poster

035. Synaptic Plasticity

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 035.05

Topic: B.04. Synaptic Transmission

Support: NIH AG034113
NIH AG057496
Korea NRF 2021R1A6A3A14045044

Title: Meningeal lymphatic's role on prefrontal cortex synaptic integrity

Authors: *K. KIM, J. KIPNIS;
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Abstract: Meningeal lymphatics is the primary external route of the cerebrospinal fluid (; CSF) flow. CSF, an ultrapure fluid produced in the choroid plexus, passing through the ventricular system, perfused into the parenchyma in the perivascular spaces. After exchanging their contents with parenchymal interstitial fluid, it is drained into the lymphatics in the dura and finally returns to the blood circulation through the deep cervical lymph node and lymphatic vessels. They convey CNS-driven antigens, immune cells, and clear CNS-originated wastes during their rapid production and transport. Meningeal lymphatics' function is associated with neurodegenerative conditions and the disease prognosis. Beneficial effects of enhancing meningeal lymphatic function were reported in animal models of neuroinflammatory diseases such as amyloidosis, brain tumor, and multiple sclerosis. However, little is known about the mechanism of action compared to its translational potential. Also, it's largely unknown how this contributes to the homeostatic brain function. In this study, we surgically aberrated meningeal lymphatics and observed electrophysiological, behavioral, and cellular consequences in multiple brain regions. Aberration of meningeal lymphatics for a month results in multifaceted adverse effects of synaptic transmission and synaptic plasticity, bifurcated by the areas, while not altering cellular compositions and intrinsic excitability of neurons. These synaptic changes lead to cognitive deficits in the animal's behavior. To investigate the direct mechanism of the synaptic changes, unbiased proteomics screening was conducted using LC-MS/MS with the CSF and synaptosome samples. These results suggested mild neuroinflammatory conditions in the lymphatics-aberrated CSF. This is accompanied by an increase in phagocytosis and morphological changes in microglia. To check the microglial contribution to the synaptic and behavioral consequences, a CSF1R antagonist was used to deplete microglia. Depleting microglia completely blocked synaptic effects of meningeal lymphatics aberration, suggesting that microglia are the primary mediator of meningeal lymphatics dysfunction.These results suggest that CSF flow neutralizes passively accumulating neuroinflammatory conditions and protects synaptic functions by regulating microglial function. In future work, these findings will be confirmed in the other accessible models to solidify and extend this work.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.06

Topic: B.04. Synaptic Transmission

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CU Medicine Endowment to VJ-T

Title: Neonatal ketamine exposure impairs infrapyramidal bundle pruning and causes lasting synaptic hyperexcitability in hippocampal CA3 neurons

Authors: O. H. CABRERA[1], S. MAKSIMOVIC[1], N. USEINOVIC[1], **M. NEAR**[1], N. QUILLINAN[1,3,4], S. M. TODOROVIC[1,3], *V. JEVTOVIC-TODOROVIC[1,2];
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Abstract: General anesthetics (GA) have been associated with widespread neurodegeneration in developing rodent and non-human primate brains. The initial cell death during peak stages of synaptogenesis is followed by localized changes in synaptic excitability and cognitive impairments that present later in life. During normal development, long axon collaterals are pruned as a part of neurogenesis. We hypothesize that this physiological axonal pruning may be disrupted by ketamine exposure early in life leading to alterations in the infrapyramidal bundle (IPB) of the hippocampus. To examine changes in CA3 neuronal synaptic excitability, mouse pups at postnatal day (PND) 7 were exposed to ketamine or vehicle then sacrificed at several developmental time points during peak stages of IPB pruning. The pups were injected intraperitoneally four times, once every 90 minutes, with 40 mg/kg of ketamine to maintain sedation over 6 hours. Following exposures, hippocampal sections were analyzed at PND 20, 30, and 40 with immunohistochemistry to quantify reshaping of the IPB. Treated groups displayed IPB extension into CA3b and CA3c regions of the hippocampus, were thicker, and were more numerous than vehicle groups. At PND 30, the peak age for stereotyped axonal pruning in mice, IPB length was 20% shorter in vehicle groups compared to ketamine while at PND 40 this difference increased to 30%. To determine the functionality of synapses in unpruned IPBs we jointly labeled tissue with synaptophysin to mark synaptic vesicles and calbindin to label mossy fibers. We found a high-degree of colocalization for both labels in ketamine groups which contrasted the minimal colocalization in vehicle groups. This indicated that unpruned neurons of the IPB in ketamine exposed mice expressed functional synapses that innervate CA3 neurons. In order to understand how abnormal pruning impacted synaptic excitability we measured the frequency of miniature excitatory postsynaptic potentials (mEPSCs) in hippocampal CA3 neurons with patch-clamp electrophysiology. We found a leftward shift in interevent intervals and a rightward shift in cumulative frequency amplitude for ketamine exposed groups compared to controls. These results suggested neonatal exposure to ketamine led to synaptic hyperexcitability in hippocampal neurons of the CA3 region in concert with the reduced axonal pruning in the IPB. Along with the well documented potential for neurodegeneration following GA, we propose that the disruption of stereotypical IPB pruning may considerably impact the maturation of young brains and lead to cognitive impairments that persist into adulthood.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.07

Topic: B.04. Synaptic Transmission

Support: CIHR Grant 211744

Title: Differential dynamic contributions of pre and postsynaptic mechanisms during single and clustered inputs to CA1 dendrites

Authors: *A. PILLAI[1], C. SOARES[2], D. TROTTER[2], R. NAUD[2], J.-C. BEIQUE[1];
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Abstract: A growing literature is showing that information processing in cortical networks is enriched by the ability of individual neurons to distinguish spatiotemporal features of their inputs distributed across their dendritic arbor. For canonical CA1 synapses, burst inputs of a synaptic population leads to facilitation, while postsynaptic currents at single synapses are expected to be depressed due to receptor desensitization. However, the relative contributions of these opposing pre and postsynaptic mechanisms on overall gain modulation, and the time scale of their interactions, are less clear. Here, we addressed this question in a combination of whole-cell electrophysiology, two-photon imaging and uncaging and computational approaches. We first examined quantal properties and presynaptic dynamics of glutamate release at single spines in CA1 pyramidal neurons expressing the genetically-encoded optical glutamate sensor iGluSNFR. We found that the amount of glutamate release at single synapses was dynamically modulated, with strong facilitation occurring with 20Hz inputs. This was accompanied by an expected increase in release probability, but also in quantal size and in the number of vesicles released per stimulation, as estimated using a statistically grounded binomial model. We next compared these dynamics of presynaptic release with the temporal and spatial constraints of postsynaptic mechanisms probed by uncaging of MNI-glutamate at visually identified spines in CA1 radial oblique dendrites while separately measuring EPSPs and EPSCs, both for single and clustered inputs. In direct opposition to presynaptic facilitation, paired EPSPs at single spines were depressed at short inter-pulse intervals. Interestingly, both the magnitude and time course of this post-synaptic depression was enhanced for clustered inputs. Direct examination of postsynaptic currents revealed a differential desensitization timescale for AMPA and NMDA receptor that was consistent with the increased contribution of NMDARs to the EPSPs during clustered inputs. Thus, our results highlight the role of two contrasting pre- and postsynaptic mechanisms that, together, provide a means to distinguish the information content of synaptic inputs based on their spatiotemporal features.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.08

Topic: B.04. Synaptic Transmission

Support: Colorado State University
R01-MH126017

Title: Induction of synapse formation by de novo neurotransmitter synthesis

Authors: *S. CHANDA[1], T. C. SUDHOF[2], M. A. XU-FRIEDMAN[3], T. P. CAST[1], M. GHEBRIAL[4], O. BENNER[1], C. D. S. PASSOS[1], L. LUBOW[1], L. PETERKIN[1], N. F. WONG[5], S. R. BURLINGHAM[1];
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Abstract: A vital question in neuroscience is how neurons align their postsynaptic structures with presynaptic release sites. Although synaptic adhesion proteins are known to contribute in this process, the role of neurotransmitters remains unclear. Here we inquire whether de novo biosynthesis and vesicular release of a noncanonical transmitter can facilitate the assembly of its corresponding postsynapses. We demonstrate that, in both stem cell-derived human neurons as well as in vivo mouse neurons of purely glutamatergic identity, ectopic expression of GABA-synthesis enzymes and vesicular transporters is sufficient to both produce GABA from ambient glutamate and transmit it from presynaptic terminals. This enables efficient accumulation and consistent activation of postsynaptic GABAA receptors, and generates fully functional GABAergic synapses that operate in parallel but independently of their glutamatergic counterparts. These findings suggest that presynaptic release of a neurotransmitter itself can signal the organization of relevant postsynaptic apparatus, which could be directly modified to reprogram the synapse identity of neurons.

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Poster

035. Synaptic Plasticity

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Topic: B.04. Synaptic Transmission

Support: NIH Grant DA035430
NIH Grant DA044760

Title: Golgi satellites mediate activity-dependent trafficking of transmembrane synaptic proteins from dendrites to axons

Authors: O. JEYIFOUS, **T. A. RUSSELL**, A. P. GOVIND, L. O. VAASJO, X. ZHUANG, W. N. GREEN;
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Abstract: We have previously shown that the Golgi apparatus in neurons undergoes activity-dependent fragmentation resulting in the formation of Golgi satellites (GSats) in dendrites and somata. GSats function in local secretory and endosomal pathways and modify the N-glycosylation state of transmembrane proteins near local postsynaptic domains. Here, we describe GSats in axons. Unlike dendrites, axons to not contain Golgi outposts, as identified by the presence of Golgi structural enzymes such as GM130. However, GSats in axons are readily visualized as vesicles containing Golgi enzymes such as sialyltransferase-3 and mannosidase II. We have identified two pools of axonal GSats, one of which is mobile, and is transported primarily in the anterograde direction at a rate of ~ 1 um/sec via microtubule-based motors. The second pool is stationary and colocalizes with markers for presynaptic terminals. Increasing neuronal activity significantly increases both GSat pools in axons, whereas both axonal pools are reduced by blocking neuronal activity with tetrodotoxin. Increased activity also redirects dendritic GSat transport flow in dendrites in the retrograde direction, which parallels increases in anterograde GSats transport, suggesting that GSats transport postsynaptic cargo either directly or indirectly to presynaptic terminals in response to activity. This cargo includes neurotransmitter receptors and synaptic adhesion proteins that are initially trafficked to dendritic sites and subsequently trafficked to axon terminals, including nicotinic acetylcholine receptors (nAChRs). In in vivo studies, mice treated with nicotine chronically display a higher density of GSats in dopaminergic axons in the nucleus accumbens than untreated mice, and GSats in these axons contain nAChRs. Taken together, our findings reveal that increased activity at postsynaptic somatodendritic domains drives GSat transport of cargo to presynaptic domains. This coordination of synaptic changes in a neuron’s dendrites driving changes at its axon terminals may be critical in strengthening of neural circuits generally, and the neuronal changes that occur with during addiction with drugs of abuse such as nicotine.

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Poster

035. Synaptic Plasticity

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Title: Dysregulation of corticotropin releasing factor system in infralimbic prefrontal cortex of alcohol dependence and withdrawal male and female rats

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Abstract: Alcohol use disorder (AUD) is characterized by an impaired ability to control alcohol consumption and is accompanied by negative emotional state during abstinence. Brain hyperactive stress systems, corticotropin-releasing factor (CRF) and its cognate receptor CRF1 signaling, contribute to the compulsive nature of AUD. Pyramidal neurons in the infralimbic cortex (IL) of the medial prefrontal cortex (mPFC) predominantly express CRF1 receptors and are altered by chronic alcohol exposure. The IL innervates central amygdala (CeA) (IL◊ CeA) and this circuit regulates emotional responses. Despite evidences on IL◊ CeA connection, the role of CRF signaling in alcohol dependence and withdrawal remains to be explored. In this study, we induced ethanol dependence using the chronic intermittent ethanol vapor paradigm. Male and female rats were exposed to ethanol vapors (daily 14 h on/10 h off) in their home cages for 5-7 weeks, while age matched naïve controls were exposed to air only. A subset of dependent rats were then subjected to protracted withdrawal for 2 weeks. We performed *ex vivo* whole-cell patch clamp recordings from pyramidal neurons in layer V IL of naive, dependent and withdrawal rats and examined the effects of CRF (200nM) on spontaneous inhibitory and excitatory postsynaptic currents (sI/EPSCs). We observed that CRF decreases spontaneous GABA release in naïve male rats but has bidirectional effects (both increase and decrease) in females. CRF showed significant reduction in $GABA_A$ receptor function in a subset of neurons in naïve females, with no change in amplitude in naïve males. Tracing studies indicated that CRF predominantly decreases GABA release onto labeled specific ILàCeA neurons compared to unlabeled IL neurons. Interestingly, in dependence and withdrawal, CRF has opposing effects (both decrease and increase) on GABA release suggesting an altered sensitivity to CRF in both sexes. Additionally, CRF substantially increased glutamate release and postsynaptic function on IL pyramidal neurons of both naïve male and female rats. These results demonstrate the major role of CRF signaling on network activity in mPFC. Also, alcohol dependence and withdrawal induce changes in the modulatory role of CRF on IL neuronal activity, hence, becoming a potential target to study for therapeutic intervention for AUD.

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Poster

035. Synaptic Plasticity

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Topic: B.04. Synaptic Transmission

Support: NSERC
CIHR

Title: Developmental changes in the intrinsic properties and synaptic function of pyramidal neurons in the auditory cortex ofCntnap2KO rats.

Authors: *R. MANN, B. L. ALLMAN, S. SCHMID;
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Abstract: The contactin-associated protein-like 2 (*Cntnap2*) gene, which codes for the cell-adhesion protein CASPR2, is highly important for brain development, especially in sensory structures and areas central for language development. Disruptions in *Cntnap2* can result in neurodevelopment disorder displaying the core symptoms of autism spectrum disorder (ASD) and moderate to severe language impairments in humans. Importantly, the *Cntnap2* gene is not exclusive to humans and is expressed throughout the sensory and cortico-striato-thalamic circuits in other animals. Knocking out this gene in rodents results in ASD-like symptoms that involve auditory processing deficits. A homozygous *Cntnap2* gene functional knockout in mice and rats is known to disrupt basic functions requiring auditory processing, such as the acoustic startle response and sensorimotor gating. Though it is well established that *Cntnap2* is essential for auditory processing, its mechanisms of action at the cellular level and how it affects neural networks required for auditory processing have not yet been fully characterized. This study used *in vitro* electrophysiology to examine developmental alterations in auditory cortex pyramidal neurons of *Cntnap2*$^{-/-}$ rats, hypothesizing that *CNTNAP2* is essential for maintaining intrinsic neuronal properties and synaptic wiring in the developing auditory cortex.Whole-cell patch-clamp recordings were conducted in wildtype and *Cntnap2*$^{-/-}$ littermates at 3 postnatal age ranges (P8-12, P18-21, and P70-90). Consistent changes across age were seen in all measures of intrinsic membrane properties and spontaneous synaptic input. Intrinsic cell properties such as action potential half widths, rheobase, and action-potential firing frequencies were different between wildtype and *Cntnap2*$^{-/-}$ rats predominantly during the juvenile stage (P18-21), whereas adult *Cntnap2*$^{-/-}$ rats showed higher spontaneous (sEPSC) and mini excitatory post-synaptic currents (mEPSC) frequencies, with lower sEPSC amplitudes. These results indicate that intrinsic cell properties are altered in *Cntnap2*$^{-/-}$during the juvenile age, leading to a hyperexcitable phenotype during this stage of synaptic remodeling and refinement. While intrinsic properties eventually normalize by reaching adulthood, changes in synaptic input seem

to manifest in the adult age and are presumably responsible for the hyperreactive behavioral phenotype. These experiments will provide novel insights into how *Cntnap2* impacts auditory processing networks at a cellular level, and shed light on the neural mechanisms underlying altered auditory processing seen in *Cntnap2*$^{-/-}$ rats.

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Poster

035. Synaptic Plasticity

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Topic: B.04. Synaptic Transmission

Support: College of A&S University of Kentucky (KEB)
Summer Research Fellowship in Neuroscience (KEB)
Chellgren Fellowship (RLC)
Department of Biology University of Kentucky

Title: The effects on resting membrane potential and synaptic transmission by Doxapram (blocker of K2p channels) at the Drosophila neuromuscular junction

Authors: *K. BROCK[1], R. M. VACASSENNO[4], C. N. HADDAD[2], R. L. COOPER[3]; [2]Dept. of Biol., [3]Univ. of Kentucky Dept. of Biol., [1]Univ. of Kentucky, Lexington, KY; [4]Dept. of Biol., Eastern Kentucky Univ., Richmond, KY

Abstract: The resting membrane potential of most cells ismaintained by potassium leak channels referred to as K2p channels. Thepharmacological profile and distribution of the various K2p channel subtypesamong organisms are still being investigated. The Drosophila genome contains 11subtypes and their function and expression profiles have not yet beendetermined. Doxapram also known as Stimulex or Respiram is used clinically toenhance respiration in humans. Doxapramis a blocker of the acid sensitive K2p TASK subtype in mammals. The restingmembrane potential of larval Drosophila muscle and synaptic transmission at theneuromuscular junction are pH sensitive. Doxapram (10 mM) depolarizes themuscle and appears to depolarize the motor neuron causing an increase infrequency of spontaneous quantal events and evoked excitatory junctionpotentials ($N=6, p<0.05$ Sign Test). These changes are matched by anextracellular increase in KCl (50 mM) and are blocked by Cd2+($N=6, p<0.05$Sign Test). It is assumed the motornerve depolarizes to open voltage gated Ca2+ channels in presynaptic nerveterminals as a result of exposure to Doxapram. These findings are significantin building models to better understand the function of pharmacological agentswhich affect K2p channels and how K2P channels contribute to physiology intissues. Drosophila offers a genetic amenable model to be able to alter tissueselective expression of K2p channel subtypes to parallel known human diseasesrelated to this family of channels.

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Poster

035. Synaptic Plasticity

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Program #/Poster #: 035.13

Topic: B.04. Synaptic Transmission

Support: National Science Foundation
Overland Foundation

Title: Postsynaptic receptors regulate presynaptic neurotransmitter stability

Authors: *S. GODAVARTHI[1,2], M. HIROMOTO[3], Y. IGNATYEV[4], J. B. LEVIN[5], H. LI[1,2], M. PRATELLI[1,2], J. BORCHARDT[6], C. CZAJKOWSKI[6], L. N. BORODINSKY[5], L. B. SWEENEY[4], H. T. CLINE[3], N. C. SPITZER[1,2];
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Abstract: Robust communication systems include an acknowledgement signal from the receiver to the sender indicating that the sender's message has been received. We have tested this organization at the embryonic and larval *Xenopus* neuromuscular junction (NMJ), with neurotransmitter receptor loss-of-function (LOF) and gain-of-function (GOF) experiments. We find that LOF with focal blockade of endogenous acetylcholine receptors (AchRs) by antagonists -pancuronium or curare- results in loss of ChAT in presynaptic motor neurons (MNs). The LOF results suggest that blockade of endogenous receptors destabilizes presynaptic transmitter expression. Knockdown of the AchR co-receptor protein, Lrp4, recapitulates the loss of ChAT seen with AchR antagonists. Ectopic expression of GABA receptors ($GABA_AR\alpha\beta\gamma$) in myocytes in a GOF experiment leads to expression of GABA in presynaptic cholinergic MNs. GABA expression is seen in innervating axon terminals and in cell bodies in the spinal cord. Co-expression of the MN markers Hb9, ChAT and Lim3 confirms their primary MN identity. Consistent with the appearance of GAD67 and VGAT in these MNs, GABAergic miniature endplate potentials at the NMJs are recorded from the myocytes. Misexpression of a single $GABA_AR\alpha$ subunit, resulting in a non-functional $GABA_AR$ that is not trafficked to the postsynaptic membrane, does not lead to expression of GABAergic markers in innervating axon terminals. Because GABA is normally expressed transiently in MN processes innervating the myotome at earlier stages of development, exogenous $GABA_AR\alpha\beta\gamma$ likely prevents the loss of GABA expression. Expressing the GABA-insensitive mutant $GABA_AR\alpha\beta_{E179Q}\gamma$ reveals that $GABA_AR$ channel activity is not necessary for presynaptic expression of GABA. The GOF

results suggest that ectopically expressed postsynaptic receptors promote presynaptic transmitter stability. Knocking down Lhfpl4, a co-receptor for $GABA_AR\alpha\beta\gamma$ prevents the $GABA_AR\alpha\beta\gamma$-mediated appearance of GABA in MNs. Lrp4 and Lhfpl4 are known to interact with dystroglycans and neuroligins respectively. These in turn bind to neurexin presynaptically, forming trans-synaptic bridges. Immunostaining reveals the presence of both neurexin and neuroligin at the *Xenopus* NMJ. As the loss of coreceptors Lrp4 and Lhfpl4 is sufficient to prevent receptor-mediated stabilization of cognate transmitters, receptor-specific information for appropriate transmitter expression appears to be conveyed to presynaptic neurons via trans-synaptic bridges.

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Poster

036. Synaptic Signaling

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Program #/Poster #: 036.01

Topic: B.05. Synaptic Plasticity

Support: ERC STG 945700

Title: Data-driven model of multi-protein activity quantitatively links mutations to synaptic pathophysiology

Authors: *Y. LIU[1], J. KRUMMEICH[2], S. SCHWEIGER-SEEMANN[2], T. TCHUMATCHENKO[1,3];
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Abstract: Memory and learning are fundamental to the brain's function and development, and they depend on communication across different brain areas through synapses. To ensure the proper synaptic function, neurons need to maintain a constant supply of proteins. Furthermore, the connection between neurons can be strengthened or weakened by modifying the protein composition via synaptic plasticity. Experimental work shows that proteins are often produced locally in the dendrites to quickly satisfy the protein requirement in response to synaptic activity. Dysfunction of local translation has been associated with various symptoms of Autism Spectrum Disorder (ASD) and intellectual disability, caused by mutations of genes in signaling pathways that regulate local translation or translation initiation. To quantitatively understand how such mutations can lead to abnormal protein composition at synapses and cause impaired plasticity activity, we construct a data-driven model that elaborates the impact of the mutation on the local protein synthesis. This model can additionally serve as a computational framework that

elucidates the most effective method of correcting the aberrant protein composition or treating such disorders.
Associated with learning disabilities and symptoms of ASD, the Tuberous Sclerosis (TSC) Complex is caused by mutations in genes encoding Hamartin or Tuberin proteins, which together form a protein complex. This complex lies in the mammalian target of rapamycin (mTOR) signaling pathway that couples receptors and local protein synthesis. Using homogenate and synaptosome data obtained from the mouse model of TSC Complex, we first analyzed the difference between synaptic protein compositions in wild type vs. mutants, which helped us identify the proteins coupled with the changes in the signaling pathway. Secondly, we looked at the data across discrete timepoints during early development and discovered complex temporal dynamics shaped by interactions between synaptic proteins.
Based on our observations and the published literature on how the mTOR signaling pathway interacts with different synaptic proteins, we constructed a biologically inspired mathematical framework to (i) quantify the impact of the mutation on the local synthesis of receptors, (ii) identify the interaction between receptors, and (iii) fill in protein synthesis information at experimentally unobserved timepoints. In conclusion, our work quantitatively entails the mechanisms that link the mutation of TSC genes to abnormal synaptic protein composition and synaptic dysfunction.

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Poster

036. Synaptic Signaling

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Research to Prevent Blindness

Title: Changes in the Bipolar Cell Transcriptome During Photoreceptor Degeneration

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Abstract: A major change in retinal circuits following photoreceptor death is bipolar cell (BC) rewiring. BCs receive input from rod and cone photoreceptors and provide excitatory input to retinal ganglion cells. There are two classes: ON and OFF. As photoreceptors die in diseases like retinitis pigmentosa, BCs extend their dendrites to form new connections to surviving photoreceptors. BCs then retract their dendrites when they can no longer find nearby photoreceptors. Understanding how BCs react to the loss of synaptic input is important for predicting and reversing their synaptic rewiring during retinal degeneration as this is likely to severely disrupt visual processing.
To understand the underlying mechanisms of BC rewiring, we took a transcriptomics approach. The structural and molecular changes associated with BC rewiring are at least partly initiated by changes in gene transcription. With a transgenic mouse line (*Grm6;GFP*) that expresses GFP exclusively in ON BCs, we isolated and sequenced ON BC transcriptomes from retinal tissue. These mice also lacked expression of the rod cyclic nucleotide gated channel beta subunit ($Cngb1^{neo/neo}$), which models a human form of retinitis pigmentosa, RP45. We sampled different time points of degeneration: P30, P90 and P210. These ages exhibited the following amounts of rod (cone) survival, respectively: 66% (98%), 30% (95%) and 4% (65%).
We found that over 2000 genes were differentially expressed across progressive photoreceptor death in the $Cngb1^{neo/neo}$ ON BC transcriptome. In this list, we found that three genes that encode proteins key for signal transduction at BC dendrites were downregulated across the time course of degeneration. We also found two transcription factors required for ON BC development were continuously down regulated. Lastly, we found many gene expression changes were specific for proteins expressed in type-5 ON BCs. These genes were also analyzed across the time course of aging in controls to verify that degeneration-induced changes were not a result of aging. Taken together, the ON BC transcriptome exhibits many changes in response to photoreceptor degeneration, which may serve as avenues for treating RP.

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Poster

036. Synaptic Signaling

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Program #/Poster #: 036.03

Topic: B.05. Synaptic Plasticity

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Title: Rescuing behavioral and synaptic deficits in SHANK3 knock-out mice by pharmacological enhancement of mGlu5 receptor

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Abstract: Shank proteins (Shank1, Shank2 and Shank3) are large scaffold proteins located at post synaptic density (PSD) of excitatory synapses which are crucial for synapse development and plasticity and is not surprising that mutations or deletions of SHANK genes are associated to the onset of neurological disorders that can be indicated with the term Shankopathies. It is known that haploinsufficiency of SHANK3 is the major cause of neurological symptoms associated with Phelan-McDermid Syndrome (PMS), which includes hypotonia, global developmental delay, language and speech delay, and autistic behavior.Previously, we demonstrated that mGluR5 signalling was altered in absence of Shank3 and that the improvement of mGluR5 activity with a positive allosteric modulator (PAM), named CDPPB, ameliorated functional and behavioral defects observed in Shank3Δ11-/- mice (Vicidomini et al. 2017). These data suggest that alteration in mGluR5 signalling might be involved in the pathogenesis of PMS and a pharmacological treatment that increase mGluR5 activity may represent a novel strategy for treating patients that are affected by PMS and SHANK3 mutations. In this study, we investigated the molecular mechanisms associated with the ASD-like behaviors observed in Shank3Δ11-/- mice and test the ability of VU0409551, a novel PAM of mGluR5, to rescue behavioral and synaptic dysfunction observed in *Shank3*Δ11-/- mice. First, at the molecular level our results show a specific reduction of protein translation, assessed by SUnSET assay, in cortex and striatum of *Shank3*Δ11-/- mice at 15 PND and 30 PND when compared with WT mice. Then, from RNA seq and western blot analyses we found a reduction in both striatum and cortex of *Shank3*Δ11-/- mice of RPL3, a ribosomal protein of 60S subunits of the ribosome, which might be correlate with the reduction of the protein translation. The reduction of protein translation and RPL3 levels in *Shank3*Δ11-/- mice were rescued by chronic treatment of VU0409551.Then, we also found that acute treatment with VU0409551 rescues, sociability stereotyped behavior and learning and memory deficits found in *Shank3*Δ11-/- mice. These data were also confirmed in cortical neurons derived from iPSCs from PMS patients in which chronic treatment of VU0409551 was able to restore the protein translation and the level of RPL3 when compared with control. Our results support a casual relationship between loss of Shank3 and deficits in protein translation through RPL3 downregulation. Moreover, we have demonstrated that chronic activation of mGlu5 receptor persistently alleviates behavioral deficits in Shank3 KO mice.

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Poster

036. Synaptic Signaling

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Program #/Poster #: 036.04

Topic: B.05. Synaptic Plasticity

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Title: The nBAF (mSWI/SNF) chromatin remodeling complex regulates activity-induced neuronal gene transcription by facilitating RNA Polymerase II elongation

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Abstract: The coupling of neuronal excitation at the membrane with transcription in the nucleus leads to the induction of activity-regulated genes (ARGs), whose transcriptional products mediate many subsequent neuronal processes and are therefore critical in neurodevelopment and thereafter, in normal functioning of the brain. Recent work from our laboratory and others has shown that transcribed ARGs can be classified based on their temporal induction kinetics into three distinct classes of genes. Among them, rapid IEGs are unique among all genes transcribed in having paused RNA Polymerase II (Pol II) near their promoters at rest. Upon stimulation, the pioneer Pol II urgently undertakes productive elongation and pre-mRNA production, a process amplified by continued Pol II recruitment. However, we don't know how productively elongating Pol II overcome nucleosomal barriers within the gene body. We hypothesized that Pol II productive elongation is facilitated by co-transcriptional ATP-dependent chromatin remodeling. Such remodeling was hypothesized to be mediated by the neuronal BAF (nBAF or mammalian SWI/SNF) complex, a megadalton-sized protein complex composed of 29 genes and 12-15 subunits. We used novel pharmacological inhibitors, degraders, and RNAi of several nBAF subunits in primary cortical rat neurons to show that the nBAF complex is required for activity-induced transcription of neuronal rIEGs. Using Arc as a model rIEG to study underlying mechanisms, our ChIP assays reveal that neuronal activity triggers accumulation of BAF complex subunit SMARCC2 at the promoter and in the Arc gene body. Furthermore, inhibition, degradation, or knockdown of nBAF attenuates paused Pol II levels at Arc promoter and hinders its continued recruitment during activity-induced transcription. To uncouple transcription elongation from initiation, we performed ChIP assays after pharmacologically inhibiting Pol II recruitment. These assays revealed that in the absence of a functional BAF complex, levels of transcriptionally-competent Pol II decrease within the Arc gene body, likely due to ineffective clearance of nucleosomal barriers. Taken together, our data set suggests that the nBAF complex regulates Pol II productive elongation and provides a broader understanding of the mechanisms that regulate transcription during neuronal activity. Given that mutations in several of these BAF subunits are strongly associated with neurodevelopmental disorders, such as autism spectrum disorders, our findings may provide insights into awry transcriptional mechanisms underlying these disorders.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: CRISPR/SaCas9 elimination of alternative polyadenylation on the *Timp2* transcript increases hippocampal neuron filopodia

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Abstract: In recent years, it has become evident that environment-gene interactions likely contribute to the emergence of complex neurological pathologies. A prime example of this duality is disordered appetite. Obesity and anorexia lie on seemingly opposite ends of the behavioral phenotypic spectrum, yet both disorders are functionally mediated by common appetite circuitry in the central nervous system. This duality has been posited to result from coordinated environmental changes to multiple gene products. One mechanism of interest is alternative polyadenylation (APA), a rapid, post-transcriptional processing mechanism that regulates poly(A) tail length and crucial regulatory elements that direct transcript stability, localization, and maturity. Previous work using a diet-induced obesity model has identified a significant APA pattern change on tissue inhibitor of metalloproteinases 2 (*Timp2*), a gene implicated in the development of an obese phenotype and the regulation of extracellular matrixes.
Here, we tested the hypothesis that APA site elimination on the *Timp2* gene would alter dendritic spine density in primary cultured hippocampal neurons. To accomplish this, we designed a CRISPR/SaCas9 DNA construct designed to target and eliminate a regulatory APA site on the *Timp2* gene. Following validation, hippocampal neurons were cultured from P1 Sprague-Dawley rats. On day in vitro (DIV) 5, neurons were transfected with pCAGGS-clover-actin and an empty pCAGGS construct or a combination of an empty pCAGGS construct and a pCRISPR/SaCas9-*Timp2*-APA construct (3-5% transfection efficiency). On DIV 11, neurons (n=10/treatment) were fixed and imaged for spine analysis. Results indicate that the elimination of the *Timp2* APA site in primary hippocampal neurons significantly increases immature filopodia density but does not impact mature mushroom or stubby spine density (two-tailed t-test, $p<0.05$). This data suggests that the regulatory APA site on *Timp2* plays a functional role in hippocampal neuronal filopodia density, and future studies will assess the relevance of this change for the regulation dendritic spine density in developing and mature neurons. Collectively, these studies serve to advance our understanding of RNA regulation as a functional link between the genome and the behavioral phenome.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: Phosphorylation of FMRP regulates protein synthesis in presynaptic mossy fiber terminals

Authors: *S. C. KHAROD[1], R. H. SINGER[2], P. E. CASTILLO[1], Y. J. YOON[1];
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Abstract: The molecular mechanisms that govern long-term forms of presynaptic plasticity are largely unknown. The hippocampal mossy fiber (MF)-CA3 synapse expresses robust presynaptic plasticity and has notably large and complex boutons. The mossy fiber boutons (MFBs) contain Fragile X messenger ribonucleoprotein (FMRP), an RNA-binding protein known to repress translation. We recently reported that presynaptic FMRP is required for MF-CA3 structural and functional plasticity. However, how exactly FMRP is regulated by activity is poorly understood. FMRP interacts with polyribosomes and mRNAs to form granules, which can be trafficked in an activity-dependent manner. In addition, dephosphorylation of FMRP on serine 499 (S499) can trigger granule disassembly and lead to loss of translational repression. By utilizing a novel viral vector expressing an FMRP-Halotag fusion protein, we show that fluorescent FMRP granules can localize to MFBs. Induction of MF-long term plasticity (MF-LTP) led to disassembly of FMRP granules in the MF tract. To further investigate the mechanism of FMRP granule assembly and disassembly, we expressed phospho-deficient (S499A) or phospho-mimic (S499D) mutants of FMRP to observe the effect of phosphorylation on FMRP granule size, transport, and assembly. Our results show that the phosphodeficient mutant exhibited a reduction in FMRP puncta radius, intensity and signal-to-noise ratio. Additionally, the changes in dissociation upon activity were correlated to changes in rates of local protein synthesis within individual MFBs. Together, we provide evidence that local disassembly of FMRP granule leads to increases in presynaptic protein synthesis at excitatory synapses in the mature mammalian brain, which may have important implications in our understanding of FMRP function, local protein synthesis, and memory formation.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: Crosstalk between lysine specific demethylase 1 and dopamine neurotransmission

Authors: *M. ANDRES[1], G. MERELLO[2], J. CASTILLO[1], A. P. ESCOBAR[3], G. CARRASCO[1], M. GONZÁLEZ[1], D. VERBEL-VERGARA[1], M. OLIVARES-COSTA[1], F. RUSCONI[4], E. BATTAGLIOLI[4];
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Abstract: The transcriptional repressor complexes formed by Lysine-Specific Demethylase 1 (LSD1/KDM1A), RCOR1 (CoREST), and histone deacetylases HDAC1/2 remove activating histone posttranslational modifications to repress transcription. LSD1 has a splicing variant only expressed in neurons, called neuroLSD1. This variant differs from the ubiquitous LSD1 (uLSD1) by a four amino acid microdomain. NeuroLSD1 and uLSD1 transcripts are in a ratio of approximately 50%, which is rapidly and transiently modified by neuronal activity. Mice null for neuroLSD1 are less responsive to stress and show altered synaptic plasticity, suggesting that the uLSD1/neuroLSD1 may regulate dopaminergic neurotransmission. To test this hypothesis, we studied basal and amphetamine (AMPH) -induced dopamine efflux in the nucleus accumbens (NAc) of wild-type (WT) and neuroLSD1-null mice. Fast scan cyclic voltammetry (FSCV) and microdialysis were used to assess dopamine efflux. We found similar basal synaptic dopamine levels in the NAc between WT and neuroLSD1 null mice. However, AMPH-induced dopamine efflux was lower in NAc from neuroLSD1-null mice than in WT mice. The tissue content of dopamine in the striatum was similar in both genotypes. Our data suggest that LSD1 is involved in the dopaminergic response to psychostimulants.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

Support: NIH Grant 1R01NS114253 to G.J.B and E.T.W

Title: Muscleblind-like RNA binding protein is transported with early and late endosomes

Authors: *A. JANUSZ-KAMINSKA[1], A. SHEN[1], R. HILDEBRANDT[2], E. T. WANG[2], G. J. BASSELL[1];
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Abstract: Transport of mRNAs to distal parts of the cell and subsequent localized translation are considered crucial for neuronal development and function. Recent findings revealed a role of endosomes in RNA granule transport and as platforms for localized translation in conjunction with RNA binding proteins. Muscleblind-like (MBNL) proteins are RNA binding proteins that regulate alternative splicing in muscle and brain. MBNLs are depleted from the cytoplasm in myotonic dystrophy type 1 (DM1) by sequestration on the expanded CTG repeats in the 3' UTR of dystrophia myotonica protein kinase (DMPK) transcripts. A role for MBNLs in mRNA localization has been reported although the mechanism is not known. We hypothesized that the unstructured carboxy terminus of MBNL1 regulates endomembrane attachment. Using immunofluorescence, colocalization of MBNL1 with early (Rab5, Neep21), late (Rab7) and recycling (Rab11) endosomes was observed in n2a cells. Using live imaging in primary neurons, EGFP-MBNL1 co-transport with early endosomes (Scarlet-I-Neep21) and late endosomes/endolysosomes (Scarlet-I-Lamp1) in neuronal processes was observed. Expression of full-length EGFP-MBNL1-41, but not a carboxy terminus deletion mutant of MBNL1, revealed decreased velocity of Rab11 recycling endosomes. We conclude that MBNL1 mRNA granules are transported with early and late endosomes via piggybacking and that the C-terminus of MBNL1 acts as an anchor to restrict mobility of recycling endosomes. Work in progress is to evaluate the role of the C-terminus of MBNL1 in endosome coupled local translation of synaptic proteins. We speculate that one of the mechanisms for MBNL1 to regulate mRNA cargo distribution and localized translation in neurons is endomembrane anchoring.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: Molecular approaches to control local protein synthesis in dendritic spines

Authors: *A. GRETZINGER, J. PARK;
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Abstract: *De novo* protein synthesis is considered required for memory. Synaptic plasticity is modeled in two phases; (1) early-long-term potentiation (LTP), which increases α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) insertion with pre-existing proteins, (2) late-LTP, which requires *de novo* protein synthesis of synaptic proteins including AMPARs. Also, the significance of protein synthesis in long-term memory has been shown for many decades. Recently, local protein synthesis in dendrites and axons has been proposed to have a role in synaptic plasticity. Emerging evidence includes the dendritic translation of local mRNA pools and the presence of polysomal and monosomal ribosomes in dendritic spines. Despite the accumulating evidence supporting local protein synthesis, its roles in memory formation remain elusive. To address this question, we generated two viral constructs expressing a genetically encodable protein synthesis inhibitor (gePSI) and postsynapse-targeted gePSI. We validated the efficacy of our constructs in a heterologous cell system utilizing a puromycylation assay. We then analyzed to validate their compartment-specific inhibition of protein synthesis utilizing a puromycylation assay of *ex vivo* hippocampal slices expressing either gePSI or postsynapse-targeted gePSI. Using these molecular approaches, we tested the roles of global and local protein synthesis in the contextual fear memory of mice. These data may provide insights into the memory process mechanisms, which are of benefit in understanding the progression of many dementia-associated neurodegenerative disorders.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: The RNA binding protein CPEB3 couples neuronal activity and morphogenesis with mitochondrial energy metabolism and function

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Abstract: Cytoplasmic Polyadenylation Element Binding protein 3 (CPEB3) and its *Aplysia* ortholog ApCPEB are RNA binding proteins and translational regulators that have been shown to be critical for long term synaptic plasticity and memory maintenance in rodents and in the

invertebrate model *Aplysia californica*. We found that rodent CPEB3 is highly localized to mitochondria in distal neurites where it correlates with local protein synthesis hotspots. The CPEB3-mitochondria association was specific to the brain, but not other tissues such as liver and testes. Knockdown of CPEB3 led to dysregulated expression of two key mitochondrial proteins HSP60 and Pink1 in addition to its established plasticity related protein targets GluR2 and PSD95 and also to reduced mitochondrial membrane potential and dramatic loss of neurite branching, which is highly dependent on local mitochondrial energy and metabolic support. CPEB3 expression in the hippocampus was enriched in neurons with a higher level of basal synaptic activity such as CA3 pyramidal neurons, PV interneurons and mossy cells in the dentate gyrus and closely correlated with higher mitochondrial density in these neurons, consistent with their higher energy demands. Expression of both CPEB3 and mitochondrial markers in the hippocampus was enhanced by contextual fear conditioning. Moreover, CPEB3 expression was controlled by Brain Derived Neurotrophic Factor (BDNF) - a major activity dependent neurotrophin in the brain, providing a mechanistic link between neuronal activity, local protein synthesis and energy metabolism. Finally, we observed a very similar association of ApCPEB with mitochondria in the *Aplysia* CNS and in cultured *Aplysia* sensory and motor neurons, with ablation of ApCPEB leading to similar functional consequences - loss of mitochondrial membrane potential and neuritic branching. In addition, similarly to rodents, ApCPEB expression is controlled by the *Aplysia* ortholog of BDNF, ApNT.
Our findings are consistent with a novel, feed forward model, wherein activity dependent BDNF signaling and downstream CPEB3 mediated local protein synthesis at distal mitochondria enables mitochondrial function, which in turn supplies the energy and metabolic support needed to sustain highly energy demanding cellular processes such as local protein synthesis, neurite branching and synaptic plasticity and memory maintenance. Moreover, the coordinate local translation of mitochondrial as well as plasticity related protein targets by CPEB3 ensures precise and dynamic matching between alternating energy demands and energy supply at the synapse during transmission and plasticity.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

Support: R01NS123405

Title: Neurite outgrowth in dopaminergic neurons is improved by re-adjusting the F1 to FO ratio in the ATP synthase

Authors: *R. CHEN[1], C. MARTINEZ-ACEVES[1], P. LICZNERSKI[1], W. CARTER[1], E. GOUCHER[1], J. TANG[1], M. GRAHAM[1], W. MANDEMAKERS[2], V. BONIFATI[2], E. JONAS[1]; [1]Yale Univ., Yale Univ., New Haven, CT; [2]Erasmus MC, Rotterdam, Netherlands

Abstract: Defects of neurite outgrowth are key pathological hallmarks in neurodegenerative diseases including Parkinson's Disease (PD). DJ-1 (PARK7) mutation causes familial PD. DJ-$1^{-/-}$ mouse DN culture provides a useful technique to study early pathological alterations. In a previous report, we showed that the number of dendrites initiated directly from DJ-$1^{-/-}$ somata is decreased, that neurites fail to reach the same length as those of WT DN neurons and that the number of branch points of each neurite is reduced. We noted that the ATP synthase F1/FO ratio is relatively lower in the aging DJ-$1^{-/-}$ mouse brain and that mitochondria have an inner membrane leak causing inefficiency in ATP production. In the current study, we find that mitochondria from DJ-1 patient fibroblasts have morphological changes suggesting metabolic dysfunction. We reported that mitochondrial metabolic changes alter cellular protein synthesis rates. New protein synthesis is crucial for normal neuron growth and repair. We measured protein synthesis rate for ATP synthase β subunit by Puromycin-Proximity Ligation Assays (PLA). During four weeks of growth, the DJ-$1^{-/-}$ DN neurons had a lower rate of β subunit protein production than the WT DN, especially at the 3rd week, when neurite growth is most exuberant. The low level of ATP synthase β protein could result from low β mRNA. By using RNA Scope technique, we found that the β mRNA levels in DJ-$1^{-/-}$ DN neurites are very low, but β mRNA levels in the soma are compatible with WT. We rescued the β mRNA protein synthesis rates in patient cells by overexpressing ATP synthase β subunit, which presumably normalizes mitochondrial inner membrane coupling. In DN overexpression of β subunit increased neurites' length in both WT and DJ1 $^{-/-}$ DN, and we found that the DJ-$1^{-/-}$ DN neurites' length was completely recovered to the WT level. The number of neurites initiated directly from somata was also significantly increased in WT DN, but that was not the case for the DJ1 $^{-/-}$ DN. Our results indicate that DJ-$1^{-/-}$ DN are more vulnerable to growth defects because of abnormal ATP synthase stoichiometry caused in part by abnormal targeting of β subunit mRNA to distal neurites, and we suggest that improvement in ATP synthase stoichiometry will rescue neurodegeneration.

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Poster

036. Synaptic Signaling

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Topic: B.05. Synaptic Plasticity

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Title: Mice harboring a point mutation in TRAX that abolishes the miRNA-degrading activity of the translin/TRAX RNase show deficits in hippocampal synaptic plasticity and long-term memory

Authors: *M. SHETTY[1,2], X. FU[3], T. C. CHOWDHURY[1,2], J. M. BARABAN[3], T. ABEL[1,2]; [1]Iowa Neurosci. Inst., [2]Dept. of Neurosci. and Pharmacol., Carver Col. of Medicine, Univ. of Iowa, Iowa City, IA; [3]The Solomon H. Snyder Dept. of Neurosci., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Persistent forms of synaptic plasticity and memory require protein synthesis, either from newly transcribed mRNAs or from transcripts localized near the dendritic domains that are maintained in a translationally repressive state. MicroRNAs (miRNAs) are one of the key players in the translational repression of mRNAs, which do so by binding to the 3'-UTRs of transcripts along with many RNA-binding proteins, forming the miRNA-induced silencing complex. Mechanisms of translational repression and its rapid reversal in response to specific stimuli thus constitute an additional layer of epigenetic regulation of learning and memory. Translin and TRAX are two evolutionarily conserved proteins enriched in brain that are shown to be involved in RNA interference and translational regulation. The translin/TRAX (TN/TX) complex has been shown to exhibit RNase activity directed against precursor and mature miRNAs. We have previously shown that the translin KO mice, which lack both the translin and TRAX proteins, exhibit synaptic plasticity and memory deficits due to defective degradation of miRNAs following learning stimuli. To specifically identify the role of the RNase activity of the TN/TX complex in synaptic plasticity and memory, we have generated mice containing a point mutation (E126A) in TRAX that abolishes TN/TX RNase activity but does not alter the expression of translin or TRAX proteins in the brain, nor their ability to co-precipitate. Here we have utilized these mice to investigate multiple forms of hippocampal synaptic plasticity induced by different stimulation paradigms in the CA1 region using acute slice preparation. Our results show that the TRAX(E126A) point mutant mice exhibit deficits in a long-lasting LTP dependent on cAMP-PKA signaling and translation, as well as deficits in synaptic tagging and capture. We have also assessed hippocampus-dependent memory in a spatial object location task and the data show that the TRAX(E126A) mutant mice display impaired long-term memory compared to the WT littermates. These findings support the hypothesis that the miRNA-degrading RNase activity of the TN/TX complex mediates rapid reversal of translational silencing underlying persistent forms of hippocampal synaptic plasticity and long-term memory.

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Poster

036. Synaptic Signaling

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CIHR PG 156223 (PJS)

Title: Local translation in axons governs synapse-type-specific neurotransmission

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Abstract: Dendritic translation is well-established to regulate neurotransmission. Long-term plasticity and homeostatic plasticity, for instance, rely on the rapid and local synthesis of proteins in dendrites. Similarly, axonal translation serves as a pivotal regulator during early development and peripheral axon injury. In contrast, whether local translation happens in mature mammalian axons and plays functional roles in the central nervous system, such as neurotransmission, have been more controversial. These questions have been hard to answer since major experimental complications exist after synapses are formed, including the difficulties of (1) attributing pre- vs. postsynaptic effects in intact synapses, (2) manipulating micron-sized axonal compartments, and (3) fulfilling the spatiotemporal specificity that makes existing whole-cell genetic manipulation not feasible.

Here we uncover that local protein synthesis (PS) in axons of pyramidal cells regulates neurotransmission in mouse primary visual cortex. By taking advantage of intracellular drug loading via patch pipettes in paired recordings, we specifically manipulated pre or postsynaptic neurons. We observed synaptic response deficit and exaggerated paired-pulse ratio (PPR) after presynaptic blockade with mTOR inhibitor PP242 (EPSP: 75% ± 4%, $n = 12$, $p < 0.001$; $\Delta PPR = 0.16 \pm 0.05$, $p < 0.01$) or M7 cap analog (EPSP: 47% ± 6%, $n = 16$, $p < 0.001$; $\Delta PPR = 0.13 \pm 0.05$, $p < 0.05$), indicating synaptic release is sustained via mTOR and cap-dependent PS. Presynaptic NMDA receptor blockade with Ro 25-6981 suppressed release (65% ± 5%, $n = 8$) but was occluded by translation inhibitor cycloheximide (CHX) pre-treatment (100% ± 6%, $n = 9$, $p < 0.001$), suggesting these NMDARs act up-stream of PS. Using laser microsurgery to sever cell body from axon, we showed that axonal PS was key to sustaining activity-dependent neurotransmission (axotomy: 104% ± 3%, $n = 8$ vs. axotomy + CHX: 55% ± 4%, $n = 7$, $p < 0.001$). Live imaging of endogenous RNA revealed lasting and discrete docking patterns at individual presynaptic sites, suggesting bouton-specific regulation. In agreement, presynaptic PS was important for sustaining neurotransmission at synapses from pyramidal cells to other pyramidal cells, but not to inhibitory Martinotti cells (63% ± 6%, $n = 7$ vs. 116% ± 3%, $n = 23$, $p < 0.001$).

Taken together, our findings reveal how axonal PS governs neurotransmitter release in a synapse-type-specific manner. Recently, PS has emerged as a promising target for alleviating neuropathology. However, the focus has conventionally been on the postsynaptic side. Our results unveil the potential of leveraging local PS in axons as a novel therapeutic target.

Disclosures: **H.H. Wong:** None. **A.J. Watt:** None. **P.J. Sjöström:** None.

Poster

036. Synaptic Signaling

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 036.14

Topic: B.05. Synaptic Plasticity

Support: MoST105-2311-B-001-078-MY3
MoST108-2320-B-001-020-MY3
MoST110-2811-B-001-569-

Title: Axon-localized and activity-dependent control of CPEB2 promotes Slc17a6 translation for synaptic vesicle cycling and presynaptic plasticity

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Abstract: Remodeling the synaptic proteome to sustain long-term plasticity and memory can depend on locally translated mRNAs. However, the regulatory mechanisms have been biasedly discovered in postsynaptic (dendritic) rather than presynaptic (axonal) compartments due to the lack of distinct polyribosomes in the tiny domain of adult mammalian forebrain axons. Cytoplasmic polyadenylation element binding protein 2 (CPEB2)-controlled translation is important for postsynaptic function and spatial memory. Therefore, we investigated the presynaptic role of CPEB2 by electro-recording the Schaffer collateral-CA1 and temporoammonic-CA1 pathways and found defective fiber volley amplitude and paired pulse facilitation in CPEB2-deleted presynaptic inputs. By cross-comparing CPEB2-immunoprecipitated transcriptome with a learning-associated axonal translatome of adult cortex (GSE124592), we identified and validated that *Slc17a6*, encoding vesicular glutamate transporter 2 (VGLUT2), is translationally upregulated by CPEB2 in axons. Blocking activity-induced axonal *Slc17a6* translation by CPEB2 deficiency or cycloheximide diminished VGLUT2-dependent synaptic vesicle cycling. Together, CPEB2 promotes presynaptic translation to facilitate plasticity and memory.

Disclosures: **Y. Huang:** None. **W. Lu:** None. **Y. Chang:** None. **T. Chang:** None. **S. Chou:** None.

Poster

036. Synaptic Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 036.15

Topic: B.05. Synaptic Plasticity

Support: Grant No. SRG/2019/000382, to SJ by DST-SERB, Govt. of India

Title: Potential regulation of exercise-induced adult hippocampal neurogenesis by RNA-binding proteins: a computational analysis

Authors: *N. M J[1], S. JHA[2];
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Abstract: Adult hippocampal neurogenesis (AHN) is known to be enhanced by voluntary physical exercise. However, the underlying molecular regulators of this phenomenon are not completely understood (Overall et al. 2016; Front Neurosci. 10, p.362). RNA-binding proteins (RBPs) are predominant regulators of multiple biological processes. A number of RBPs are known to be critical in embryonic neurogenesis However, there is a limited understanding of the involvement of RBPs in AHN (Nishanth & Jha, 2022; Biochem. Genet. 1-18). The present study employed computational approaches for global identification of RBPs potentially regulating exercise-induced AHN in mice. Publicly available transcriptome data were analysed to identify differentially expressed genes (DEGs) within the hippocampi of mice, in response to wheel-running. Subsequently, RBPs potentially driving the observed differential gene expression patterns *via* untranslated regions (UTRs) were identified. Known modulators of neural homeostasis such as Musashi, Matrin3, and FXR were identified to be potentially involved in exercise-induced AHN, along with a number of other RBPs. Further, the DEGs modulated by exercise which are known to be associated with AHN, were screened to identify RBP binding sites within their UTRs. Majority of the exercise-modulated, AHN-associated genes were found to possess binding sites for RBPs such as ELAVL4, FXR1, IGFPBP2, Musashi, QKI, SFPQ, and RBMS within their 3'UTRs. Thus, the present study indexed the RBPs potentially modulating AHN in response to exercise in mice. We are currently studying the potential protein-protein interaction networks of RBPs which could influence AHN. Based on these findings, future experiments to evaluate RBP-mediated regulation of AHN could provide new insights into the molecular basis of AHN.

Disclosures: N. M j: None. **S. Jha:** None.

Poster

036. Synaptic Signaling

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Program #/Poster #: 036.16

Topic: B.05. Synaptic Plasticity

Support: NIMH Grant MH065635

Title: Insulin-like growth factor 2 regulates immediate early gene metabolism

Authors: *K. PANDEY, C. M. ALBERINI;
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Abstract: Insulin-like growth factor 2 (IGF2) is critical for the formation and enhancement of long-term memory. In fact, intrahippocampal injection of IGF2 results in a significantly stronger and more prolonged memory. This effect occurs via the high affinity receptor for IGF2, IGF2R, also known as cation-independent mannose 6 phosphate receptor (CIM6PR), but the underlying downstream molecular mechanisms remain to be determined. IGF2R regulates the trafficking of mannosylated enzymes to the lysosomes suggesting that the IGF2 effect may involve lysosomal, hence protein degradation functions. In our previous studies we showed that IGF2R plays a critical role in memory consolidation by controlling the training-induced *de novo* protein synthesis, a fundamental and conserved mechanism of long-term memory consolidation. Furthermore, memory enhancement evoked by IGF2 requires *de novo* protein synthesis. Here we investigated whether IGF2 treatment regulates protein synthesis and/or degradation, mechanisms regulated by learning and required for long-term memory. Using episodic learning paradigm inhibitory avoidance (IA) in rats, we found that intrahippocampal administration of IGF2 significantly blocked the learning-induced hippocampal increase in protein synthesis, measured *in vivo* by surface sensing of translation (SUnSET). We also found that, while, as expected, IA training increased the levels of proteins encoded by immediate early genes arc, c-fos, and egr1, IGF2 hippocampal injection blunted this increase, without affecting the upregulation of mRNA levels. Finally, using a pH-sensitive reporter, we found that IGF2 treatment significantly upregulated the learning-induced autophagic flux *in vivo*. Collectively, these data suggest that a potential mechanism by which IGF2 exerts its effect as a memory enhancer is to regulate the protein metabolism homeostasis of immediate early genes by either reducing their learning-dependent mRNA translation or by increasing their degradation via autophagic flux.

Disclosures: K. Pandey: None. **C.M. Alberini:** None.

Poster

036. Synaptic Signaling

Location: SDCC Halls B-H

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Program #/Poster #: 036.17

Topic: B.05. Synaptic Plasticity

Support: Leverhulme 94035

Title: A new Translating Ribosome Affinity Purification (TRAP) mouse line to characterize the translatome in cortical dendrites

Authors: *T. SMITH[1], C. CARNELL[1], H. KING[2], J. C. HUNT[2], A. GERBER[2], J. SEIBT[1];
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Abstract: Neuroplasticity, especially during learning, relies on molecular mechanisms that are localised within specific compartments (i.e. soma and dendrites, specifically synapses). Post-transcriptional changes regulate expression of proteins via synapse-specific translational regulation, but these are poorly understood. Therefore, the identification of actively translated mRNAs, the "translatome", within neuronal dendrites during learning paradigms could lead to better therapeutic interventions for multiple neurological pathologies. After a literature review, we chose to generate a mouse line (C57 background) that expresses hemagglutinin (HA) tags on the large ribosomal protein 22 in a subset of cortical neurons (Layer V). This particular population of neurons possess dendrites that show greater calcium fluctuations than the soma during a neuroplasticity-inducing learning paradigm. With this targeted expression of HA, we can perform the Translating Ribosome Affinity Purification (TRAP) protocol to study the translatome. Our methods use both sexes and utilise blinding/automated analysis to exclude bias. Immunofluorescent staining confirmed co-localisation of the HA-tag with a neuronal specific marker in ~80% of neurons (n = 1 mouse). Now, we are currently optimising the TRAP procedure to maintain high quality RNA in extracts and during affinity isolations for up to 24-hours. To determine the best total RNA extraction protocol, we compared two commercially available kits and a phenol-chloroform extraction (n = 4). While total RNA quantity was highest with phenol-chloroform extraction (n.s.), the commercial kits provided better quality, as evident from RNA integrity number values determined with the Bioanalyzer (n.s.). Our TRAP protocol will be refined until we obtain > 100 ng of dendritic RNA from a single cortical hemisphere that we can use for RNA sequencing. Overall, our mouse model and associated protocol for the characterization of the dendritic translatome of L5 cortical neurons from single mouse brain will advance our knowledge of complex brain function, including cognition, sensory integration, learning and memory.

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Poster

036. Synaptic Signaling

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Program #/Poster #: 036.18

Topic: B.05. Synaptic Plasticity

Support: NIH grant 5F31MH124355
NIH grant 1R01NS111162

Title: Precise synaptic regulation by a locally translated activity dependent transcription factor.

Authors: *D. A. HEINZ[1], B. L. BLOODGOOD[2];
[1]Div. of Biol. Sci., UCSD, San Diego, CA; [2]Divsion of Biol. Science, Neurobio., UCSD Dept. of Neurosciences, La Jolla, CA

Abstract: The activity induced transcription factor NPAS4 acts as a molecular hub, coordinating a dynamic interplay between neuronal excitation and inhibition. In mouse CA1 pyramidal neurons NPAS4 is induced by two discrete forms of excitation, with AP trains leading to de novo transcription and translation of NPAS4, and dendritic EPSPs driving local dendritic translation of an alternate Npas4 transcript with a long 5'UTR. Using Cas9 to disrupt the local dendritic translation of NPAS4 in vivo and precise ex vivo stimulation of inhibitory synapses, we examine the synaptic consequences of NPAS4 from these different sources, with two key findings: 1) While experientially induced NPAS4 is necessary for both an increase in somatic and decrease in dendritic inhibition, EPSP induced local translation of NPAS4 in the stratum radiatum (SR) dendrites is specifically necessary for the experience dependent reduction in inhibition on the same region of dendrite, and 2) the NPAS4 dependent destabilization of dendritic inhibitory synapses is restricted to a genetically defined subpopulation of interneurons known to gate dendritic spikes and long term plasticity. Together these findings demonstrate a remarkable precision in the experience-driven, transcriptionally-regulated coordination of excitation and inhibition.

Disclosures: D.A. Heinz: None. **B.L. Bloodgood:** None.

Poster

036. Synaptic Signaling

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Program #/Poster #: 036.19

Topic: B.05. Synaptic Plasticity

Support: 1R01NS111162
1F31MH123112-01A1

Title: The effects of activity-dependent reorganization of inhibition on CA1 pyramidal cell in vivo activity

Authors: *A. PAYNE[1], S. LEUTGEB[3], B. L. BLOODGOOD[2];
[2]Divsion of Biol. Science, Neurobio., [1]UCSD Dept. of Neurosciences, La Jolla, CA; [3]Neurobio., Univ. of California San Diego, La Jolla, CA

Abstract: NPAS4, an immediate early gene that is expressed transiently following PN activity, has been linked to changes in inhibitory synaptic connectivity. Specifically, NPAS4 leads to recruitment of somatic CCK basket cell synapses and destabilization of dendritic inhibitory synapses. This sophisticated reorganization of inhibition uniquely positions NPAS4+ PNs within a local microcircuit. Somatic inhibition from CCK basket cells likely shapes the action potential output of these PNs while a decrease in dendritic inhibition will likely foster a state that is amenable to various forms of plasticity. To investigate the role of NPAS4 in this microcircuit, NPAS4 was knocked out from a sparse population of optically tagged CA1 PNs in the mouse hippocampus, allowing for simultaneous recordings from knock out (KO) and wild-type (WT)

neurons while an animal is freely moving on a track. Intriguingly, KO neurons had overall higher firing rates than their WT counterparts but were less likely to fire in bursts. These opposing effects in the pattern of activity of NPAS4 KO cells translated to deficits in their spatial tuning. Furthermore, NPAS4 KO neurons were less theta modulation due to spurious firing outside of the preferred theta phase. These results demonstrate that synergistic reorganization of somatic and dendritic inhibition, mediated by the activity-dependent transcription factor NPAS4, is important in shaping the activity of CA1 PNs across an environment as well as at fine temporal scales.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.01

Topic: B.07. Network Interactions

Support: Fondecyt 1210069

Title: CVE topography: A map of the truly synchronous populations underlying EEG oscillations

Authors: *V.-M. HIDALGO[1,2], J. DIAZ[3], J.-C. LETELIER[1,2], F. J. FLORES[4,5];
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Abstract: EEG topography is a common tool to study the spatial organization of neural dynamics using the spectral power and frequency of signals recorded on the scalp. The presence of oscillations tends to be highlighted in these analysis, as they often present a distinctive high power peak in a narrow frequency band. Oscillations are also often assumed to be the result of synchronous activity in the underlying neural populations. However, it is common knowledge in telecommunications that high-amplitude oscillations can arise from asynchronous, Gaussian processes in a phenomenon known as Rayleigh fading. Rayleigh fading can be detected by analyzing the coefficient of variation of the signal's envelope (CVE), which summarizes the amplitude modulation pattern of the signal. We have previously shown that brain oscillations that arise from asynchronous processes display a CVE close to 0.523, while sub-Gaussian and super-Gaussian values are related to different kinds of synchronization. Here we develop CVE topography, a spatial map of amplitude modulation patterns across sites in scalp EEG recordings. We assess the effect of filtering, sampling rate, and epoch length, as well as the effects of different referencing schemes on the CVE topography. We explore this new tool on human EEG recordings obtained during resting-state, eyes-closed conditions as a case study and show that the

amplitude modulation of the human alpha rhythm coexists with both Gaussian and synchronous amplitude modulation patterns over the scalp surface. Crucially, CVE is independent of the power and frequency of a signal but is instead related to the phase component of the Fourier decomposition, thus complementing traditional spectral topography. CVE topography constitutes a powerful tool for brain state tracking.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.02

Topic: B.07. Network Interactions

Support: R01MH128235

Title: Deficits in endogenous neurosteroidogenesis contributes to network and behavioral consequences of chronic stress

Authors: ***N. L. WALTON**, P. ANTONOUDIOU, A. EVANS-STRONG, J. MAGUIRE; Tufts Univ. Sch. of Med., Tufts Univ. Sch. of Med., Boston, MA

Abstract: Depression remains the leading cause of morbidity worldwide. A major risk factor for depression is chronic stress yet, the mechanisms whereby stress induces illness remains unclear. Lack of insight into these mechanisms further complicates treatment of depressive disorders, with less than 50% of individuals with depression achieving any form of symptom relief with first- and second-line antidepressants. Zulresso, a proprietary formulation of the endogenous 5α-reduced neurosteroid allopregnanolone, was recently approved for the treatment of postpartum depression. Here we investigate whether deficits in endogenous 5α-reduced neurosteroid signaling contribute to the pathophysiology of depression by examining behavioral impairments following chronic unpredictable stress (CUS) in mice. We demonstrate a reduction in allopregnanolone levels and expression of the rate-limiting enzymes involved in neurosteroid synthesis, 5α-reductase type 1 and 2, in the basolateral amygdala (BLA) of mice subjected to CUS compared to controls. Further, the expression of δ-$GABA_A$Rs is decreased in the BLA following CUS. Collectively, these results suggest that neurosteroid signaling is compromised in the BLA following CUS. To determine whether deficits in endogenous neurosteroid signaling contributes to the behavioral deficits associated with CUS, we used a CRISPR approach to knockdown 5α-reductase type 1 and 2 (5αR1/2), in the BLA. Knockdown of 5αR1/2 in the BLA mimicked the behavioral deficits associated with CUS, increasing avoidance, and learned helplessness behaviors. Lastly, we demonstrated that lentiviral overexpression of 5αR1/2 in the BLA rescues behavioral impairments induced by CUS. These findings suggest that deficits in endogenous neurosteroid signaling contribute to the behavioral deficits associated with chronic stress. Further, these studies suggest that the therapeutic efficacy of 5α-reduced neurosteroid-

based treatments may be due to their ability to directly target the underlying pathophysiology of depressive disorders.

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Poster

037. Oscillations and Synchrony: EEG Studies

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Program #/Poster #: 037.03

Topic: B.07. Network Interactions

Support: NRF grant 2015R1D1A1A02061486
NRF grant 2019R1A2C1009674

Title: Changes in functional connectivity during propofol-induced unconsciousness in human ECoG

Authors: ***M. CHOE**[1], S.-H. JIN[3], J. KIM[2], C. CHUNG[1,3,4];
[1]Dept. of Brain and Cognitive Sciences, Col. of Natural Sci., [2]The Res. Inst. of Basic Sciences, Col. of Natural Sci., Seoul Natl. Univ., Seoul, Korea, Republic of; [3]Neurosci. Res. Inst., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of; [4]Dept. of Neurosurg., Seoul Natl. Univ. Hosp., Seoul, Korea, Republic of

Abstract: Anesthetic loss of behavioral response is not necessarily accompanied by an even block of all cortical regional activities although anesthetic drugs suppress global central nervous system. It remains unclear that the network-level explanation underlying unconsciousness induced general anesthesia although the molecular effects of general anesthesia have been well known. In this study, we investigated the functional connectivity changes in human electrocorticography (ECoG) during propofol-induced loss of consciousness. We recorded the ECoG data of 72 patients (38 males and 34 females; age: mean = 33.6, SD = 10.7 years). The recordings were obtained during awake and unconscious state during propofol-induced anesthesia. The average recording time during awake and unconscious state were 106.30 and 233.33 seconds (standard deviation, 70.74 and 127.57 seconds). Anesthesia was induced using target-controlled infusion of propofol and remifentanil. The average propofol and remifentanil infusion rate were 0.142 mg/kg/min and 0.155 mcg/kg/min (standard deviation, 0.032 mg/kg/min and 0.038 mcg/kg/min). We investigated cortico-cortical functional connectivity as measured with coherence. Wilcoxon signed rank test was performed to estimated functional network changes of cortical areas at corrected $p < 0.05$. The functional connectivity increased during unconscious state between auditory areas (auditory cortex and auditory association cortex) and inferior parietal lobe (IPL) and between temporal lobe and auditory areas, IPL, and limbic

system in delta frequency band. The connectivity also increased during unconscious state between visual cortex and auditory areas, IPL, limbic system, somatosensory cortex, and temporal lobe, and between prefrontal and IPL in delta frequency band. The connectivity increased between visual cortex and temporal lobe in theta, alpha, and beta frequency bands. In low gamma frequency band, the connectivity increased between auditory cortex and temporal lobe, and between visual cortex and IPL and temporal lobe. In high gamma frequency band, the connectivity increased between visual cortex and IPL and somatosensory cortex and between prefrontal cortex and temporal lobe and visual cortex.The functional connectivity changes induced propofol anesthesia indicated altered networks of visual and auditory processing during the loss of consciousness. Therefore, we concluded that loss of consciousness induced by general anesthesia results from disrupted communication between self and external world, with changed communication of the cortical areas.

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Poster

037. Oscillations and Synchrony: EEG Studies

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Topic: B.07. Network Interactions

Support: The National Research Foundation of Korea grant (NRF-2022R1A2C3003901)
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Title: Delineation of Alzheimer's disease pathogenesis by the downregulation of parvalbumin-positive neurons in the basal forebrain

Authors: *E. HWANG[1], H. SHIM[2], H. LEE[3], S. HYEON[3], H.-J. KIM[3], J. KIM[4], M.-H. NAM[3], S. KIM[3], H. PARK[2], J. LEE[5], Y. LEE[6], E. HWANG[3], T. D. STEIN[7], J. CHOI[3], H. RYU[3];
[1]Lablup Inc, Seoul, Korea, Republic of; [2]Stand up Sci., Seoul, Korea, Republic of; [3]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [4]Korea Brain Res. Inst., Daegu, Korea, Republic of; [5]Dept. of Neurol., Boston Univ. Sch. of Med., Boston, MA; [6]Dept. of Computer Sci. and Enginee, Dongguk University-Seoul, Seoul, Korea, Republic of; [7]Boston VA Med. Ctr., Boston, MA

Abstract: Association between Alzheimer's disease (AD) and the basal forebrain (BF) has been studied mostly via cholinergic pathway. While the proportion and function of parvalbumin (PV) positive neurons in BF is not negligible, its role in the development AD is hardly studied. When we examined the early-stage AD brains of human and mice, BF PV levels were significantly reduced compared to the healthy control. With the hypothesis that BF-PV loss could be one of the potential initiators for AD symptoms, we investigated the consequence of BF-PV knockdown (KD) in the behaviors, electrophysiology, and hippocampal pathology in comparison to AD mice model (APP/PS1). We observed the impairment of the social aggression and spatial memory, as

well as EEG theta rhythms and their coupling to fast oscillations in the BF-PV-KD mice. While tracking down the cause of changes in hippocampus-related behaviors and oscillations after the BF-PV-KD, we found the enhanced inhibitory synaptic transmissions in CA1 pyramidal neurons, reduced BDNF and Arc expression levels, and intensified amyloidosis in CA1 and CA3 regions. Meta-correlation analysis between the behavioral, electrophysiological, and pathological features showed that theta-gamma coupling and cleaved caspase-3 expression in BF were the significant correlates of spatial memory impairment. In the feature analysis using artificial neural network, we found that the features that play important role in distinguishing AD and normal mice also contribute to distinguishing BF-PV-KD mice and sham control, implying that AD mice share multiple pathological, electrophysiological, and behavioral features with BF-PV knockdown mice. Taken together, our integrative approach revealed the impact of BF-PV loss in emergence of AD-like phenotypes and its pathophysiological mechanism.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

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Program #/Poster #: 037.05

Topic: B.07. Network Interactions

Title: Investigation of the correlation between subjective well-being and frontal alpha asymmetry on group and single-subject level

Authors: *B. WUTZL[1], K. LEIBNITZ[3], M. MURATA[2];
[1]Osaka Univ., [2]Osaka Univ., Suita, Japan; [3]Ctr. for Information and Neural Networks, Suita, Japan

Abstract: Previous studies with EEG have revealed that subject well-being (SWB) can be correlated to frontal alpha asymmetry (FAA). However, those studies only considered a single SWB value for each trial and subject. Our goal is to find out if SWB can also be dynamically modeled in response to changing environmental conditions (i.e., temperature and humidity) on small time scales. 30 subjects (ages 22.3 ± 4.2 years, 14 females, 2 left-handed, 28 right-handed) were included in this study. An Emotiv EPOC X EEG headset was placed on the subject's head and the environment was set to one of six temperature-humidity conditions. Each recording took approximately six minutes, and the subject was asked to evaluate his/her SWB every 30 seconds on a scale from 1 (worst) to 10 (best). After performing preprocessing steps in EEGLAB, we computed the FAA for each 30-second interval centered around the timings when the SWB values were given by the subjects. Since there was a disparity in the sampled SWB values, the resulting FAA-SWB pairs were also non-uniform, with many values for SWB of 6, 7, and 8, but only few for 1, 2, 3, and 10. To overcome this imbalance, we reduced all FAA-SWB pairs from

all subjects having the same SWB value to a single representative FAA-SWB pair using the mean over these FAA. We performed a linear interpolation over all representative FAA-SWB pairs and found a significant correlation with a coefficient of determination of R^2=0.708 and p-value of p=0.002. We also performed the same analysis on individual subjects. We excluded 12 subjects due to not having sufficient data available, i.e., when the subject had too low diversity in SWB values or had just a few artifact-free EEG recordings. We found a positive linear correlation between FAA and SWB with a coefficient of determination of $R^2 > 0.2$ for 12 subjects, while the other 6 subjects showed a negative linear correlation with a coefficient of determination of $R^2 < 0.1$. In summary, our study showed that there is a positive correlation on the group level between SWB and FAA when changing the temperature and humidity of the environment. We also conclude that this relationship not only holds for a single SWB value extracted over the entire time course of the experiment, but also when observing much shorter time scales, such as 30 seconds. Finally, we could also observe a similar relationship on a single-subject level in 67% of the cases.

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Poster

037. Oscillations and Synchrony: EEG Studies

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Topic: B.07. Network Interactions

Support: Tata Trusts Grant
Wellcome Trust/DBT India Alliance
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Title: Healthy aging and cognitive impairment alter EEG functional connectivity in distinct frequency bands

Authors: *S. WUPADRASTA, S. RAY;
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Abstract: Functional connectivity (FC) measures indicate the interdependencies between brain signals recorded from spatially distinct locations and have been extensively studied during resting state as well as during cognitive tasks such as decision making and working memory. Here, we studied changes in FC in EEG signals with healthy aging and with mild cognitive impairment (MCI), while a large cohort (N=247) of elderly subjects (>49 years) viewed large cartesian gratings that induced strong gamma oscillations in two frequency bands, termed slow (20-34 Hz) and fast gamma (36-66 Hz). We have previously shown that with both healthy aging and with MCI/AD, power in both gamma bands decreases significantly, while power in the alpha (8-12 Hz) band does not. We first considered healthy subjects (N=218) in two age groups: middle-aged (50–65 year, N=91) and elderly (>66 year, N=127) and computed FC values using

Pairwise Phase Consistency functional connectivity measure with reference electrodes from occipital electrodes {P3, P1, PO3, P2, P4, PO4, POz, O1, Oz, and O2}. We found that with healthy aging, FC decreased in the alpha band (K-W test, p=0.007), but not in either the slow or fast gamma band (slow gamma: p=0.08; fast gamma: p=0.21, K-W test). These results held even when we subsampled each group for each frequency band such that the power was matched for the two groups (alpha: p=0.057; slow gamma: p=0.01; fast gamma: p=0.35, K-W test). However, the results were different when we compared MCI subjects (N=11) with their age and gender matched controls (N=70; since the sample sizes were not balanced, results were averaged across controls for each MCI subject before statistical comparison). Here, alpha FC remained unchanged, but there was a reduction in gamma FC, especially in the slow gamma band (alpha: p=0.14, slow gamma: p=0.02, and fast gamma: p=0.35, WSR test). As before, the results held even when we subsampled the controls so that the power was matched between the two groups (alpha: p=0.16, slow gamma: p=0.009; fast gamma: p=0.26, WSR test). Results remained similar when other measures of FC, such as coherence, were used. Overall, our results point towards differences in connectivity patterns with aging and disease and suggest that EEG FC can be used to distinguish healthy aging from MCI onset.

Disclosures: S. Wupadrasta: None. **S. Ray:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.07

Topic: B.07. Network Interactions

Support: K08MH080239

Title: Functional connectivity between right lateral frontal and medial frontal cortices during reward and loss processing

Authors: *M. A. PUOPOLO, A. A. VIVINO, B. C. MCNICHOL, E. M. BERNAT;
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Abstract: Intro: Theta and delta EEG/ERP amplitudes during gambling feedback have been related to loss and reward processing, respectively. Substantial evidence now demonstrates functional connectivity in theta between medial-frontal and lateral frontal regions as part of this processing, understood to subserve control. However, there is little information about functional connectivity in the delta band during gambling feedback. To address this, the current project assesses functional connectivity in delta, in a dataset where theta effects have already been demonstrated. We hypothesized that functional connectivity in delta would be enhanced relative to reward feedback, in contrast to theta effects which are increased after losses. **Method**: 145 participants completed a gambling task, choosing between two money values and receiving feedback on each trial indicating a gain or loss of the chosen amount. 128-channel EEG data was

recorded. Data in feedback-locked epochs were band-pass filtered and one-factor principal components analyses were applied within theta and delta, yielding one measure of loss-gain amplitude condition differences in each band. Interchannel phase-synchrony (ICPS) measures of functional connectivity were calculated between medial-frontal and lateral prefrontal sites. **Results**: Replicating previous work, centroparietal delta amplitude was greater after gains (Wilcoxon's $V = 8275$, $p < .001$, rank-biserial correlation $r_b = .56$), while midfrontal theta amplitude was greater after losses ($V = 272$, $p < .001$, $r_b = .95$). Similarly, there was greater ICPS in theta activity between midfrontal and right prefrontal electrodes after losses ($V = 1488$, $p < .001$, $r_b = -.72$). In contrast, as hypothesized, ICPS in delta was greater after gains between the midfrontal site and a right-lateral prefrontal site ($V = 7502$, $p < .001$, $r_b = .42$). ICPS measures of functional connectivity in theta and delta were not correlated ($\rho = .03$, $p = .7$). **Discussion**: Findings support the view that right-frontal regions are functionally connected more broadly than previously observed, with engagement for both loss and reward processing.

Disclosures: M.A. Puopolo: None. **A.A. Vivino:** None. **B.C. McNichol:** None. **E.M. Bernat:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.08

Topic: B.07. Network Interactions

Support: Fondecyt 1210069

Title: Assessing Synchronous/Arrhythmic EGG through Gaussianity determination: The CVE Method

Authors: *J.-C. LETELIER[1], V. HIDALGO[2], J. DIAZ[3];
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Abstract: The idea that some EEG traces are similar to Gaussian noise has been asserted and questioned for many years. With the renewed focus on the functional role of arrhythmic EEG activity, interest in this Gaussian similarity has resurfaced. In this context, the spectral profile of neural signals could be a decisive reference point to detect the presence of event-related synchronization (ERS). However, because neural synchronization has been linked to peaks in a particular EEG frequency band in the power spectrum, this linkage has led to a neural synchronization event being defined by the magnitude of peaks in the spectrum. Rather than the peaks in power spectrum as the ERS estimator, here we show that an alternative ERS estimator is the amplitude modulation of the signal as characterized by the coefficient of variation of the envelope (CVE). We demonstrate that while a spectral peak is a mark of an increase of power in a given frequency band, the underlying generative process can be either synchronous or

asynchronous (Gaussian), and this difference can be detected by characterizing the amplitude modulation using the CVE. Our method based on the analysis of signal envelopes is detailed here. First introduced to analyze the olfactory peripheral waves in fish, our method uses signal envelopes. In effect, the envelope of zero-mean Gaussian noise follows the Rayleigh distribution, which has the crucial property that the ratio between its standard deviation (σ) and mean (μ) — its coefficient of variation (CV=σ/μ) — is constant and equal to $\sqrt{(4-\pi)/\pi} \approx 0.523$ (Diaz et al. 2007, DOI:10.1523/JNEUROSCI.4512-06.2007). Thus, when the envelope of a neural signal is considered as a random variable, its CV (called the coefficient of variation of the envelope, CVE) is a Gaussianity fingerprint. We have used this property to study the Gaussianity of a) the peripheral waves in the olfactory epithelium of fish (DOI:10.1523/JNEUROSCI.4512-06.2007), b) the rat EEG (DOI:10.1016/j.neuroimage.2018.01.063), and c) human alpha rhythm (DOI:10.1101/2021.03.31.437785). Our Gaussianity detection algorithm is easy to implement and serves to classify behavioral states. Additionally, by using Fourier transform phase randomization, we reveal the links between CVE, Gaussianity and modulation profiles. Moreover, the CVE method allows the similarities and differences between neural signals and Gaussian noise to be studied within a solid mathematical framework that unshackles neural synchronization from Lord Adrian´s 1930 metaphor.

Disclosures: J. Letelier: None. **V. Hidalgo:** None. **J. Diaz:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.09

Topic: B.07. Network Interactions

Support: Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI
Magnificent Michigan Summer Research Fellowship from the University of Michigan Neuroscience Innovators to Kanakaharini Byraju

Title: Effect of intravenous delivery of psilocybin on sleep-wake states in rat

Authors: *K. BYRAJU[1], T. GROENHOUT[1], B. H. SILVERSTEIN[1,2], N. KOLBMAN[1,2,3], T. LIU[1], G. A. MASHOUR[1,2,3,4], G. VANINI[1,2,4], D. PAL[1,2,4,5];
[1]Dept. of Anesthesiol., [2]Ctr. for Consciousness Sci., [3]Dept. of Pharmacol., [4]Neurosci. Grad. Program, [5]Dept. of Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: There is a resurgence of interest in psychedelic drugs, including psilocybin, as potential therapeutic agents for the treatment of psychiatric disorders. There has also been increasing trend of decriminalization of psilocybin for recreational and medicinal use. However, there are limited data on the effect, acute and long-term, of psilocybin on sleep, which is important to understand because of the therapeutic and public health implications. A recent

human study showed that daytime peroral psilocybin increased the latency to onset of nightly REM sleep (REMS). Intraperitoneal psilocin in mice was shown to produce short-term increase in wakefulness (WAKE) and decrease in slow-wave sleep (SWS) and REMS. Preclinical animal models are critical from a mechanistic point of view, but intraperitoneal route lacks translational validity and there are no data on the effect of psilocybin on sleep-wake states in rat. Therefore, in this study, we investigated the effect of intravenous psilocybin on sleep-wake states in adult male Sprague Dawley rats (N=4). Under isoflurane anesthesia, animals were instrumented with a chronic catheter in jugular vein, and electrodes to record electroencephalogram (EEG) across the cortex and electromyogram (EMG) from dorsal nuchal muscles. All rats received two doses of psilocybin (low: 2.5 mg/kg, and high: 10 mg/kg) and 0.9% saline (vehicle control) as a bolus at the beginning of the light cycle (8:00 am), after which EEG and EMG data were collected across 12 h light and 12 h dark phases. The infusions were counterbalanced with 5-7 days between each infusion. The EEG and EMG data were analyzed in 4-s epochs as WAKE, SWS, and REMS. The data are reported as mean ± standard deviation. Psilocybin at low dose does not appear to have an appreciable effect on WAKE during the first 12 hours post-infusion, but the high-dose psilocybin appears to reduce the time spent awake (Saline: 41.24% ± 8.11 vs. Low dose: 41.82% ± 13.10 vs. High dose: 35.22% ± 13.00). Similarly, low dose psilocybin did not impact SWS, but the high dose appears to increase the time spent in SWS (Saline: 49.66% ± 7.97 vs. Low dose: 51.66% ± 9.59 vs. High dose: 58.14% ± 9.86). In contrast, both low dose and high dose psilocybin reduced the time spent in REMS during the first 12 hours post-infusion (Saline: 9.11% ± 5.08 vs. Low dose: 6.52% ± 4.89 vs. High dose: 6.64% ± 5.80). Further studies are needed to understand whether high-dose psilocybin has a significant impact on post-drug sleep architecture, and whether there are changes in the EEG microarchitecture of sleep-wake states.

Disclosures: **K. Byraju:** None. **T. Groenhout:** None. **B.H. Silverstein:** None. **N. Kolbman:** None. **T. Liu:** None. **G.A. Mashour:** F. Consulting Fees (e.g., advisory boards); Serves as a consultant for TRYP Therapeutics (unrelated to this study). **G. Vanini:** None. **D. Pal:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.10

Topic: B.07. Network Interactions

Support: Tryp Therapeutics, Kelowna, BC, Canada
Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI

Title: Intravenous Psilocybin Alters Brain Network Dynamics in Rat

Authors: ***B. H. SILVERSTEIN**[1,2], N. KOLBMAN[1,2,3], T. LIU[1], P. GUZZO[6], J. GILLIGAN[6], G. A. MASHOUR[1,2,3,4], G. VANINI[1,2,4], D. PAL[1,2,4,5];
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Program, [5]Dept. of Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI; [6]Tryp Therapeut., Kelowna, BC, Canada

Abstract: Psilocybin is a naturally occurring psychoactive compound that is being increasingly explored for therapeutic use in psychiatric disorders. Several studies have characterized the neurophysiological effects of psilocybin in human subjects. However, the effect of psilocybin on brain dynamics in animals is not well studied but is imperative for the development of models that can advance mechanistic understanding. Under isoflurane anesthesia, adult male Sprague Dawley rats (N=4, 300-350 g) were implanted with a jugular vein catheter, and electrodes to record electroencephalogram (EEG) across the cortex. After 7-10 days of post-surgical recovery, each rat received 10 mg/kg psilocybin or 0.9% saline (vehicle control group) as a continuous infusion over a period of 60 minutes while the EEG (0.1-300 Hz, sampling rate at 1 kHz) data were simultaneously collected. The EEG data were also collected for 20 minutes before and 120 minutes after psilocybin or saline infusion. The psilocybin and saline infusions were counterbalanced with 5-7 days between each infusion. We computed the absolute power spectrum (PSD), magnitude-squared coherence, and Lempel-Ziv Complexity (LZC) of the EEG time series during 5-minute epochs selected from 1) just before the start of infusion, 2) during psilocybin infusion (beginning, middle, and end), and 3) during the post-psilocybin recovery period. Statistical analyses were conducted using a linear mixed model with treatment condition and epoch as fixed factors, a random effect of rat, and PSD, coherence, or LZC as the outcome variables. Previous human studies have shown the psilocybin-induced psychedelic effect to be associated with increase in 1-30 Hz LZC. In rat, subanesthetic dose of ketamine has been shown to produce sustained increases in 0.5-175 Hz LZC and enhance gamma (65-175 Hz) power and coherence. In contrast, the results from the current study demonstrate the most salient LZC (temporal) changes in 95-150 Hz band, which showed a greater increase relative to pre-infusion baseline during psilocybin vs. saline infusion ($p<0.0001$); there was no significant change in LZC in 1-30 Hz band ($p=0.94$). These complexity changes were accompanied by decrease in low frequency (4-10 Hz) PSD ($p=0.002$) and coherence ($p=0.008$). No significant changes were found in high frequency gamma (95-150 Hz) PSD ($p=0.37$) or coherence (coherence: $p=0.44$). Further studies and fine-grained analysis of these data are needed to understand the translational validity of psilocybin infusion rat models.

Disclosures: B.H. Silverstein: None. **N. Kolbman:** None. **T. Liu:** None. **P. Guzzo:** A. Employment/Salary (full or part-time):; Tryp Therapeutics. **J. Gilligan:** A. Employment/Salary (full or part-time):; Tryp Therapeutics. **G.A. Mashour:** Other; Consultant for Tryp Therapeutics. **G. Vanini:** None. **D. Pal:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.11

Topic: B.07. Network Interactions

Support: Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI

Title: Intravenous DOI, a 5-HT2A/2C agonist, induces active emergence from propofol anesthesia in rat

Authors: *C. W. FIELDS[1], E. R. HUELS[1,2,3], G. A. MASHOUR[1,2,3,4], D. PAL[1,2,3,5];
[1]Dept. of Anesthesiol., [2]Neurosci. Grad. Program, [3]Ctr. for Consciousness Sci., [4]Dept. of Pharmacol., [5]Dept. of Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: General anesthesia is a pharmacologically induced coma characterized by reduced spatiotemporal electroencephalographic complexity, decrease in cortical acetylcholine, and a shift in spectral power to low frequency bands. In contrast, the psychedelic state induced by 5-HT2A agonists is associated with increase in spatiotemporal electroencephalographic complexity, elevated cortical acetylcholine, and broadband decrease in spectral power. Taken together, psychedelics and anesthetics induce profound alterations in consciousness with contrasting neurochemical and neurophysiological effects. DOI (2,5-dimethoxy-4-iodoamphetamine) is a synthetic psychedelic that acts via 5-HT2A/2C receptors. A recent study showed that intracerebroventricular delivery of DOI facilitated passive emergence from isoflurane anesthesia. However, the effects of psychedelics on brain dynamics during anesthesia are not well-characterized, and it is not clear if the arousal promoting effect of DOI is specific to isoflurane (a halogenated ether) or can be generalized to anesthesia induced by different anesthetics. In this study, we determined the effect of intravenous DOI on electroencephalogram (relative power, corticocortical coherence) and behavioral arousal in propofol-anesthetized rat. Under isoflurane anesthesia, adult male Sprague Dawley rats (N=3) were implanted with electrodes to record electroencephalogram (EEG) across cortex, and a chronic double lumen jugular vein catheter for simultaneous delivery of propofol and DOI. After at least 1 week of recovery, baseline EEG was recorded for 30 minutes. Thereafter, general anesthesia was induced via intravenous propofol (1000 ug/kg/min) for 20 minutes. After the 20-minute induction period, propofol flowrate was reduced to a maintenance dose of 600 ug/kg/min for 20 minutes, at which point intravenous DOI (1 mg/kg) was delivered over a 1-minute period while continuing the propofol infusion. Intravenous DOI during continuous propofol anesthesia induced pronounced behavioral arousal (orofacial/limb/torso movements, head-twitch response, and attempts at return of righting reflex), and 1 out of 3 rats regained the righting reflex. The most salient changes in EEG were concomitant activation with behavioral arousal and increase in high gamma (85-125 Hz) power (mean ±standard deviation: 1478.30% ± 936.66) and coherence (mean ±standard deviation: 136.2% ± 28.01). These results provide preliminary evidence for a role of DOI in reversing propofol anesthesia, modulating behavioral arousal, and increasing the power and coherence of high-frequency oscillations.

Disclosures: C.W. Fields: None. **E.R. Huels:** None. **G.A. Mashour:** None. **D. Pal:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.12

Topic: B.07. Network Interactions

Support: Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI

Title: Neurochemical and neurophysiological correlates of intravenous N,N-dimethyltryptamine in rat

Authors: *N. GLYNOS[1], E. HUELS[1], T. LIU[1], R. KENNEDY[2], G. A. MASHOUR[1], D. PAL[1]; [1]Dept. of Anesthesiol., [2]Dept. of Chem., Univ. of Michigan, Ann Arbor, MI

Abstract: *N*,*N*-Dimethyltryptamine (DMT) is a short acting serotonergic psychedelic that elicits profound alterations in consciousness and is currently being investigated clinically for the treatment of mental health disorders. Although neurophysiological effects of DMT in humans are well characterized, similar data from animal models are lacking. Furthermore, there are scant data on the effect of DMT on cortical neurochemistry, which can inform mechanism of action. Therefore, in this study, we investigated the effect of intravenous delivery of DMT (low dose: 0.75 mg/kg, high dose: 7.5 mg/kg) on cortical neurophysiology and simultaneous changes in cortical neurotransmitter release. Under isoflurane anesthesia, adult male Sprague Dawley rats (N=4, 300-350 g) were implanted with 1) electrodes to record electroencephalogram (EEG) from across the cortex, 2) a chronic jugular vein catheter for DMT infusion, and 3) guide tubes in prefrontal cortex (PFC) and somatosensory barrel field (S1BF) for neurotransmitter sampling with open flow microperfusion. The samples collected from both cortical sites were analyzed using liquid chromatography tandem mass spectrometry to quantify simultaneous changes in multiple analytes including acetylcholine (ACh), serotonin, and dopamine (D). The changes in neurotransmitter levels and EEG (relative spectral power and corticocortical coherence) after DMT infusion are reported as % change (mean ± standard deviation) from the pre-DMT baseline values. Intravenous DMT produced dose-dependent increase in neurotransmitter levels in both PFC (ACh [low]: 12.2% ± 13.7; ACh [high]: 113.4% ± 42.3; serotonin [low]: 16.2% ± 34.9; serotonin [high]: 1,485.5% ± 1,828.9; D [low]: 81.3% ± 102.2; D [high]: 664.3% ± 820.4) and S1BF (ACh [low]: -5.2% ± 25.4; ACh [high]: 84.4% ± 75.1; serotonin [low]: 90.8% ± 88.4; serotonin [high]: 326.9% ± 228.7; D [low]: 78.2% ± 68.5 ; D [high]: 70.2% ± 112.7). Global EEG analyses of the high-dose DMT data during the same period showed 1) attenuation of spectral power [-40.81% ± 12.96] and coherence [-15.37% ± 4.51] in theta band. Simultaneously, there was an increase in spectral power and coherence in medium gamma (85-125 Hz; power: 31.72% ± 32.29; coherence: 15.88% ± 9.78) and high gamma (125-175 Hz; power: 9.17% ± 50.03; coherence: 10.77% ± 10.21) bandwidth. To our knowledge, this is the first study to characterize the effects of DMT on simultaneous changes in cortical neurochemistry and EEG dynamics in rat. Further extension of this study will help inform translational validity of the rat model and enable mechanistic investigations of DMT.

Disclosures: **N. Glynos:** None. **E. Huels:** None. **T. Liu:** None. **R. Kennedy:** None. **G.A. Mashour:** F. Consulting Fees (e.g., advisory boards); Serves as a consultant for TRYP Therapeutics (unrelated to this study). **D. Pal:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.13

Topic: B.07. Network Interactions

Support: University of Michigan Department of Anesthesiology
University of Michigan Neuroscience Graduate Program
NIH Grant R21GM143521

Title: Effect of reversible inactivation of prefrontal cortex on EEG dynamics in rat

Authors: *E. R. HUELS[1,2,3], M. KIM[1,3], T. LIU[1], G. A. MASHOUR[1,2,3], D. PAL[1,2,3,4], U. LEE[1,3];
[1]Dept. of Anesthesiol., [2]Neurosci. Grad. Program, [3]Ctr. for Consciousness Sci., [4]Dept. of Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: We recently demonstrated that tetrodotoxin (TTX)-mediated inactivation of prefrontal cortex (PFC) in rats decreased the time to anesthesia-induced loss of righting reflex (LORR) and increased the time to return of righting reflex (RORR), which are surrogates for loss and return of consciousness in rodents. While these findings demonstrate a role for PFC in regulating arousal, the neurophysiological changes underlying these behavioral effects and the impact of PFC—a neural hub —inactivation on brain dynamics remains unknown. Here we analyzed the high-density (30 channels) EEG (0.1-500 Hz, 1 kHz sampling rate) in 9 rats from our published study to determine the effect of TTX-mediated PFC inactivation on brain dynamics. Baseline EEG data were collected for 30 minutes, after which TTX (156 μM) or saline (control) was bilaterally infused into PFC. Each rat received TTX and saline on different days in a counterbalanced order, with an interval of 5-7 days. The EEG recording continued during a 90-minute post-TTX injection period, after which the rats were anesthetized with 2.5% sevoflurane for 45 minutes while the EEG recording continued uninterrupted. EEG data were collected for 60 minutes during the post-anesthetic period. We analyzed relative spectral power and corticocortical coherence after TTX infusion (PFC inactivation) and following anesthetic emergence (RORR). A linear mixed model was used to compare the EEG measures between TTX and saline conditions. PFC inactivation increased delta (1-4 Hz) power ($p < 0.05$), which remained elevated following RORR ($p < 0.001$). While there was no immediate change in theta (4-10 Hz) power after TTX infusion, theta power decreased after RORR ($p < 0.05$). PFC inactivation after TTX injection also decreased delta coherence ($p < 0.05$), and delta ($p < 0.01$) and theta ($p < 0.001$) coherence were reduced after RORR. Preliminary network analyses show that PFC inactivation reduces explosive synchronization (i.e., network configurations that induce abrupt state transitions) (pre-TTX vs. post-TTX, mean ± standard deviation: 0.54 ± 0.22 vs. 0.04 ± 0.36), which suggests that disruption of the PFC decreases the brain's sensitivity as well as the capacity for state transitions, causing delayed recovery of consciousness. The comparison of neurophysiological changes after PFC inactivation with behavioral changes (increase in anesthetic sensitivity) is expected to provide mechanistic insights into network processes that

determine the trajectory of behavioral recovery from pharmacological and pathological states of unconsciousness.

Disclosures: E.R. Huels: None. **M. Kim:** None. **T. Liu:** None. **G.A. Mashour:** None. **D. Pal:** None. **U. Lee:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.14

Topic: B.07. Network Interactions

Title: Crowdsourced investigation of the relationship between dermatological and psychiatric symptoms.

Authors: *Z. STEWART[1,2], S. KORSAPATHY[3,2], F. FROHLICH[3,2];
[1]Dept. of Psychiatry, Univ. of North Carolina Chapel Hill, CHAPEL HILL, NC; [2]Carolina Ctr. for Neurostimulation, [3]Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Preliminary studies suggest that infection with *Bartonella spp.* can not only cause a diagnostic rash but also neuropsychiatric symptoms. To date, this association has only been reported in small sample case studies and it remains unclear if this association generalizes to other inflammatory skin symptoms. We used Amazon's Mechanical Turk to crowdsource a large sample (N=996) of individuals to ascertain the extent to which the presence of bartonella-associated cutaneous lesions (BACL) were associated with anxiety, depression, and schizotypy. Individuals completed surveys in which they were asked to identify dermatological symptoms (DS) that appeared on their own skin by selecting pictures followed by the Generalized Anxiety Disorder 7-item (GAD-7), the Patient Health Questionnaire-9 (PHQ-9) and the Schizotypal Personality Questionnaire-Brief Revised Updated (SPQ-BRU). The pictures were unlabeled to mask the phenomena associated with each photograph and included common DS as well as photographs of BACL. Point-biserial correlational analyses were run to determine the association between the presence of dermatological phenomena and neuropsychiatric symptomology. Participants were 37.5 ± 10.94 years of age, 81.5% White, 47.7% cisgender male, 41.5% cisgender female, 2.8% transgender male, 3.4% transgender female, 0.8% gender nonconforming and 3.7% Prefer not to answer. Measures of neuropsychiatric symptomology were high in the sample: anxiety (GAD-7; M=10.05 ± 5.60), depression (PHQ-9; M=12.73 ± 6.94) and schizotypy (SPQ-BRU; M=104.16 ± 29.79) and scores on all three measures were highly correlated. The selection of BACL photographs (n=194, 19.7%) was positively and significantly correlated with symptoms of anxiety (r=.185, p<.001), depression (r=.178, p<.001) and schizotypy (r=.123, p<.001). A photograph of an insect bite was used as a control and was not associated with symptoms of anxiety (r=-.042, p=.197), depression (r=-.028, p=.376) or schizotypy (r=-.026, p=.423). Furthermore, associations between non-BACL DS and neuropsychiatric symptomology were similar, with significant, positive associations between 9

out of 11 other conditions. While no causal inferences can be made, these preliminary findings are consistent with previous *Bartonella spp.* research and broadens evidence for the association between *Bartonella spp.* and neuropsychiatric illness. Non-BACL findings suggest the existence of shared biological processes such as inflammation and autoimmune reactions that drive both neuropsychiatric and dermatological symptoms.

Disclosures: Z. Stewart: A. Employment/Salary (full or part-time):; University of North Carolina at Chapel Hill. **S. Korsapathy:** None. **F. Frohlich:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); lead inventor of IP filed on the topics of noninvasive brain stimulation by UNC. F. Consulting Fees (e.g., advisory boards); paid consultant for Electromedical Products International. Other; honoraria from the following entities in the last twelve months: Academic Press, Insel Spital, University of Michigan.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.15

Topic: B.07. Network Interactions

Title: Effects of transcranial alternating current stimulation (tACS) on a spatial working memory training

Authors: *T. SCHWIPPEL[1,2], S. GALEFSKI[2], F. FROHLICH[1], C. PLEWNIA[2];
[1]Dept. of Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Dept. of Psychiatry and Psychotherapy, Univ. of Tübingen, Brain Stimulation Center, Ctr. for Mental Hlth. (TüCMH), Tübingen, Germany

Abstract: Introduction
Holding and manipulating information in the conscious mind requires synchronization between prefrontal and parietal cortices. Theta oscillations have been linked to the prefrontal cortex exerting top-down control over the parietal cortex and enabling phase synchronization during working memory tasks. Accordingly, the entrainment of prefrontal and parietal theta oscillations by means of transcranial alternating current stimulation (tACS) led to an increase in working memory performance in elderly and healthy volunteers. The present study is the first exploring the effects of fronto-parietal theta tACS on a working memory training. In addition, we investigate near transfer to verbal working memory, EEG functional connectivity and individual theta peak frequency before and after the training. **Methods**
This is a randomized, sham-controlled, pre-registered trial in a three-arm parallel design in 40 participants (https://osf.io/e5zhc). The experiment consists of six sessions including pre-training session, three training sessions with concurrent tACS, a post-training session and a 4-week follow-up session. Participants trained an adaptive spatial n-back task. During the training sessions, we applied 27 minutes of 1,5 mA peak-to-peak high-density 5Hz tACS to the right

dorsolateral prefrontal cortex (dlPFC, F4) and parietal cortex (P4), either in-phase (O°), anti-phase (°180) or sham. **Results**
In line with our preregistration, Likelihood-ratio tests indicated that the best model encompassed the interaction of session*condition, performance in the pre-training session and a random intercept for participant. The model RT_log ~ RT_log.pre + session * condition + (1 | id) yielded a significant effect for RT_log.pre (β = 0.55), session (β = -.04), session*anti-phase tACS (β = 0.04, *SE* = 0.02, *t* = 2.62) and session*in-phase tACS (β = 0.05, *SE* = 0.02, *t* = 3.08) indicating that anti-phase and in-phase tACS slowed response times dependent on session in comparison to sham. This is further supported by pairwise comparisons for each training session showing a significant slowing of response times in the anti-phase condition in session 1 and 3. Our second preregistered outcome, the baseline adjusted response time after the training (session 4) showed no difference between stimulation conditions, *p* = .69. **Discussion**
This study corroborates the immediate phase-specific effects of theta tACS on a behavioral outcome in a cognitive training underlining the intricate interplay between learning processes, baseline performance and tACS. Future studies will explore tACS enhanced trainings in neuropsychiatric or elderly populations.

Disclosures: **T. Schwippel:** None. **S. Galefski:** None. **F. Frohlich:** F. Consulting Fees (e.g., advisory boards); Electromedical Products International. Other; Academic Press, Insel Spital, University of Michigan. **C. Plewnia:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.16

Topic: B.07. Network Interactions

Support: UNC Department of Psychiatry

Title: Neural Signatures of Alternate Nostril Breathing: An Observational Study

Authors: A. FROHLICH[1], M. ZHANG[1], H. LAGARDE[1], J. RIDDLE[1], *F. FROHLICH[1], M. SKLEROV[2];
[1]Dept. of Psychiatry, [2]Dept. of Neurol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Breathing techniques play a central role in ancient mind-body practices such as yoga. Focus on breathing is also as part of mindfulness in third wave cognitive behavioral therapy. Despite their emerging clinical promise, very little is known about how breathing techniques modulate cortical activity patterns. Understanding this relationship will enable the design of targeted interventions such as the combination of breathing techniques with non-invasive brain stimulation like transcranial magnetic stimulation (TMS) for the synergistic treatment of psychiatric disorders.
One of the fundamental but poorly understood biological principles of breathing is that nasal

breathing usually does not occur bilaterally through both nostrils, but naturally alternates between the right and left nostril. This phenomenon is called the nasal cycle. We hypothesize that lateralization of breathing modulates the relative activation of the two hemispheres. Indeed, previous electroencephalography (EEG) studies have linked the nasal cycle to alternating cerebral hemispheric activation and have found that nasal airflow correlated with a contralateral increase in brain activity. Overall, no conclusive evidence exists for how the nasal cycle modulates cortical excitability due to the lack of adequately powered studies.
To close this gap, we investigate EEG signals associated with three different breathing patterns: right nostril breathing, left nostril breathing, and alternate nostril breathing. Healthy participants are recruited for a single session observational study, in which resting state EEG, blocks of paced slow breathing (left, right, alternating), and a visual working memory task are interleaved. In addition to 128-channel high-density EEG, continuous EKG and chest expansion signals are collected. EEG signals will be preprocessed and spectral analysis after removing of the aperiodic background signal will be performed to contrast spatial activity patterns between conditions. We hypothesize that oscillations and functional connectivity (phase-locking value) in the alpha frequency band will be modulated by the breathing condition.
If we find a relationship between neural oscillations and lateralization of breathing, future studies will examine how targeted breathing exercises could be of clinical use for disorders such as panic disorder, generalized anxiety disorder, and chronic pain.

Disclosures: **A. Frohlich:** None. **M. Zhang:** None. **H. LaGarde:** None. **J. Riddle:** None. **F. Frohlich:** Other; Electromedical Products International, Insel Spital, University of Michigan. **M. Sklerov:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.17

Topic: B.07. Network Interactions

Title: Variability in the individual alpha frequency as measured by a mobile EEG device correlates with trait anxiety

Authors: *L. SIDELINGER[1], M. ZHANG[3], F. FROHLICH[3], S. B. DAUGHTERS[2];
[1]Carolina Ctr. for Neurostimulation, [2]Dept. of Psychology and Neurosci., Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [3]Dept. of Psychiatry, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: The individual alpha frequency (IAF) has previously been identified as a unique neural signature of the alpha (8-12Hz) frequency band. However, the day-to-day stability of this feature is unknown. To accomplish this, we conducted a pilot study recruiting healthy participants without psychiatric disorders to record data at home using the Muse headband, a consumer-grade mobile electroencephalography (mEEG) device (n = 18). Preliminary testing of

the device revealed that the signal quality was too poor to identify alpha oscillations, likely due to the dry electrodes used. We thus used electrode gel to increase the recording quality. Resting-state recordings of all participants using high-density (HD) EEG were also collected before and after the at-home recording period, in addition to behavioral self-report assessments. To evaluate the data quality, spectral correlations were calculated between sessions for all location-matched electrodes. For frontal electrodes on the mEEG, there were low correlations between sessions both within the device and compared to HD EEG whereas the electrodes from the temporal regions, TP9 and TP10, had high correlation values. Because of this, only TP9 and TP10 were used for evaluating IAF stability. We then conducted a paired t-test between the IAF captured by the mEEG and the HD EEG, which rendered all insignificant results (TP9 session 1: $t(17) = 1.73$, $p = 0.10$; TP9 session 2: $t(17) = -0.24$, $p = 0.82$; TP10 session 1: $t(17) = -0.66$, $p = 0.52$; TP10 session 2: $t(17) = 1.53$, $p = 0.14$). Furthermore, we observed that IAF values were stable over one month for both EEG devices (mEEG TP9: $t(17) = -0.30$, $p = 0.77$; mEEG TP10: $t(17) = -0.56$, $p = 0.58$; HD EEG TP9: $t(17) = -0.88$, $p = 0.39$; HD EEG TP10: $t(17) = 1.45$, $p = 0.17$). Interestingly, exploratory analyses revealed a relationship between mEEG IAF day-to-day variability, as assessed by standard deviation (SD), and trait anxiety (TP9 SD vs. first session trait anxiety score: $rho = 0.50$, $p = 0.036$; TP9 SD vs. second session trait anxiety score: $rho = 0.57$, $p = 0.014$; TP10 SD vs. first session trait anxiety score: $rho = 0.51$; $p = 0.029$). Locally measured IAF varied across the scalp, often distinct from the global IAF (highest power peak across all channels). Nevertheless, local and global IAFs were strongly correlated and may be used to predict each other. Altogether, these results show that mEEG devices are useful for studying IAF stability and that the IAF is stable over time. The relationship between the day-to-day variability of region-specific IAF and the dynamics of psychiatric symptoms should be further investigated.

Disclosures: **L. Sidelinger:** None. **M. Zhang:** None. **F. Frohlich:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Academic Press, Insel Spital, University of Michigan. F. Consulting Fees (e.g., advisory boards); Electromedical Products International. **S.B. Daughters:** None.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.18

Topic: B.07. Network Interactions

Support: R01MH124387
R01MH122477

Title: Causal role of theta oscillations in the frontoparietal network during sustained attention

Authors: ***G. ROSS**[1,2], A. HUANG[1,2], P. SIEKIERSKI[1,2], Q. FANG[1,2], M. ZHANG[1,2], S. RADTKE-SCHULLER[1,2], F. FROHLICH[1];

[1]Dept. of Psychiatry, Univ. of North Carolina Chapel Hill, Chapel Hill, NC; [2]Carolina Ctr. for Neurostimulation, Univ. of North Carolina at Chapel Hill, Chapel Hill, NC

Abstract: Introduction: The frontoparietal network, including dorsal frontal cortex (dFC) and posterior parietal cortex (PPC), generates top-down control signals during cognitive tasks. Theta oscillations orchestrate frontoparietal connectivity during tasks requiring cognitive control, including sustained attention. Earlier studies have been primarily observational, thus the directionality and causal role of theta oscillations in this network remain unknown.
Methods: To investigate the causal role of theta oscillations in the frontoparietal network during sustained attention, we combined simultaneous multisite electrophysiology recordings with frequency-specific rhythmic optogenetic stimulation in the freely moving ferret (Mustela furo). To investigate sustained attention, we employed the 5-choice serial reaction time task (5-CSRTT), a preclinical analog of a common human sustained attention task. Low-theta (5 Hz; experimentally determined peak of network synchronization) and high-theta (8 Hz; control frequency) optogenetic stimulation was administered to the dFC during the 5-CSRTT while recording local field potential and single-unit activity from dFC and PPC.
Results and Discussion: We successfully modulated frontal cortical neural activity at 5 Hz and 8 Hz with temporal precision during the sustained attention period of the 5-CSRTT, with no observable effect on PPC oscillatory activity. Functional connectivity was assessed by calculating phase locking value (PLV) between network nodes and was found to be task modulated. PLV was strongest at 5 Hz around trial onset and substantially reduced by the end of the delay period. There was no observable change in PLV during either low- or high-theta stimulation. This could be explained by the lack of pronounced theta oscillations during the optogenetic stimulation period. Optogenetic enhancement of low- but not high-theta activity in frontal cortex was associated with an increased fraction of correct trials ($F(2,24) = 1.747$, $p = 0.04$) and reduced omitted trials ($F(2,24) = 0.887$, $p = 0.01$).
Conclusion: This finding supports previous results that theta oscillations in the frontoparietal network are modulated during a sustained attention task and demonstrates that local theta oscillation power in the dFC may drive behavioral performance in the 5-CSRTT.

Disclosures: G. Ross: None. **A. Huang:** None. **P. Siekierski:** None. **Q. Fang:** None. **M. Zhang:** None. **S. Radtke-Schuller:** None. **F. Frohlich:** Other; FF receives payments as a consultant from Electromedical Products International, Insel Spital, and the University of Michigan.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.19

Topic: B.07. Network Interactions

Support: NIH Grant K99MH126161
NIH Grant R01MH124387
Foundation of Hope Seed Research

Title: Causal role of delta-beta coupling for goal-directed behavior in anhedonia

Authors: J. RIDDLE[1], A. MCFERREN[2], M. SMOSKI[3], C. SCHILLER[1], D. RUBINOW[1], F. FROHLICH[1];
[1]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [2]Univ. of Rochester, Rochester, NY; [3]Duke Univ., Durham, NC

Abstract: Recent evidence suggests that symptoms of major depressive disorder (MDD) comprise distinct dimensions of anhedonia and anxiety/rumination. In a previous study, we investigated how symptom expression in participants with MDD was related to altered behavior in a reward-based decision-making task. Participants with elevated symptoms of anhedonia exhibited reduced willingness to exert physical effort for an uncertain reward, referred to as goal-directed behavior, and reduced oscillatory coupling from prefrontal to motor cortex (delta-beta phase-amplitude coupling). By contrast, participants with anxious rumination showed increased propensity to incorporate the monetary incentive into decision-making and increased coupling between prefrontal and visual areas (theta-gamma coupling). However, our previous study was observational and the neural circuits underlying these electrophysiological signals was unknown. In the present study (NCT05084924), participants with MDD (projected N=48) performed the same task during EEG as cross-frequency transcranial alternating current stimulation (tACS) was delivered in delta-beta, theta-gamma, or sham in a parallel-arm RCT. In addition, functional MRI was acquired during performance of the task. At baseline, we replicated the association between symptoms of anhedonia and goal-directed behavior ($r=-0.27$) independent from the anxious dimension (partial-correlation, $r=-0.44$). In an exploratory analysis, we found that this effect was driven by reduced goal-directed behavior after receiving the reward ($r=-0.54$; partial-$r=-0.63$). Furthermore, functional MRI revealed increased activation in the left anterior middle frontal gyrus, member of the executive control network, as a function of goal-directed behavior ($p<0.05$, $k>100$). By contrast, the incentive increased activation in superior frontal junction, member of the dorsal attention network ($p<0.05$, $k>100$). These findings suggest that distinct brain network may underly different symptom dimensions in MDD. Changes in task performance in response to tACS in this study will enable the identification of the causal role of these phase-amplitude coupled signals. Ultimately, targeting these symptom-specific functional networks may represent a promising approach to the personalized treatment of depression.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.20

Topic: B.07. Network Interactions

Support: NIH Grant R01MH124387
NIH Grant R01MH122477

Title: Claustrum Drives Frontoparietal Network During Sustained Attention

Authors: *P. SIEKIERSKI, A. HUANG, G. ROSS, Q. FANG, M. ZHANG, S. RADTKE-SCHULLER, F. FROHLICH;
Psychiatry, Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: The claustrum, linked to higher order cognitive functions, is an enigmatic subcortical structure that is highly anatomically interconnected with other brain regions, notably the frontoparietal network, composed of the frontal cortex (dFC) and posterior parietal cortex (PPC). This dorsal network exhibits rhythmic synchronization during sustained attention, a top-down process, yet the causal role of oscillatory activity in the claustrum and how it synchronizes cortical networks is unknown. We investigated the functional and effective connectivity of the claustrum, dFC, and PPC in ferrets during a sustained attention task, the five-choice serial reaction-time task (5-CSRTT), by multisite electrophysiological recordings and frequency-specific, excitatory optogenetic stimulation in the claustrum (5 Hz for peak functional connectivity targeting, and 8 Hz as a control). We used phase-locking value (PLV) as a measure of inter-region functional connectivity, and conditional Granger causality to assess effective connectivity. PLV of the CLA-dFC and CLA-PPC connections increased in the theta frequency band (4.5-7 Hz) during the delay period (CLA-dFC: $F(1,26) = 5.58$, $p=0.03$; CLA-PPC: $F(1,26) = 5.73$, $p=0.02$). Effective connectivity in CLA-dFC and CLA-PPC connections during the delay period was driven by the claustrum in the theta frequency band. Suppression of the alpha frequency band driven by the PPC was also observed during the delay period. Optogenetic stimulation in the claustrum locally increased the targeted frequency for both stimulation conditions (theta: $F(2,42) = 62.99$, $p < 0.001$; control: $F(2,42) = 272.95$, $p < 0.001$). Frequency-specific engagement was demonstrated in the CLA-dFC connection (theta: $F(2,42) = 15.76$, $p < 0.001$; control: $F(2,42) = 183.90$, $p < 0.001$). Peak theta stimulation increased behavioral performance by decreasing omission trials compared to stimulation at the control frequency ($F(2,34) = 3.89$, $p=0.03$). Our results show that (1) the claustrum drives functional connectivity with the frontoparietal network by theta oscillations during a sustained attention task and (2) claustral theta oscillations may causally contribute to increasing sustained attention by omission reduction.

Disclosures: P. Siekierski: None. **A. Huang:** None. **G. Ross:** None. **Q. Fang:** None. **M. Zhang:** None. **S. Radtke-Schuller:** None. **F. Frohlich:** E. Ownership Interest (stock, stock

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.21

Topic: B.07. Network Interactions

Support: NIH Grant DK056350
State of North Carolina
Grants from Cohen Foundation
Grants from the Shared Research Foundation

Title: Infection and Inflammation in Mild Cognitive Impairment: Does Infection with Bartonella spp. Play a Role?

Authors: *V. GUIRGUIS[1], F. PUPILLO[1], Y. VARDHAN[1], R. BERHANA[1], S. RODRIGUES[1], D. A. STEWART[3], S. J. SUMNER[3], C. E. LIEDIG[4], R. G. MAGGI[4], E. B. BREITSCHWERDT[4], N. WALKER[2], F. FROHLICH[1];
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Abstract: *Bartonella* spp. is an emerging zoonotic bacteria that has been implicated in a spectrum of CNS diseases. We have previously shown with digital droplet PCR that people with psychotic disorders were more likely than healthy participants to be infected with *Bartonella* spp. More broadly, *Bartonella* spp. is associated with vascular dysfunction that may cause a spectrum of neurological symptoms such as slowed memory processing, memory deficit, as well as mood and personality changes. The purpose of this study is to expand on previous research to determine if an association exists between *Bartonella* spp. infection and Mild Cognitive Impairment (MCI), a neuropsychiatric disorder associated with chronic inflammation and neurodegeneration. We hypothesized that people with MCI are more likely to have laboratory findings consistent with *Bartonella spp.* infection compared to healthy controls.
We are performing an observational study that contrasts healthy controls and people with MCI in terms of cognitive function, mood symptoms, peripheral blood markers for current and past infection with *Bartonella* spp., and peripheral inflammatory markers. The recruitment target is 50 healthy controls and 50 people with MCI. We here report preliminary results from the pilot feasibility stage of this study consisting of PCR and serology testing for 13 healthy controls, as well as scores for the clinical and cognitive assessments. The average age in years was 72 ± 5.9, with a female to male ratio of 8/5. The scoring of clinical and cognitive assessments was 1.08 ± 1.26 for PHQ-9 (depression), 1.69 ± 1.32 for GAD-7 (anxiety), 6 ± 2.55 for the Epworth

Sleepiness Scale, 59.9 ± 4.91 for the Q-LES-Q-SF (quality of life), and 27.4 ± 1.50 for the Montreal Cognitive Assessment. All 13 participants tested negative for the presence of *Bartonella* spp. by quantitative PCR (qPCR). One participant, however, tested positive with qPCR after culturing using *Bartonella* alphaproteobacterial growth medium (BAPGM), and also presented the highest antibody levels via serology testing for various strains of *Bartonella* spp. (highest titer: 1:1024 for *Bartonella vinsonii subsp. berkhoffii*). Of the 13 healthy controls, 4 presented evidence of past *Bartonella* infection due to high IGG antibody titer (≥ 1:128) for more than one *Bartonella* strain. These preliminary results support a low but non-zero prevalence of potentially active *Bartonella* spp. infection in healthy controls and a measurable fraction of people with a history of exposure. This indicates that further data collection is justified and required to investigate the potential link between *Bartonella* spp. infection and mild cognitive impairment.

Disclosures: V. Guirguis: None. **F. Pupillo:** None. **Y. Vardhan:** None. **R. Berhana:** None. **S. Rodrigues:** None. **D.A. Stewart:** None. **S.J. Sumner:** None. **C.E. Liedig:** None. **R.G. Maggi:** F. Consulting Fees (e.g., advisory boards); RGM has led research efforts to optimize the BAPGM platform, droplet digital PCR, and is the scientific-technical advisor for Galaxy Diagnostics. **E.B. Breitschwerdt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); EBB, in conjunction with Sushama Sontakke and North Carolina State University, holds US Patent No. 7,115,385, Media and Methods for Cultivation of Microorganisms, which was issued October 3, 2006., EBB is a founder, shareholder and the chief scientific officer for Galaxy Diagnostics, a company that provides diagnostic testing for detection of Bartonella spp. and other vector-borne pathogens. **N. Walker:** None. **F. Frohlich:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); FF is the lead inventor of IP filed on the topics of noninvasive brain stimulation by UNC. F. Consulting Fees (e.g., advisory boards); FF is a paid consultant for Electromedical Products International. Other; FF has received honoraria from the following entities in the last twelve months: Academic Press, Insel Spital, University of Michigan.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.22

Title: WITHDRAWN

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.23

Topic: B.07. Network Interactions

Support: NIH grant DK056350
The State of North Carolina
Cohen Foundation
Bay Area Lyme Foundation

Title: Network and immunological dysfunction in tic disorders?

Authors: *H. LAGARDE[1,3], J. RIDDLE[1,3], P. SIEKIERSKI[1,3], M. ZHANG[1,3], D. A. STEWART[4], S. J. SUMNER[4], C. E. LIEDIG[5], R. G. MAGGI[5], E. B. BREITSCHWERDT[5], S. TRAU[2], F. FROHLICH[1];
[1]Psychiatry, [2]Neurol., Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Carolina Ctr. for Neurostimulation, Chapel Hill, NC; [4]Nutr., Nutr. Res. Inst. UNC - Chapel Hill, Chapel Hill, NC; [5]Col. of Vet. Med., Intracellular Pathogens Res. Laboratory, Ctr. for Comparative Med. - North Carolina State Univ., Raleigh, NC

Abstract: The etiology of Chronic Tic Disorders and Tourette's Syndrome (CTD/TS) is thought to be multifactorial and likely involves dysfunction of multiple cortical and subcortical pathways, and immunologic dysregulation. The objective of this research is to elucidate the network level cerebral circuity and immunologic markers associated with CTD/TS. We aim to establish potential biomarkers both at the macroscopic scale of electric network activity patterns and at the microscopic scale of infectious and inflammatory markers that have been suggested to be involved in the pathogenesis of tic disorders. CTD/TS patients and healthy controls (ages 10-17) undergo psychological assessments and EEG recording of resting state and voluntary movement (blink, throat clearing, and saying a word) in an event-based task format. Additionally, CTD/TS patients undergo recording during tic suppression. We use high-density EEG to map pathological network signatures onto anatomical brain structures through source localization to provide further insights about the structural-functional substrate of these disorders. Phase-amplitude coupling will be computed with primary focus on coupling of frontal delta and central beta oscillations given their hypothesized role in cortico-striatal synchronization and inhibitory control. With the advent of increasingly sensitive molecular and microbiological diagnostic assays, Bartonella spp. infections have now been implicated in a spectrum of central nervous system (CNS) diseases. Individual case reports have suggested that infection with vector-borne illnesses such as bartonellosis can cause striatal dysfunction and associated tic symptoms. It remains unknown if infection is associated with CTD/TS. We examine markers of inflammation and infection using an expanded 80-cytokine panel together with a validated, novel assay to detect emerging infectious agents, notably Bartonella spp. We hypothesize that CTD/TS corresponds to pathological dysregulation of top-down cortico-striatal control mediated by delta-beta phase-amplitude coupling and that CTD/TS is associated with elevated activation of the inflammatory processes and increased probability of active infections with CNS-targeting pathogens. By connecting network-level signatures with potential molecular inflammatory markers we lay the foundation for future multi-scale investigations. Our long-term goal is that the biomarkers discovered through this research guide the development of novel, individualized treatments, such as non-invasive brain stimulation and novel medication therapy, for patients with CTD/TS.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.24

Topic: B.07. Network Interactions

Support: NIMH Grant MH105574
the North Carolina Translational and Clinical Sciences (NC TraCS) Institute supported by NCATS UL1TR002489

Title: Frontotemporal alpha-frequency transcranial alternating current stimulation (tACS) reduces depressive symptoms in people with schizophrenia and auditory hallucinations in a double-blind placebo controlled clinical trial

Authors: *M. ZHANG[1,2], R. B. FORCE[1,2], C. WALKER[1], S. AHN[1,3], L. F. JARSKOG[1], F. FROHLICH[1,2];
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Abstract: Rhythmic brain stimulation is a new form of treatment for psychiatric disorders by specifically targeting relevant brain oscillations. Reduced alpha oscillations and frontotemporal functional connectivity have been observed in people with schizophrenia, which may reflect insufficient top-down inhibition of spurious activity in the auditory cortex, leading to auditory hallucinations. Here we examine the efficacy of 10-Hz tACS in enhancing alpha oscillations and

treating auditory hallucinations in people with schizophrenia in a double-blind, randomized, placebo-controlled clinical trial. The trial consisted of a treatment week and a maintenance period. For the treatment week, 25 participants were randomly assigned to receive daily 40-min tACS or sham for five consecutive days. After the treatment week, participants were re-randomized to receive weekly maintenance tACS or sham for two months. For tACS, 10 Hz AC was administered in-phase at dorsolateral prefrontal cortex and temporoparietal junction, with the return current at Cz. For sham, the same electrode placement was used, and a brief stimulation was used to create the skin sensation of tACS onset. Blinding was successful. We found that daily tACS treatment significantly increased alpha power in the target region at 1- and 2-month follow up (23.1 ± 8.2 %, 34.1 ± 14.5 %) compared to the sham group (−3.0 ± 10.4 %, −7.3 ± 7.2 %; between-group, $p<0.05$). Daily tACS also increased the peak frequency of global functional connectivity towards 10 Hz (0.43 ± 0.22 Hz for tACS, -0.36 ± 0.37 Hz for sham; between-group, $p<0.05$). Both observations are consistent with findings in our previous clinical trial, demonstrating successful and reproducible target engagement. We did not observe a significant change in auditory hallucinations in either tACS or sham group. However, tACS group showed a significant decrease in general psychopathology symptoms from baseline to Day 5 of the treatment week (−3.29 ± 4.76, $p=0.011$) compared to the sham group (−0.45 ± 3.21, $p=0.324$; between-group, $p<0.05$). Among general psychopathology symptoms, depression showed the greatest decrease in the tACS group (-0.79 ± 0.97) compared to the sham group (0.00 ± 1.00; between-group $p<0.05$). Importantly, the decrease in symptom scores significantly correlated with the increase in the peak frequency of global functional connectivity (rho=-0.44, $p=0.026$ for general psychopathology, rho=-0.45, $p=0.026$ for depression). In light of recent work demonstrating the effectiveness of frontal alpha-tACS in treating major depressive disorder, this work provides initial evidence for using alpha-tACS as a transdiagnostic treatment for depression.

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Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.25

Topic: B.07. Network Interactions

Support: Medical Research Council grant MC_UU_00003/1
Medical Research Council grant MC_UU_00003/3

Title: How to entrain a selected neuronal rhythm but not others: open-loop dithered brain stimulation for selective entrainment

Authors: *B. DUCHET, J. J. SERMON, G. WEERASINGHE, T. DENISON, R. BOGACZ; Univ. of Oxford, Oxford, United Kingdom

Abstract: While brain stimulation therapies such as deep brain stimulation for Parkinson's disease can be effective, they have yet to reach their full potential across neurological disorders. Entraining neuronal rhythms using rhythmic brain stimulation has been suggested as a new therapeutic mechanism to improve patients' symptoms in conditions such as chronic pain, depression, and Alzheimer's disease. However, theoretical and experimental evidence indicate that brain stimulation can also entrain neuronal rhythms at sub- and super-harmonics, far from the stimulation frequency. Crucially, inadvertently entraining these rhythms can be harmful to patients. For example, in patients with Parkinson's disease, entraining narrow-band gamma oscillations (60-80Hz) at the first sub-harmonic of deep brain stimulation may trigger debilitating involuntary movements. We therefore propose a principled approach to selectively promote rhythms close to the stimulation frequency, while avoiding potential harmful effects by preventing entrainment at sub- and super-harmonics. Our open-loop approach to selective entrainment, dithered stimulation, consists in adding white noise to the stimulation period. We establish a mathematical basis for the ability of dithered stimulation to selectively entrain a given brain rhythm, and confirm its effectiveness in computational models of coupled neural oscillators. Additionally, we test our selective entrainment approach in healthy human subjects using electroencephalographic recordings and flashing visual stimulation at alpha frequency. Compared to periodic stimulation, we show that our approach can entrain the alpha rhythm while drastically reducing entrainment at stimulation harmonics. Finally, we demonstrate that dithered stimulation could be implemented in neurostimulators with limited capabilities by toggling within a finite set of stimulation frequencies. Likely implementable across a variety of existing brain stimulation devices, dithering-based selective entrainment has potential to enable new brain stimulation therapies, as well as new neuroscientific research exploiting its ability to modulate higher-order entrainment.

Disclosures: B. Duchet: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ongoing IP application. **J.J. Sermon:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ongoing IP application. **G. Weerasinghe:** None. **T. Denison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ongoing IP application, Stock ownership (<1%) in Bioinduction Ltd.. F. Consulting Fees (e.g., advisory boards); Advisor for Synchron, Advisor for Cortec Neuro, Advisor for Bioinduction. **R. Bogacz:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Ongoing IP application.

Poster

037. Oscillations and Synchrony: EEG Studies

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 037.26

Topic: B.07. Network Interactions

Support: MRC Confidence in Concept (Ref No 0005001)
Onassis Foundation (Ref No FZK 085-2)

Title: Physiological or Supraphysiological gamma titration? Targeting ascending arousal networks for modulation of sleep and wakefulness

Authors: *A. DELI[1], B. DUCHET[2], S. HE[2], Y. HUANG[1], T. AZIZ[1], H. TAN[2], V. VYAZOVSKIY[3], T. DENISON[4], A. L. GREEN[1];
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Abstract: Neuromodulation using gamma frequency stimulation is proposed as a means of entraining cortical activity linked with enhanced cognition and alertness. Surgical neurostimulation of subcortical arousal networks has been used in pilot trials for disorders of consciousness. However, parameter selection in human clinical populations remains empirically driven in the majority of cases. The pedunculopontine (PPN) nucleus, part of ascending brainstem arousal circuits, is crucial in sleep wake regulation, autonomic and motor functions. Here we compare two gamma stimulation protocols based on intrinsic nucleus activity parameters, deployed during slow-wave sleep in a cohort of four patients with deep brain stimulators for treatment of multiple systems atrophy. We characterised baseline circuit dynamics using simultaneous electroencephalogram (EEG) and brainstem local field potentials (LFP). The two protocols (including sham trials) were administered in selected lead contacts while recording EEG and contralateral LFPs during slow-wave sleep. Effects on brainstem cortical circuit dynamics were explored (imaginary coherence and Granger causality). The two protocols were compared in terms of induced cortical power spectral changes as well as phase-locking value (PLV) during stimulation periods. Statistical analyses included a nonparametric cluster-based permutation procedure and analysis of variance, controlling for multiple comparisons. Physiological (40 Hz) brainstem gamma stimulation more effectively increased cortical alpha and beta oscillations in the post-stimulation period compared to a higher (100 Hz) frequency protocol (both $p < 0.0001$, CI: 0.058 0.075 and 0.007 0.015 respectively). These were accompanied by changes in circuit dynamics that mirrored differences between sleep and wake. High frequency stimulation however was superior in slow wave activity reduction ($p<0.0001$ CI 0.915 1.774) and increases in cortical gamma content ($p =0.001$ CI: -0.014 -0.002). Differences in PLV between the two stimulation protocols were explored as a potential underlying mechanism, coupled with both brainstem and cortical oscillators. Overall, gamma brainstem stimulation effectively changes circuit dynamics and induces lighter sleep or wakefulness. However frequency titrations (and design based on physiological range of nucleus activity) can affect sleep depth in different ways. These highlight the importance of PPN targeting where wakefulness is reduced, furthering our understanding of intrinsic dynamics and facilitating evidence-based parameter selection for human closed-loop protocols, in disorders of sleep and wakefulness.

Disclosures: **A. Deli:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder. **B. Duchet:** None. **S. He:** None. **Y. Huang:** None. **T. Aziz:** None. **H. Tan:** None. **V. Vyazovskiy:** None. **T. Denison:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder. **A.L. Green:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); receipt of intellectual property rights/patent holder.

Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.01

Topic: B.08. Epilepsy

Support: Conacyt-Mexico (scholarship No. 003236 to CVMP).

Title: Type 2 diabetes increases brain injury induced by lithium-pilocarpine status epilepticus in male rats.

Authors: *C. V. MERIDA PORTILLA, K. RAMOS RIERA, F. CHENA BECERRA, L. BELTRAN PARRAZAL, C. MORGADO VALLE, M. LÓPEZ MERAZ;
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Abstract: Type 2 diabetes mellitus (DM2) characterizes by the presence of hyperglycemia associated with insulin resistance and dysfunction in its production. Clinical and experimental evidence links DM2 with the development of epileptic seizures, including a higher incidence of status epilepticus (SE), a neurological emergency with a high mortality rate and characterized by continuous epileptic activity for along 30 minutes. However, this relationship has not been fully characterized, and it has not described if there is brain damage in other areas besides the hippocampus. Therefore, this study aimed to analyze the brain injury after SE in male Wistar rats with DM2. Three-days-old Wistar rat pups were given a subcutaneous injection of streptozotocin (STZ; 100mg/kg; n=8) to induce DM2 (STZ group); control rats (CTRL group) received an equal volume of citrate buffer (pH 4.5; n=8). SE was induced with the lithium-pilocarpine model (3mEq/kg, LiCl; pilocarpine hydrochloride, 30mg/kg) at postnatal day 90. One day later, rats were perfused transcardially under anesthesia, their brains were embedded in paraffin, cut into 10 um thinness sections, and stained with hematoxylin-eosin and fluoro-Jade B, whereas glia and microglia were detected by immunohistochemistry. The results showed that the STZ group had fewer neurons and glia, a higher percentage of tissue disintegration, and an increased number of cells in neurodegeneration and microglial cells in the hippocampus (CA1, CA2, CA3 fields), the dorsomedial nucleus of the thalamus, the basolateral nucleus of the amygdala, and the piriform cortex in comparison with the CTRL group. All data together suggest that the presence of DM2

increases neuronal brain injury due to SE in limbic structures. Supported by Conacyt-Mexico (scholarship No. 003236 to CVMP).

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.02

Topic: B.08. Epilepsy

Support: 1F31NS124290-01 (L.D.)
R01NS097750 (V.S.)
R01NS069861 (V.S.)

Title: Seizure induced changes in Synaptic Inputs to Hippocampal Dentate Semilunar Granule Cells

Authors: *L. DOVEK[1,2], A. HUANG[2,3], A.-T. NGUYEN[2], M. ASSAF[2], D. SUBRAMANIAN[5], V. SANTHAKUMAR[4];
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Abstract: The hippocampal dentate gyrus (DG) plays a major role in formation of episodic memories by processing complex cortical inputs into sparse granule cell (GC) activity mediated by layer-specific inhibition of GCs by functionally distinct GABAergic interneurons. Temporal lobe epilepsy leads to extensive reorganization of DG inhibitory circuits and results in memory impairments by mechanisms that are not fully understood. Semilunar granule cells (SGCs), a subtype of DG projection neurons with expansive molecular layer dendritic arbors and persistent firing, have been proposed to shape memory processing by supporting feedback inhibition (Larimer et al., 2010). SGCs differ from GCs in their intrinsic physiology and synaptic inhibition and are preferentially recruited in memory representations. However, whether SGC intrinsic physiology and synaptic inputs are altered in epilepsy is not known. Here we examined synaptic inputs to SGCs and how they are altered in experimental epilepsy. Adult male and female (4-7 weeks) C57BL/6 mice were treated with pilocarpine to induce status epilepticus (SE). Development of spontaneous recurrent seizures was confirmed in video-EEG recordings in a cohort of mice 1-month post-SE. In studies using a Barnes Maze task, we identified significant impairments in spatial memory function in mice 1-week after SE. Whole-cell recordings were obtained from GCs and SGCs in hippocampal slices from mice 1-month after pilocarpine-SE and saline-injected or naïve controls. Cell types were confirmed based on post-hoc recovery of

biocytin fills. In naïve mice, dendritic reconstruction revealed a higher density of dendritic spines in SGCs than GCs. Consistently, the frequency of spontaneous excitatory postsynaptic currents (sEPSCs) in SGCs was higher than in GCs. However, EPSCs evoked by hilar and perforant path stimulation were not different between cell types. 1- month after SE, sEPSC frequency and amplitude was increased in GCs and. SGCs, unlike GCs, received significantly larger sIPSCs after SE. SGC intrinsic parameters including resting membrane potential and input resistance were not altered after SE. These findings suggest that synaptic inputs to SGCs are altered after SE which could impact their contribution to memory processing.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.03

Topic: B.08. Epilepsy

Support: NIH NS050229 to NPP
AES Seed grant to NPP

Title: Progressive dysregulation of tau phosphorylation in an animal model of temporal lobe epilepsy

Authors: N. A. EKSTROM, F. A. CONCEPCION, M. N. KHAN, O. O. ESTES, ***N. P. POOLOS**;
Univ. of Washington, Seattle, WA

Abstract: Tau is an intracellular protein known to undergo an increase in phosphorylation (hyperphosphorylation) and subsequent neuro-toxic aggregation in Alzheimer's disease. Genetic deletion of tau in animal models of epilepsy reduces seizure frequency, suggesting that tau expression exerts a pro-epileptic action. In post-mortem studies of brain from patients with refractory temporal lobe epilepsy using immunohistochemical methods, it has been unclear whether tau expression and phosphorylation are altered in the hippocampus where seizures originate. We investigated tau expression and phosphorylation at the S202/T205 human locus, known to be hyperphosphorylated in Alzheimer's disease, in the rat pilocarpine post-status epilepticus (SE) model of temporal lobe epilepsy. We used western blotting to measure tau expression at two time points of chronic epilepsy: 6-9 weeks post-SE and 14-17 weeks post-SE. When co-staining with the anti-3R tau and anti-4R tau antibodies in lysates from the entire hippocampal formation, four out of six tau isoforms (0N3R, 0N4R, 1N4R, and 2N4R) were readily observed, while the other two isoforms, 1N3R and 2N3R, were present only at low levels. In the entire hippocampus at 6-9 weeks post-SE, we observed modestly reduced total tau levels (20.6 ± 3.10% lower than naïve controls; p=<0.001) but no significant reduction of

S202/T205 phosphorylation levels. However, in whole hippocampi from 14-17 week post-SE rats, total tau expression was unchanged, but there was a significant reduction in S202/T205 phosphorylation levels (45.0 ± 7.57% decrease; $p<0.001$). We further investigated tau within two hippocampal subregions. Both CA3 and CA1 showed loss of S202/T205 phosphorylation in chronic epilepsy, with CA3 showing larger changes than CA1. Unlike in CA1, area CA3 showed a modest loss of total tau expression in both periods of epilepsy. A region in somatosensory cortex outside of the seizure onset zone in hippocampus showed no changes in tau expression or phosphorylation. Immunohistochemical analysis showed S202/T205 tau phosphorylation was most strongly observed in the cell body layers of areas CA1 and CA3, and additionally in the mossy fiber projections to CA3, with less expression in the dendritic regions of pyramidal neurons. We conclude that tau phosphorylation at a canonical site involved in Alzheimer's is reduced in the hippocampus in an animal model of temporal lobe epilepsy rather than increased as is seen in Alzheimer's. Further study is needed to understand how this change in tau expression impacts neuronal excitability and the development of epilepsy after a neural insult.

Disclosures: **N.A. Ekstrom:** None. **F.A. Concepcion:** None. **M.N. Khan:** None. **O.O. Estes:** None. **N.P. Poolos:** None.

Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.04

Topic: B.08. Epilepsy

Title: Expression of synaptic vesicle glycoprotein 2A in patients with temporal lobe epilepsy and during experimental epileptogenesis in three rat models

Authors: *J. D. MIKKELSEN[1], B. A. PAZARLAR[4], S. S. ARIPAKA[2], C. MADSEN[2], K. KHODOSEVICH[3], L. PINBORG[2], J. BASTLUND[5], B. BANG-ANDERSEN[5], P. BASCUÑANA[6], M. BANKSTAHL[6], J. P. BANKSTAHL[7];
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Abstract: Synaptic vesicle glycoprotein-2A (SV2A) is an anti-seizure drug target and likely a biomarker of synaptic density. In this study, we analyzed the expression of SV2A in the temporal neocortex by using single nucleus-RNA sequencing and quantitative PCR in temporal lobe epilepsy (TLE) patients and healthy controls. Further, autoradiography using the SV2A selective radiotracer [^{3}H]-UCB-J was performed to analyze the correlation between the SV2A mRNA transcript and SV2A binding capacity . Our data from RNAseq revealed that the SV2A transcript is expressed in all glutamatergic and GABAergic cortical neuronal subpopulations indicating that virtually all cortical neurons express SV2A. The level of [^{3}H]-UCB-J binding and the

concentration of SV2A mRNA is strongly correlated in each patient, and the expression is lower in the TLE patients compared to control. We also examined the spatial and temporal regulation of [^{3}H]-UCB-J binding in three rat models of TLE: a) systemic kainic acid, b) intrahippocampal kainic acid, and c) systemic pilocarpine. Brain tissues were sampled at different time points after the initial status epilepticus. In all three models, a decrease in [^{3}H]UCB-J binding was observed in the temporal cortex as well as several other brain areas in the acute phases. Reductions in binding was most prominent in the neocortex and the hippocampus and occurred within 3-15 days. The time course was slightly different between models. Interestingly, in the two systemic models, a full restoration in the binding level 30 days after the treatment was observed in all areas probably reflecting neuronal reorganization under epileptogenesis. However, after the local injection of kainic acid, the binding in the hippocampus, and in temporal and piriform cortices did not return to basal levels. The time-course profile also displayed lateralization in the focal model. These results obtained in brains from both TLE patients and from experimental models of epileptogenesis demonstrate changes in the amount of presynaptic SV2A binding both during epileptogenesis and chronic epilepsy. This suggests that SV2A has importance in eliciting spontaneous seizures and might also be a promising biomarker for epileptogenesis.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.05

Topic: B.08. Epilepsy

Support: 1R37NS115439

Title: Tracking the evolution of synaptic dysplasticity after early life seizures

Authors: *B. XING[1], B. BABROWICZ[2], X. LI[3], D. M. TALOS[1], F. E. JENSEN[2];
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Abstract: Early life seizures (ELS) can result in long-term cognitive deficits as well as spontaneous seizures. We have previously shown that ELS increases neuronal excitability and increases calcium-permeable (CP) AMPARs (PMID: 27521497; 30952813). In addition, ELS can block critical period cortical plasticity, and prevent synaptic long-term potentiation (LTP) in hippocampal CA1 (PMID: 22171027; 18685023). The increased neuronal excitability, altered synaptic plasticity, cognitive deficits, and long-term seizure can be attenuated by early post-treatment with AMPAR and CP-AMPAR antagonists (PMID: 24117347; 29738885). Here we aimed to use a FosTRAP mouse model to identify specific neuronal populations that are recruited during ELS, and explore the specificity of long-term modifications in AMPA glutamate

receptor-mediated synaptic function. Using activity-dependent genetic labeling (FosTRAP**)** transgenic mouse model, we induced ELS (kainate i.p) at postnatal day (P)10 followed by 4-OHT to activate Cre-dependent tdTomato (tdTom). We compared glutamate receptor-mediated synaptic function and plasticity in tdTom+ principal CA1 neurons in hippocampal slices removed after ELS at P30, within slice tdTom- neurons and also CA1 neurons from non-seizure controls. At P30, tdTom positivity was primarily found in neurons (93.5 ± 8.9%), versus astrocytes (6.9% ± 2.1%). At P15, whole-cell patch clamp recording of tdTom+ CA1 neurons (td+) revealed increases in postsynaptic AMPAR function (spontaneous EPSC amplitude at P15 36.57 ± 3.32vs. saline 25.15 ± 1.69 and Td- neurons 29.06 ± 2.00, $p < 0.05$) and reduced fraction of NMDAR-only silent synapses (25.5% compared with same slice Td- neurons 63.8% or slices from saline controls 73.8% , $p < 0.05$). AMPAR amplitude was persistently increased at P30 in the tdTom+ neurons, with P30 38.58 ± 3.87, $p < 0.05$ vs. saline 24.61 ± 2.01 and Td- neurons 24.62 ± 2.97), and these cells uniquely revealed increased inward rectification consistent with synaptic insertion CP-AMPARs (rectification index at P30 td+: 3.58 vs td-: 1.84 and saline: 1.64, $p < 0.05$, one-way ANOVA). Moreover, tdTom+ neurons show significant LTP deficits using a pairing protocol which induce robust LTP in saline-control and tdTom- neurons, indicating a long-term synaptic plasticity impairment after ELS (td+: 72.22 ± 6.35% vs td-: 125.7 ± 18.9% and saline: 127.4 ± 15.6%, $p < 0.05$). In conclusion, this study reveals that ELS causes early and long-lasting changes in only a subset of neurons, and the TRAP technique should have future utility in tracking subcellular processes that might represent new therapeutic targets to prevent epileptogenesis and network dysfunction due to ELS.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.06

Topic: B.08. Epilepsy

Support: NIH

Title: Understanding the Role of Lipid Metabolism during Seizure in the Adult Dentate Gyrus

Authors: *A. AHAMAD[1,2], S. GE[1];
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[2]Clin. Lab. Sci., Sch. of Hlth. Professions, Stony Brook Univ., Stony Brook, NY

Abstract: The dentate gyrus (DG), a neurogenic niche, is a metabolically dense subregion of the hippocampus. Continuous production and integration of new neurons in the existing circuit accompanied by neuron-glia coupling is essential to maintain hippocampal homeostasis throughout adulthood. Cell metabolism regulates the homeostatic balance of the hippocampal

network and its associated functions, such as memory and cognition. An imbalanced circuit activity results in mesial temporal lobe epilepsy (MTLE), impairing the overall network function. Although altered lipid metabolism has been reported in status epilepticus, the role of lipid droplets (LDs), the minuscule metabolically active organelle known to provide a substrate for cellular energy has not been explored in DG during seizure. LDs are composed of neutral lipids and surrounded by phospholipid monolayer studded with a structural Perilipin family of proteins 1-5 (PLIN 1-5), reported to be involved in lipid metabolism. To understand the role of lipid metabolism in DG during seizure, we used the pilocarpine-induced seizure model. To study LDs in the brain, we used a novel approach by injecting Bodipy, a lipid dye in the tail vein of mice, and successfully labeled the LDs with spatial and temporal resolution in the DG. We sacrificed mice at three timepoints, 0.5, 1-, and 3 hours post-seizure induction. We found a significant increase in Bodipy signal and Perilipin 4, LDs specific marker expression at three timepoints post-seizure than in the control cohort. To elucidate the role of neuron-glia metabolic coupling in DG, we measured LDs in glia and found a significant increase in LDs in seizure mice than in the control group suggesting seizure dysregulates lipid droplets metabolism in neurons and glia in DG. Overall, this novel study will highlight the undiscovered role of LDs in the DG during seizure and, in the future, can be used as a therapeutic target to alleviate the MTLE phenotype.

Disclosures: **A. Ahamad:** None. **S. Ge:** None.

Poster

038. Epilepsy: Post-Seizure Modifications

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Topic: B.08. Epilepsy

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Georgia Research Alliance
Biolocity
NIH grant R01NS112350

Title: Neuroinflammation and cognitive deficit following status epilepticus - role of the EP2 receptor for prostaglandin E2

Authors: N. H. VARVEL[1], R. AMARADHI[1], C. ESPINOSA-GARCIA[1], S. DUDDY[2], R. FRANKLIN[3], A. BANIK[1], C. ALEMAN-RUIZ[1], W. WANG[1], L. BLACKMER-RAYNOLDS[1], T. HONORE[4], T. GANESH[1], **R. J. DINGLEDINE**[1];
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Abstract: Among neurological diseases, epilepsy accounts for the highest disability-adjusted life years lost. Epilepsy is frequently associated with cognitive and psychiatric comorbidities. In

many pharmacoresistant patients these issues can be more debilitating than the seizures themselves. Because cognitive comorbidities can substantially reduce the quality of life in people with epilepsy, their mitigation represents a major goal in the epilepsy community. Inflammation is a component of all chronic diseases including epilepsy, and is the consequence of dysregulation of several broad signaling cascades including cyclooxygenase-2 (COX-2). We have shown that activation of the EP2 receptor for PGE2 appears responsible for blood-brain barrier leakage and much of the inflammatory reaction, neuronal injury and cognitive deficit that follows seizure-provoked COX-2 induction in brain. The mechanistic basis of cognitive comorbidities is suggested to involve sclerosis-associated neuronal death and/or neuroinflammation. Here we show that brief exposure of mice to TG11-77, a potent, selective, orally available and brain permeant EP2 antagonist, reduces delayed mortality and eliminates the profound cognitive deficit in Y-maze performance tested weeks after status epilepticus (SE). Improved performance of TG11-77 treated mice in the Y-maze after SE was not due to a difference in motor ability. TG11-77 reduced SE-induced microgliosis in the amygdala, CA1 hippocampus, and neocortex four days after SE, with a minimum effective dose of 8.8 mg/kg. Interestingly however, astrocytosis and neuronal injury were not significantly alleviated by this EP2 antagonist. Plasma and brain pharmacokinetics guided these efficacy studies. Plasma exposure in the rat was dose-linear between 15 and 1000 mg/kg dosing. The absence of neuroprotection by TG11-77 redirects attention towards its alleviation of neuroinflammation as a possible mode of action to improve cognition and lessen delayed mortality. We are developing TG11-77 as adjunctive therapy to attenuate SE-driven cognitive deficits, with its administration starting several hours after SE onset. All in vitro studies that can be included in an investigational new drug (IND) application have been completed, and non-GLP dose range-finding toxicology in the rat reveals no overt, organ or histopathology signs of toxicity after 7 days' oral administration of 1000 mg/kg/day. TG11-77 thus appears poised to continue development towards the initial clinical test of the hypothesis that EP2 receptor inhibition after status epilepticus can provide the first preventive treatment for one of the chief comorbidities of epilepsy.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.08

Topic: B.08. Epilepsy

Support: The São Paulo Research Foundation, FAPESP: 2018/18014-1

Title: How does homeostatic plasticity modulate excitability after electrically-induced seizures?

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Abstract: Homeostatic plasticity is essential to maintain neuronal activity within physiological ranges. It acts as a negative feedback mechanisms counterbalancing situations of hypo- or hyperactivity. Among the homeostatic mechanisms, synaptic scaling is quite evident. It modulates the synaptic strength and is mediated by modifications on dendritic spines density and morphology, as well as subunit composition and trafficking of AMPA receptors in the postsynaptic membrane. Here, we hypothesized that hyperactivity induced by seizures recruit homeostatic plasticity mechanisms to stabilize brain activity. Partial seizures were induced in free-movement animals through an electrical stimulation (50Hz, 10sec) delivered in the perforant pathway. Local field potentials (LFPs) and field evoked potentials (fPSPs) were recorded in the hippocampal dentate gyrus. We observed that a single electrically-induced seizure that causes hyperexcitability induces counterbalancing reduction in the fPSP that lasts for at least one hour. The electrophysiological reduction on synaptic strength was supported by a decrease in the postsynaptic density of pGluA1 and GluA1 subunits of AMPA receptors one hour after seizure induction. It was also concomitant to morphological changes in dendritic spines. Next, we assessed how synaptic strength was altered when recurrent seizures (3x, 24h interval) were delivered. We observed that all stimulations cause an instantaneous decrease in the fPSP. Interestingly, fPSPs returned to baseline levels following different temporal patterns. After the third induced seizure, we observed a faster return and subsequent increase (reverse point) in the fPSP levels when compared to baseline. During the 3 hours recording after first seizure, fPSP did not reach the reverse point. It occurred around 3h after the second seizure and about 1.5h subsequent to the third one. The anticipation in the reverse point suggests that homeostatic plasticity is losing effectivity when seizures are induced in a recurrent way. We speculate that recurrence of electrically-induced seizures could lead to a point where homeostatic plasticity would not be able to keep neuronal excitability within an adequate range and then the seizures would be more likely to occur, featuring an epilepsy-like condition.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.09

Topic: B.08. Epilepsy

Support: MEXT Grant-in-Aid for Scientific Research (21H02585)

Title: Hibernation-like state presents a new approach to understanding and treating temporal lobe epilepsy

Authors: *T. M. MOKHOTHU[1], A. HIRANO[2,3], T. SAKURAI[2,3], K. Z. TANAKA[1];
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Abstract: Temporal Lobe Epilepsy (TLE) is a debilitating neurological disease with no cure. Treatments reportedly work in only two-thirds of the patients. Although much research is being done to develop more treatment lines, there is also a growing research interest focused on preventative measures to stop the disease before it becomes fully-fledged chronic epilepsy. To have effective targeted prevention strategies, we should first understand the changes in each stage of the disease progression and pinpoint the therapeutic windows. One such stage of the disease with immense potential for targeted therapy is the latent stage, a silent/ seizure-free period between the initial insult and the first spontaneous seizure. The consensus is that this period comprises neuronal plasticity changes, aberrant gene expression, and changes in the resting-state functional connectivity between regions and hemispheres. The current study probes the question of whether the presence of a latent period is a determiner of chronic epilepsy or not. We, therefore, ask, if these processes could be stopped, could chronic epilepsy be prevented? We pause the latent stage processes in the brain by inducing an artificial hibernation-like state in mice following the pilocarpine induction of epilepsy. Using a transgenic mouse line, Qrfp-*iCre, which* expresses iCre in Qrfp-expressing neurons in the hypothalamus, we can excite these neurons in a DREADD mediated paradigm, thus initiating a rapid reduction of overall metabolism, which effectively suppresses neuronal activity. Thereafter, we compare the seizure outcome through statistical analyses of spontaneous seizure onset, frequency, and severity in hibernated mice versus typical pilocarpine-induced mice with no hibernation. Our results show firstly that mice can stay hibernated for over 48 hours. A comparison of hibernated vs non-hibernated animals shows an increase in the duration of the latent period in those hibernated than in those who were not. Further analysis of the results in this experiment will allow us to evaluate using other parameters whether a state of hibernation that halts the metabolic activities, which we believe governs the development of acute seizures into chronic seizures, is enough to impact the severity and duration of spontaneous recurrent seizures. This work will further increase our understanding of the latent period and forge new research paths to understand the mechanisms underlying epileptogenesis further and expand on the intervention timelines before chronicity.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.10

Topic: B.08. Epilepsy

Support: Supported by the fund from the Department of Anesthesia, Critical Care and Pain Medicine at Massachusetts General Hospital to HJF

Title: Vanillin Suppresses Seizure-Induced Respiratory Arrest in the DBA/1 Mouse Model of SUDEP

Authors: E. K. FARRELL[1], ***H.-J. FENG**[2];
[1]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Boston, MA;
[2]Massachusetts Gen. Hosp. and Harvard Med. Sch., Boston, MA

Abstract: Background: Sudden unexpected death in epilepsy (SUDEP) is a lethal complication of epilepsy, rendering a significant burden on public health. Seizure-induced respiratory arrest (S-IRA) has been demonstrated to be the primary event leading to death after generalized seizures. Vanillin, a compound extracted from the herb, vanilla, was reported to stimulate noradrenergic signaling, and enhancing noradrenergic neurotransmission reduces S-IRA in DBA/1 mice. Therefore, we hypothesized that vanillin prevents S-IRA in DBA/1 mice. **Methods**: DBA/1 mice of both sexes were primed by applying acoustic stimulation once daily for 3-4 days starting at postnatal day 26-28. Primed DBA/1 mice exhibit consistent susceptibility to S-IRA following audiogenic seizures characterized by wild running and generalized tonic-clonic seizures. S-IRA was evoked by acoustic stimulation using an electrical bell (96 dB SPL) in primed DBA/1 mice. S-IRA was always confirmed 24 hr prior to experiment. Vanillin or vehicle (10% DMSO in saline) was administered intraperitoneally 30 min prior to acoustic stimulation, and the effects of vanillin and the vehicle on audiogenic seizures and S-IRA was examined and digitally recorded for offline analysis. Statistical comparison of S-IRA between the vanillin treatment group and the control group was performed using the Chi-square test. **Results**: The incidence of S-IRA evoked by acoustic stimulation was significantly reduced by vanillin at 300 mg/kg (50%, $n = 10$; $p < 0.01$) and 400 mg/kg (12.5%, $n = 8$; $p < 0.001$) as compared with vehicle control (100%, $n = 12$) in primed DBA/1 mice. Compared with vehicle control, vanillin at 100 mg/kg (100%, $n = 8$) and 200 mg/kg (77.8%, $n = 9$; $p = 0.086$) did not significantly alter S-IRA. Vanillin selectively suppressed S-IRA without interfering with audiogenic seizures in 11.1% of mice tested at 200 mg/kg, 30% at 300 mg/kg and 25% at 400 mg/kg. Vanillin blocked generalized tonic seizures in 11.1% of mice tested at 200 mg/kg, 20% at 300 mg/kg and 62.5% at 400 mg/kg. **Conclusions**: Our study demonstrate that vanillin can specifically prevent S-IRA and produce anticonvulsant effect in DBA/1 mice. These data suggest that vanillin has the merit as a potential therapy for SUDEP.

Disclosures: E.K. Farrell: None. **H. Feng:** None.

Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.11

Topic: B.08. Epilepsy

Support: FRM: Fondation pour la recherche médicale en France

Title: Protective role of microglia and T lymphocytes in a mouse model of temporal lobe epilepsy

Authors: *M. MORENO-MONTANO[1], P. NOBILI[1], L. MARTINS[1], A. VIRENQUE[2], V. DARDALHON[3], H. HIRBEC[1], V. GARCIA[1], J. MATEOS-LANGUERAK[4], N. MARCHI[1], E. AUDINAT[5];
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Abstract: Temporal lobe epilepsy (TLE) is a chronic neurological disease affecting brain structures implicated in cognitive functions. The disease is manifested by the appearance of disabling, spontaneous, recurrent seizures caused by the hyper-excitability and hyper-synchronization of neurons in the temporal lobe structures. Seizures are accompanied by neuronal death, gliosis, structural and synaptic reorganization. Seventy five percent of patients do not respond to anti-epileptic drugs and the only curative intervention is the surgical resection of the epileptic focus. It is therefore urgent to better understand the mechanisms involved in pathogenesis of TLE in order to identify new therapeutic targets. It is now well documented that neuroinflammation is critically involved in this disease. Accumulated data suggest that microglia develop a dual profile, pro and anti-inflammatory, and that the balance between these two states determines the evolution of the disease. Using a TLE model in mice lacking T cells (CD3 -/-), we observed a loss of the microglial anti-inflammatory response and an increase of neuronal death in the hippocampus together with an increase of spontaneous seizure frequency. Regulatory T cells (Treg) have been shown to promote anti-inflammatory reactions in different models of CNS disorders. We therefore inactivated Treg by administrating anti-CD25 antibodies during early epileptogenesis and this reproduced the loss of the anti-inflammatory response in microglia, the increased neuronal death and the increase of spontaneous seizure frequency. In preliminary experiments, we depleted microglial cells during early epileptogenesis and we observed an increased neuronal death. Together these data suggest that interactions between microglia and Treg cells drive an anti-inflammatory response that protects the hippocampus from damage and limits seizure activity. These results will be confirmed and completed by transcriptomic and proteomic analyses of pro- and anti-inflammatory markers in the hippocampus These findings may allow the identification of new therapeutic targets to enhance the endogenous anti-inflammatory response and ultimately improve the treatment of TLE.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.12

Topic: B.08. Epilepsy

Support: RCSI Funding

Title: Exploring epigenetic dysfunction in the development of temporal lobe epilepsy

Authors: *A. HARNETT[1,2], E. LANGA[1], N. DELANTY[3], D. O'BRIEN[3], D. HENSHALL[1,2], G. P. BRENNAN[4,2];
[1]Royal Col. of Surgeons In Ireland, Royal Col. of Surgeons In Ireland, Dublin, Ireland; [2]FutureNeuro SFI Res. Ctr., Dublin, Ireland; [3]Neurol., RCSI Beaumont Hosp., Dublin, Ireland; [4]Univ. Col. Dublin, Dublin, Ireland

Abstract: Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults with 1/3 of patients refractory to anti-epileptic drugs. Acquired TLE often develops following a brain insult e.g. traumatic brain injury, stroke or status epilepticus (SE), triggering widespread gene expression changes which have so far been poorly characterized. These changes may spur various pathogenic processes involved in the development of the disease, including comorbidities. Changes in gene expression occurring in the latent period between SE and the generation of spontaneous recurrent seizures can play a major role in the establishment of epilepsy and are often driven by epigenetic mechanisms.Epigenetics broadly refers to processes such as DNA methylation, histone modifications and non-coding RNAs that control gene expression by influencing the accessibility of the chromatin to transcriptional machinery. All three major epigenetic processes have been found to be altered in both experimental and human epilepsies. Epigenetic modifications can have far-reaching consequences on cellular function, influencing the susceptibility of neuronal networks to seizure generation.EZH2 and Brd4 are two such epigenetic enzymes which can have profound effects on gene expression patterns in the brain. EZH2 and Brd4, respectively, are epigenetic writers and readers, that modify histones thus altering chromatin accessibility and ultimately controlling gene expression patterns. In this project, we explore dysregulation of PRC2 and BET proteins in epileptogenesis and chronic epilepsy.Preliminary results identified altered expression of EZH2 and Brd4 protein levels and various other epigenetic enzymes in the hippocampus following SE suggesting widespread epigenetic reorganization of the genome initiated by epilepsy-inciting events. Additionally, we broadly mapped the protein-DNA binding events involving EZH2 and Brd4 using CUT&RUN, enabling us to further analyze the dynamic interactions between these epigenetic enzymes and DNA driving gene expression changes in epileptogenesis and chronic epilepsy.Investigation into the role of EZH2 and Brd4 will enhance our understanding of how these mechanisms contribute to brain function homeostasis and how they are altered in epilepsy. This understanding of basic mechanisms of neuronal function is key to the development of novel anti-epileptic therapeutics.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.13

Topic: B.08. Epilepsy

Support: NIH/NINDS Grant R21 NS112779
AstraZeneca supplied SAR

Title: The effects of Fyn/Src kinase inhibition (Sarcatinib) on spontaneous seizures and epilepsy-associated behavioral deficits in a rat kainate model of temporal lobe epilepsy

Authors: *N. RAO, M. PUTRA, C. MAFUTA, M. BUNNY, S. CHENEY, A. ALMANZA, T. THIPPESWAMY;
Biomed. Sci., Iowa State Univ., Ames, IA

Abstract: One-third of epilepsy patients are intractable to currently available anti-seizure drugs (ASD), prompting a quest to discover novel therapies for drug-resistant epilepsy. Epilepsy is often also associated with behavioral impairments. Of the several mechanisms of hyperexcitability in experimental models of epilepsy, we investigated the role of Fyn, a Src family tyrosine kinase. In this study, we investigated the impacts of Saracatinib (SAR), a Fyn/Src kinase inhibitor, on epileptic rats and epilepsy-related behavioral changes in the KA-induced temporal lobe epilepsy rat model. We observed a significant reduction of spontaneous recurrent seizures (SRS) during the 14 days of SAR treatment and a week later in epileptic rats. There was a 40% reduction in epileptiform spikes in the SAR-treated group but the difference was not significant compared to the vehicle group. In Zero Maze, the control and SAR-treated groups spent more time in closed arm than open arm, while epileptic rats spent equal time in both arms. In Fear Conditioning Test, both control and SAR-treated groups did not show significant differences in freezing time between conditioning and probe trials, while epileptic rats froze significantly longer in probe test. Interestingly, in Novel Object Recognition test, epileptic rats showed a significant positive discrimination index, at 2h post-familiarization, in contrast to the control and SAR-treated groups. In Morris Water Maze, there was significantly increased latency to the platform in both KA-treated groups relative to the control during the learning phase. Overall, our study demonstrates that SAR treatment for two weeks after the onset of epilepsy significantly reduces SRS, but only during the treatment period and a week later, indicating the anti-epileptic potential of SAR. The two-week SAR treatment demonstrated limited effect on epilepsy-induced anxiety-like behavior and cognitive deficit. These findings suggest that SAR can be a potential disease-modifying agent but may require treatment for greater than two weeks. SAR-in-diet approach for longer duration is under investigation.

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Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 038.14

Topic: B.08. Epilepsy

Support: NIH K12NS080223
Indiana CTSI

Title: Canonical Wnt pathway transcriptional dysregulation in temporal lobe epileptogenesis

Authors: *M. D. MARDONES, K. GUPTA;
Stark Neurosciences Res. Inst., Indianapolis, IN

Abstract: The dentate gyrus of the hippocampus undergoes neuronal network remodeling during temporal lobe epileptogenesis (TLE); changes in this area include dentate granule neuron dispersion, decreased neurogenesis, increased migration and dendritic complexity. These remodeling events contribute to the pathogenesis of temporal lobe epilepsy, however, the underlying pathways responsible for this remodeling are unknown. Previously, we determined that some components of Wnt signaling are dysregulated in the mouse kainate model of temporal lobe epilepsy 3-days after Kainate injection, here we hypothesized that throughout epileptogenesis there is a continuous dysregulation of the Wnt signaling pathway. Focal temporal lobe epilepsy was induced by unilateral CA3 kainate (KA) injection (19nM) in 8-weeks-old C57BL/6J mice, bilateral dentate gyri were collected 3, 7 and 14-days post injection (dpi) and the dorsal portion processed for RNA extraction. Libraries were prepared by Illumina TruSeq and sequenced to average depth of 11.2 million read pairs. Differential expression analysis was performed using DESeq2, functional analysis by Database for Annotation, Visualization and Integrated Discovery (DAVID) version2022q1 and Kyoto Encyclopedia of Genes and Genomes (KEGG)-pathways tools. Genes with FDR<0.01 and FC>+-1.5 were considered for analysis. N=4/group. DAVID analysis demonstrated that dysregulation of genes associated with GO:Signaling Mechanism are most represented in the ipsilateral epileptogenic zone (EZ) after 3 and 7-dpi, in the contralateral seizure network (SN), signaling mechanism genes are most represented at 3dpi and few genes are dysregulated at 14dpi. KEGG pathway analysis indicates that canonical Wnt pathway is differentially dysregulated in the dentate gyrus, in both the ipsilateral EZ and in the contralateral SN as soon as 3-days after KA injection and the changes of expression of Wnt pathway components remain throughout all time points. We determined that in the KA induced TLE mouse model there is abnormal gene expression across the EZ and SN in epileptogenesis, and specifically we observed dysregulation in canonical Wnt signaling. This suggests that the Wnt pathway may play a role in the neuronal network remodeling of the dentate gyrus and represents a potential therapeutic target in epileptogenesis.

Disclosures: M.D. Mardones: None. **K. Gupta:** None.

Poster

038. Epilepsy: Post-Seizure Modifications

Location: SDCC Halls B-H

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Program #/Poster #: 038.15

Topic: B.08. Epilepsy

Support: MOE2018-T1-002-033
MOE2017-T3-1-002
NTU Nanyang Assistant Professorship

Title: Seizure enhances protein SUMOylation and zinc finger transcriptional repression in neuronal nuclei

Authors: H. SOON[1], J. GAUNT[1], V. BANSAL[1], S. SZE[2], ***T. CH'NG**[1];
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Abstract: A single episode of pilocarpine-induced status epilepticus in rodents can trigger long-term changes in gene expression and alter other nuclear processes that may contribute toward the development of recurring seizures. We are interested in uncovering the underlying changes in these nuclear events during the initial phases of acute seizures. To that end, we used a transgenic mouse model to isolate excitatory neuronal nuclei and profiled the seizure-induced nuclear proteome and transcriptome. Apart from robust enrichment for all AP-1 complex subunits along with bZIP interacting partners, we also observed an enrichment of SUMO2/3 and several proteins associated with the SUMOylation pathway which we validated by Western blotting, immunohistochemistry both in brain sections and in stimulated neuronal cultures. In parallel with nuclear proteome, we also characterized nuclear gene expression at both exon and intron levels by RNA-seq. Besides a robust induction of MAPK signaling genes, we also report widespread Zinc-mediated nuclear events including activation of zinc finger transcription factors and transcriptional repression of genes containing zinc finger motifs that harbor the KRAB repressor domain. Our results indicate that this methodology is applicable to different sequencing platforms, but also particularly sensitive for profiling the nuclear proteome, which can be used to examine other cell types and in different disease models.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.01

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

Support: University of Turin (Italy)
Compagnia di San Paolo
MIUR

Title: Dysregulation of calcium released from ryanodine receptors induces synaptic impairments during Alzheimer's disease onset

Authors: *A. MARCANTONI, E. HIDISOGLU, G. CHIANTIA, G. TOMAGRA, E. CARBONE, V. CARABELLI;
Univ. of Turin, Univ. of Turin, Torino, Italy

Abstract: Synaptic dysfunction is one of the earliest hallmarks of Alzheimer's Disease (AD). It is known that the oligomeric form of amyloid beta protein Abeta42 contributes to the development of synaptic abnormalities and cognitive impairments associated with AD. We have already reported that Abeta42 increases intracellular calcium concentration released through calcium permeable ryanodine receptors (RyRs) and opposite effects have been described on AMPA and NMDA dependent glutamatergic synapses. In particular we have observed that, while the former are inhibited, the latter are potentiated. To date, there is an impressive lack of information on how Abeta42 affects the elementary parameters regulating inhibitory GABAergic synaptic function. We found that Abeta42 increased the frequency and amplitude of GABAergic miniature currents (mIPSCs) as well as the amplitude of evoked inhibitory post synaptic currents (eIPSCs). Noise analysis of mIPSCs revealed a postsynaptic remodeling of the number and permeability of GABA receptors that agree with the increased mIPSCs amplitude observed. At presynaptic site, we did not observe any significant change on *paired pulse depression* (PPD) and GABA *release probability* (p) induced by Abeta42 while both the size of readily releasable pool responsible for synchronous release (RRP_{syn}) and the number of release sites (N) were increased. We further tested whether ryanodine receptors (RyRs) contributed to exacerbate these changes by stabilizing the interaction between RyRs and the accessory protein calstabin. We observed that the RyRs calstabin interaction stabilizer S107 restored the GABAergic synaptic parameters to values comparable to those of control conditions. In conclusion, our findings clarify the mechanisms of potentiation of GABAergic synapses induced by Abeta42 and suggest that RyRs are involved in the control of synaptic activity during the early stage of AD onset. Their stabilization rather than inhibition could represent a new therapeutical approach for AD treatment.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.02

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

Title: An in vitro study of the exosomal cargo implication on the pathogenesis of Alzheimer´s Disease

Authors: *S. E. OCHOA HUGO[1], M. A. HERNÁNDEZ SAPIÉNS[1], E. E. REZA ZALDÍVAR[1], B. D. MINJAREZ VEGA[2], V. J. SÁNCHEZ GONZÁLEZ[3], Y. K. GUTIÉRREZ MERCADO[3], A. A. CANALES AGUIRRE[1];
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Abstract: Alzheimer´s disease (AD) is a neurodegenerative, progressive, and irreversible disorder that gradually leads to a total loss of cognition. AD´s microenvironment is characterized by the presence of beta-amyloid protein (βA) and Tau protein in its hyper-phosphorylated form (p-Tau), along with other signaling and transporting proteins such as exosomes, all of them interacting with neurons and glial cells on different development states. For AD to be manifested, cells must communicate with each other, on that context the use of neural precursor cells (NPCs) presents the opportunity to study cell communication through exosomes and its pathological events. This work aimed to study NPCs-derived exosomes, with A431E mutation in gen PSEN1, and its effects on the induction of neurodegenerative processes related to AD. This is the first research work on exosomes of induced pluripotent stem cells (iPSC)-derived NPCs with this mutation. The iPSCs culture (previously reprogrammed from AD´s patient's skin fibroblasts by our research group) was induced to NPCs for five days, sub-cultured eight times, and conservated its morphological characteristics such as individual, pyramidal, and long perimeters. The established NPCs culture was nestin-positive expressive cells which indicate its neural stem cell identity. We characterized NPCs-derived exosomes. NPCs-derived exosomes were extracted from the medium of a cell density of 9x106 cell/ml from which we obtained 0.503 μg/μl exosomes with a size of 157 nm on average that showed an expression of the trans-membranal exosomal marker CD81-positive, which indicates its identity as exosomes. We also evaluated the cytotoxic effect of NPCs-derived exosomes on astrocytic cell culture. The astrocytic cell line U138MG, with the expression of glial marker GFAP-positive, was used for the treatment and cytotoxicity assays with the NPCs-derived exosomes for 24 h, 48 h, 72 h, and exosome concentrations of 30 μg/μl, 60 μg/μl and 90 μg/μl. The cytotoxicity assay indicated no cell density or morphological changes post-treatment, cell viability percentages were statistically analyzed by ANOVA test with values $p>0.05$ which indicates no statistical differences between the treated groups. Finally, immunodetection of AD´s related proteins such as βA and p-Tau on NPCs and NPCs-derived exosomes was negative. We suggest further analysis of the presence of these proteins on the NPCs-derived exosomes by implementing more sensitive techniques, delving into the study of human exosomes from iPSC-derived NPCs, and analyzing their real functions throughout AD´s development on *in vivo* models.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.03

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

Support: NIH/NIA 1R01AG069433

Title: Neural stem cells-derived exosomes promote synaptic resistance to amyloid oligomers by modulating the neuroinflammatory tone in the hippocampus via specific miRNAs.

Authors: *M.-A. MICCI[1], O. ZOLOCHEVSKA[2], X. WU[3], Q. CHENG[3], G. TAGLIALATELA[4];
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Abstract: Alzheimer's disease (AD) is the most common and severe age-associated neurodegenerative dementia. While aging is the most significant risk factor for AD, disruption of synapses by oligomers of both amyloid beta (Aβ) and tau, via converging synergistic mechanisms, is one of the earliest events leading to memory dysfunctions. We previously found that exosomes secreted by hippocampal neural stem cells, and not mature neurons, act via specific miRNAs to provide synaptic protection against amyloid oligomers. Here we aimed to determine the mechanism of action of NSC-derived exosomes (NSC-exo) and their bioactive miRNA cargoes, in comparison to MN-derived exosomes (MN-exo), by perfoming transcriptomics analysis using machine learning approaches.Male and female adult wild-type mice were injected ICV with NSC-exo or MN-exo, or a combination of the three selected miRNA cargoes (17, 322, 485). Twenty-four hours after the ICV injections, synaptic fractions were prepared from the hippocampus and total RNA was isolated for deep-sequence analysis. Transcriptomics data were analyzed using machine learning algoritms to select and identify genes differentially affected in the experimental groups and further analyzed using Gene Set Enrichment Analysis (GSEA). Statistical analysis was performed using analysis of variance (ANOVA).The transcriptomics analysis revealed that immune response pathways were selectively activated after treatments with miRNAs or NSC-exo. These data suggest that specific miRNA cargoes enriched in NSC-derived exosomes modulate the neuroinflammatory tone which plays an important role in synaptic plasticity, thus promoting synaptic resilience to Aβ and tau oligomers.

Disclosures: M. Micci: None. **O. Zolochevska:** None. **X. Wu:** None. **Q. Cheng:** None. **G. Taglialatela:** None.

Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.04

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

Support: NIH Grant R01NS105680

Title: Transmembrane protein 184B (TMEM184B) promotes expression of synaptic gene networks in the mouse hippocampus

Authors: E. G. LARSEN, **E. B. WRIGHT**, H. R. HART, M. R. BHATTACHARYA; Neurosci., Univ. of Arizona, Tucson, AZ

Abstract: In Alzheimer's Disease (AD) and other dementias, hippocampal synaptic dysfunction and loss contribute to the progression of memory impairment. Recent analysis of human AD transcriptomes has provided a list of gene candidates that may serve as drivers of disease. One such candidate is the membrane protein TMEM184B. To evaluate this hypothesis, we asked whether loss of TMEM184B in mice causes gene expression or behavior alterations. Because one major risk factor for AD is age, we compared 5 month and 15 month old wild type and TMEM184B mutant mice to assess the dual contributions of age and genotype. Across all datasets, we identified disruption of pre- and post-synaptic transcripts, including those involved in maintaining synapses and neuronal processes. Wnt-activated transcription factor binding were enriched among differentially expressed genes, suggesting an intersection with this pathway. Both young and aged TMEM184B mutant mice showed synaptic gene expression alterations from wild type. Fewer differences existed between young and aged mutants, suggesting that most transcriptional effects of TMEM184B occurred earlier in life. To understand how TMEM184B disruption may impact behaviors, we evaluated memory using the novel object recognition test and anxiety using the elevated plus maze. Six-month-old TMEM184B mutant mice show normal object discrimination, suggesting a lack of spatial memory impairment at this age. However, mutant mice showed decreased anxiety, a phenotype seen in neurodevelopmental disorders. Taken together, our data suggest that TMEM184B is required for proper synaptic gene expression and function early in life but may not be causal for AD and related dementias.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.05

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

Support: RF1AG033570

Title: A profile of immature and mature granule neurons recruited into memory circuits in Alzheimer's disease

Authors: *P. KUMAR[1], Z. MORRISSEY[1], T. X. PHAN[3], R. MISHRA[1], M. GUPTA[2], C. HOLLANDS[1], A. SHETTI[2], K. L. LOPEZ[4], M. MAIENSCHEIN-CLINE[5], H. SUH[6], R. HEN[7], O. LAZAROV[5];
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Abstract: Title: A profile of immature and mature granule neurons recruited into memory circuits in Alzheimer's disease
Pavan Kumar[1#], Zachery Morrissey[1,2,3#], Trongha Phan[1#], Rachana Mishra[1#], Muskan Gupta[1], Carolyn Hollands[1], Aashutosh Shetti[1], Kyra Lauren Lopez[1], Mark Maienschein-Cline[4], Hoonkyo Suh[5], Rene Hen[6], Orly Lazarov[1]*.
Alzheimer's disease (AD) is characterized by progressive memory impairments. Deficits in hippocampal neurogenesis have been observed in human patients as well as in mouse models of familial Alzheimer's disease (FAD). However, it is not clear whether impairments in neurogenesis play a role in memory deficits. To address this, we examined the recruitment of immature neurons into memory circuits using viral vectors expressing activation- induced reporter. We observed that fewer immature neurons were recruited following behavior into the neuronal ensemble in FAD mice compared to wild type. In addition, those cells that were recruited exhibited compromised morphology and reduced density of dendritic spines. To examine the molecular underpinning of these impairments we utilized in situ sequencing in brain sections of these mice. An array of genes critical for neuronal function was upregulated in recruited neurons compared to non-recruited. Neurogenesis-related genes showed the most significant differences between recruited neurons in the wild type and FAD mice. Interestingly, several AD- related genes, such as amyloid precursor protein (App) and A Disintegrin and metalloproteinase domain-containing protein 10 (Adm10) were modulated in engram neurons. Genes that were vastly upregulated in the wild type engram were not modulated in the engram in FAD mice, while a separate set of genes, not greatly modulated in the wild type, was upregulated in the FAD engram. Importantly, enhancing levels of neurogenesis in FAD partially rescued this phenotype. This study suggests that deficits in new neurons lead to an impaired profile of new and mature neurons participating in memory formation in FAD.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 039.06

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

Support: National Health and Medical Research Council of Australia (NHMRC) grant (APP1125796)
A.R.W. is a recipient of an NHMRC Senior Research Fellowship (APP1118452)

Title: An Alzheimer's disease patient-derived olfactory cell model identifies gene expression changes associated with neural function

Authors: *L. M. RANTANEN[1,2], M. BITAR[1], R. LAMPINEN[3], E. PENTTILA[4], J.-M. LEHTOLA[5,6], T. SAARI[5], S. HANNONEN[5,6], H. LOPPONEN[4], A. M. KOIVISTO[5,6,7], A. MACKAY-SIM[8], A. S. CRISTINO[8], K. KANNINEN[3], A. R. WHITE[1];
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Abstract: Alzheimer's disease (AD) is an incurable degenerative brain disorder with an increasing incidence worldwide. There are currently no disease-modifying treatments in clinical use, aside from the recent approval of Aduhelm, an anti-amyloid antibody. To address this shortage, future therapeutic paradigms should include cellular model systems that co-relate with early clinical features observed in AD. A deteriorated sense of smell is one of the earliest symptoms and a significant predictor of conversion to AD from mild cognitive impairment (MCI), a prodromal AD condition. However, the molecular and cellular basis of cognitive decline and loss of olfaction remains elusive. Found deep within nares, olfactory stem cells provide a window into the brain. Their inherent ability to form neuroglia makes these cells an ideal model system to examine the early pathophysiological changes that take place in AD. We have generated human olfactory neurosphere-derived (ONS) cell lines using olfactory mucosal biopsies from age-, gender- and ApoE genotype-matched cognitively healthy individuals (HC) (n=6), patients with AD (n=6), and individuals with MCI (n=6). We performed global RNA sequencing to determine gene expression changes in AD, MCI and HC ONS cells. Our transcriptomic results revealed several differentially expressed genes associated with cognitive changes, A-kinase anchoring protein 6 (*AKAP6*) being the most significantly altered in AD compared to MCI and HC. The greatest change in the gene expression of all differentially expressed genes occurred between AD and MCI. Pathway analysis indicated defects in multiple cellular processes with aging and alternative splicing being the most significantly dysregulated in AD ONS cells. In the present study, we have demonstrated that ONS cells can provide a cellular model for AD that recapitulates disease-associated differences. We have revealed potential novel genes, especially *AKAP6* that may have a role in AD, particularly MCI to AD transition, and should be further examined.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 039.07

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

Support: NIH/NIA grant R01AG069433

Title: Single-nuclei analysis of hippocampi from wild-type mice after intracerebroventricular injection of neural stem cell-derived exosomes

Authors: *S. SAIEVA[1], J. GUPTARAK[1], A. FRACASSI[1], W.-R. ZHANG[1], K. M. JOHNSON[2], M.-A. MICCI[3], G. TAGLIALATELA[1];
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Abstract: Neural stem cells (NSC) and adult neurogenesis are known to modulate synaptic plasticity and cognitive function. Decreased number of NSC have been reported in Alzheimer's Disease (AD), whereas their increase is associated with improved learning and memory. However, an increased neurogenesis does not correlate with preserved cognition, thus suggesting that other mechanisms involving increased numbers of NSC promote the preservation of memory. NSC are also known for their capacity to secrete exosomes, which are microvesicles containing cell-specific cargos of biomolecules, that can be taken up by other cells, thus modulating their function. We and others have shown that NSC-derived exosomes (NSCexo) injected in brains of AD transgenic mice models ameliorated the cognitive decline and decreased the binding of toxic oligomers to synapses. The exact mechanisms by which NSCexo provide protection to synapse is still under investigation, although initial deep sequencing analyses show that elements of neuroinflammation seem to be involved in NSCexo-mediated neuroprotection. Our long-term goal is to find the specific genes modulated by NSCexo that may be responsible for the cognitive resilience and the overall improvement of CNS function. To shed light on this mechanism, we show the results of our single-nuclei gene expression investigations on hippocampi isolated from mice injected with NSCexo and compared them to not-injected mice. In parallel, here we determine cell-specific changes in gene expression after NSCexo injection, thus indicating which cell types are more likely to drive exosomes-mediated neuroprotection.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

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Program #/Poster #: 039.08

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

Support: NIH Grant AG053740
NIH Grant AG070255

Title: Global electrophysiological Excitatory to Inhibitory imbalance in hippocampus and temporal cortex in mild cognitive impairment and Alzheimer's disease

Authors: P. SCADUTO, W. RUSSELL, ***A. LIMON**;
UTMB, UTMB, Galveston, TX

Abstract: Individuals at distinct stages of Alzheimer's disease (AD) show abnormal electroencephalographic activity which has been linked to network hyperexcitability and cognitive decline. Using electrophysiology and proteomics of human synapses from the hippocampus and temporal cortex of control, mild cognitive impaired (MCI) and AD individuals we aimed to determine the global synaptic balance in hippocampus and temporal cortex at distinct stages of neuropathology. Electrophysiological synaptic E/I ratios in post-mortem samples from the temporal cortex of individuals with MCI (n = 6) or AD (n = 6) compared to non-demented controls (n = 6), and the hippocampus (MCI, n = 8; AD n = 11, CTRL = 8) were assessed by microtransplantation of synaptic membranes (MSM). Proteomics of synaptosomes from temporal cortex were analyzed in the context of their electrophysiological responses using Electrophysiologically-anchored Dataset Analysis (EDA). We found that the higher the amplitude of $GABA_A$Rs currents the better the cognitive performance score (R^2=0.152; p=0.044) in the hippocampus. A similar association was observed for AMPARs currents (R^2=0.133; p=0.06) also in the hippocampus. The eE/I ratio was significantly higher in the temporal cortex (TCx) of AD subjects and was negatively associated with the MMSE in the TCx (R^2= 0.205; p=0.059) but not in the hippocampus. The synaptoproteome revealed the impact and directionality of protein alterations and neuropathology on the amplitude of synaptic receptors responses and cognitive MMSE scores.These findings indicate that early shifts of the E/I balance contribute to the loss of cognitive capabilities in the continuum of AD symptomatology.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

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

Support: Basal Center of Excellence in Aging and Regeneration AFB 170005-ANID to NI
FONDECYT 1221178 to CTR
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ANID Fellowship 21211882 to AKT

Title: Canonical Wnt signaling regulates the expression of mtUPR-related proteins in neuronal cells by a mechanism that requires beta-catenin-dependent transcription

Authors: A. K. TORRES[1], C. TAPIA-ROJAS[3], ***N. C. INESTROSA**[2];
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Abstract: Canonical Wnt signaling is essential for the maintenance of neuronal function. Its activation induces the accumulation and translocation of β-catenin to the nucleus to bind TCF/LEF transcription factors and transcriptional coactivators CBP/p300, inducing Wnt target genes. This signaling is downregulated in Alzheimer's Disease (AD), and its activation reduces Aβ and tau pathology and improves mitochondrial function, however, this regulation is still poorly understood. Mitochondrial unfolded protein response (mtUPR) is a mitochondrial stress response activated by abnormal proteins in the mitochondria, which induces the transcription of chaperones and proteases and their import to the mitochondria. Interestingly, the expression of these mtUPR-related genes also requires CBP/p300 coactivators, and mtUPR overactivation also reduces AD pathology. Here, we propose a new regulatory mechanism for controlling the expression of mtUPR genes induced by the transcription of Wnt target genes. We evaluated whether canonical Wnt signaling regulates the expression of mtUPR-related proteins. For this objective, we pharmacologically activated Wnt signaling in mouse primary hippocampal neurons of 14 DIV during 24 hours using Andrographolide (a GSK-3β inhibitor) and we observed that its activation increased the expression of ATF5 and Hsp60 by 20%, and the proteases Oma1 and Lonp1 in 30%. Otherwise, the inhibition of Wnt signaling with ICG001 (a CBP/β-catenin inhibitor that blocks Wnt gene transcription) resulted in an opposite effect. In addition, bioinformatic analysis shows that all mtUPR-related genes have TCF/LEF elements in their promoters, suggesting they correspond to new Wnt target genes. Moreover, those mtUPR-related genes that present a low number of TCF/LEF elements in their promoters correspond to: Oma1 (3) Lonp1 (3) and ATF5 (6), the same molecules that are modulated by Wnt signaling activity, suggesting that a reduced number of TCF/LEF elements would predict a greater regulation effect. We also observed the same Wnt-dependent mtUPR regulation in 14-month-old SAMP8 mice, a non-transgenic AD mice model. These findings support the idea that canonical Wnt signaling regulates mtUPR-related protein expression by activating their β-catenin dependent transcription.

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Poster

039. Mechanisms of Synaptic Dysregulation in Alzheimer's Disease

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Program #/Poster #: 039.10

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

Support: National Science Foundation Graduate Research Fellowship
Royalty Research Fund
Alzheimer's Association

Title: Circular RNAs localize to synapses in the human brain implying critical roles for synaptic function and could be important components of neurodegeneration.

Authors: *S. N. SMUKOWSKI[1], C. DANYKO[1], M. M. COURSE[1], K. GUDSNUK[1], N. POSTUPNA[2], C. D. KEENE[2], P. N. VALDMANIS[1];
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Abstract: Circular RNAs (circRNAs) are unique transcript isoforms that may play critical regulatory roles in the human brain which could have implications in the progression of neurodegenerative disorders such as Alzheimer's Disease (AD). CircRNAs are produced by back-splicing a later exon to a preceding exon creating a closed-loop structure. They can perform a variety of functions, in addition to being translated, including acting as microRNA "sponges" and sequestering RNA binding proteins. CircRNAs also persist in the cytoplasm due to exonuclease resistance. Previous research demonstrated circRNAs preferentially localize to synapses and expression of many are perturbed in AD. We corroborate this evidence with the addition of many novel insights using new techniques and statistical approaches. To discover and quantify RNA transcripts localized to the synapse in AD, we acquired more than 30 postmortem human brain samples including AD patients and controls. From homogenized brain tissue, we fractionated synaptic particles (synaptosomes) using sucrose gradient ultracentrifugation. Next, we extracted RNA from both the synaptosome fraction and homogenate, then acquired RNA sequencing data using a ribo-removal total RNA strategy which captures non-canonical isoforms, in contrast to polyA pulldown. By comparing synaptosome to homogenate, we confirmed that the overwhelming majority of circRNAs are enriched in synapses. Furthermore, by comparing synaptosomes between AD and control, we discovered that there are also many circRNAs that differentially localize to synapses in AD. We hypothesize that these differences in circRNA synaptic localization influence dynamics of synaptic-localized protein translation likely contributing toward neurodegenerative pathology. Among the most significantly differentially localized circRNAs, two isoforms of the gene *GSK3B* stood out. One isoform is a circle of exons 7, 8, and 9, and the other isoform is a circle of exons 8b, 9, and 10. Most interesting is that the former isoform is upregulated in AD with a log2 fold change of 1.5 whilst the later is downregulated in AD with a log2 fold change of -1.6. The discovery that there are two unique circular isoforms of *GSK3B* each differentially localized at synapses in AD is noteworthy since *GSK3B* has an established role in tau hyperphosphorylation. We also see significant differences in isoforms of *AKT3*, *HOMER1*, and *WDR7*, which each could have their own implications in disease progression. Here we present our work to uncover the significance of these findings which represents a novel investigation into this class of understudied molecules.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.01

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

Support: National Institute for Neurological Disorders and Stroke (NINDS) Grant R21NS106640 (S.V.)
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Houston Methodist NeuralCODR Fellowship program (S.S.)

Title: Effect of the fecal microbiota transplantation from young wild-type controls in aged Alzheimer's disease mice after traumatic brain injury

Authors: *S. SORIANO[1], Y. LIU[2], H. FLINN[1], M. HOLCOMB[1], E. CHOW[1], L. PATRICK[1], T. J. TREANGEN[2], S. VILLAPOL[1,3];
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Abstract: Traumatic brain injury (TBI) is one of the largest risk factors for the later development of neurodegenerative diseases such as Alzheimer's disease (AD). The gut microbiome is an essential modulator of the crosstalk between the intestine and the brain and intestinal microbiota dysbiosis has been linked to numerous neurological disorders, including both TBI and AD. In a recent publication, we found that gut microbiota from AD mice aggravated the neuroinflammatory response and neurological outcomes after TBI in young wild-type (WT) controls. Specifically, WT mice exhibited larger lesion, increased activated microglia/macrophages, and reduced motor recovery after receiving a fecal microbiota transplantation (FMT) from aged AD mice compared to the recipients of an FMT from young controls. Given the detrimental effect of AD gut microbiota following TBI, we hypothesize that restoration of the AD microbiome to a healthy state could improve the neuropathological consequences of brain injury. Therefore, the aim of this study was to characterize the effect of an FMT from WT mice administered to aged AD mice after TBI. Eight- to nine-month-old 5XFAD transgenic males and females, and their WT littermates, underwent a controlled cortical injury (CCI) as model of TBI. Prior to the surgeries, all mice received a gut microbiota depletion treatment consisting of an antibiotic cocktail delivered by oral gavage for three days. We prepared FMTs from the cecum of 2- to 3-month-old C57BL/6 wild-type mice that were administered orally 24 hours after injury. We characterized the microbiota composition of fecal samples collected at baseline, after the antibiotic regimen, and at 1-, 3- and 11-days post-injury.

16S rRNA gene sequencing analysis revealed changes in the gut bacteria community induced by the microbiome depletion and the FMT treatment. As expected, the 5XFAD mouse model showed a significant increase in inflammatory brain response at 3 days post injury. However, there was no major effect of the FMT treatment on lesion volume or neuroinflammatory markers after CCI in the 5XFAD mice. Similarly, the 5XFAD mice that received FMTs showed no differences in the motor recovery following TBI, or in the memory and anxiety-like behaviors. In summary, microbiota depletion followed by a single FMT at 24h post-TBI could not limit the neuropathology associated to brain injury in 5XFAD mice, and future studies will focus on testing alternative approaches of microbiota restoration.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.02

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

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Canada Research Chairs Program Tier 1 in Brain Repair and Regeneration
Canadian Institutes of Health Research (137064, 166184, 168906, 154272)
National Institutes of Biomedical Imaging and Bioengineering of the National Institutes of Health (RO1-EB003268)
Temerty Chair in Focused Ultrasound Research

Title: Engineered adeno-associated viruses combined with focused ultrasound-mediated blood-brain barrier modulation leads to gene delivery to large and multiple connected brain regions

Authors: *R. KOFOED[1], K. NOSEWORTHY[1], C. L. DIBIA[1], L. M. STANEK[3], B. ELMER[3], L. S. SHIHABUDDIN[3], K. HYNYNEN[2], I. AUBERT[1];
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Abstract: Disease modifying treatments are lacking for disorders of the central nervous system such as Alzheimer disease, Parkinson disease, and amyotrophic lateral sclerosis. Gene therapy can provide long-lasting clinical benefits with a single administration; however, the blood-brain barrier (BBB) limits the entry of intravenously administered recombinant adeno-associated viruses (AAVs), commonly used gene therapy vectors, to the brain. Therefore, high intravenous doses or invasive intracranial injections are required to deliver sufficient quantities of AAVs to the brain, which increase the risk of unwanted side-effects. Magnetic resonance image-guided

focused ultrasound in the presence of intravenous microbubbles (MRIgFUS) temporarily increases the permeability of the BBB and in preclinical studies can enable the delivery of intravenous AAVs to targeted sites of the brain and spinal cord. While MRIgFUS can deliver naturally occurring AAVs with millimeter precision to targeted brain areas, the treatment of diseases with brain-wide pathology may require gene transduction to large or multiple regions. Here, we have used MRIgFUS to deliver two engineered AAVs to the mouse brain: 1) AAV2-HBKO with an increased ability to spread in brain tissue compared to the parental AAV2, and 2) AAVrg with retrograde transport properties from axonal terminals to the soma of neurons. MRIgFUS targeting 30% of the striatum and thalamus combined with $1.7x10^{13}$ GC/kg AAV2-HBKO led to transgene expression in 13% and 21% of all neurons in each brain region, respectively. AAVrg was transported retrogradely from the MRIgFUS-targeted areas to brain regions distal from the delivery sites where transgene expression was observed. Novel AAVs are being developed with an increased ability to cross the BBB after intravenous administration; however, these vectors do not permit targeted delivery to subregions of the brain. Here we demonstrate that genes can be delivered (1) to defined brain areas, small or large, by using AAV2-HBKO and MRIgFUS and (2) to specific neuronal networks using AAVrg and MRIgFUS targeting brain regions containing axonal terminals. Non-invasive gene therapy designed for the treatment of diseases affecting small, large or multiple brain areas remains an unmet need and our results demonstrate that engineered AAVs combined with MRIgFUS can provide new strategies for gene delivery to the brain.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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

Support: NIA/NIH 5R01AG06943302
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Title: Continuous wave 670nm LED light therapy improves memory, reduces phosphorylated tau burden and activates plaque-associated microglia in 3xTg-AD mice

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Abstract: BACKGROUND Non-invasive therapeutic alternatives for Alzheimer's Disease (AD) are desirable due to their accessibility and sustainability of use. Photobiomodulation therapy uses 620-1100nm red to near-infrared light to modulate a variety of biological processes including neuroinflammation, amyloid and tau oligomerization, and apoptosis. 670nm red light reduces synaptic vulnerability to amyloid-β oligomers in wild-type (WT) mice and reduces tau oligomerization in multiple mouse models of tauopathy. The 3xTg-AD mouse model of AD neuropathology develops an abundance of amyloid plaques and neurofibrillary tangles causing progressive cognitive dysfunction starting around 12 months of age. Here we investigated if 670nm light therapy could rescue cognitive and motor dysfunction and reduce amyloid and tau pathology in 3xTg-AD mice. **METHODS** 14-to-18-month-old 3xTg-AD mice were treated with continuous-wave 670nm LED light applied directly to the head for 90s per day, 5 days per week for 4 weeks. 3xTg-AD sham and WT control animals were held under the LED device with the light turned off for the same amount of time. Animals were tested for working memory in the Y maze spontaneous alternation task and short-and long-term memory in the novel object recognition task during the last 2 days of treatment. At the completion of behavioral testing on treatment day 20, brains were collected for western blot analysis of total and phosphorylated tau, and immunofluorescence analysis of microglial reactivity around amyloid plaques. **RESULTS** Light-treated 3xTg-AD animals showed significant short-term object recognition (DI 21.83 ± 7.96, one sample t-test $p<0.05$) at 2 hours post-training, whereas sham animals did not. Light treatment also reduced the ratio of hippocampal pTau/Tau in 3xTg-AD mice as compared to sham (0.11 ± 0.05 vs. 0.32 ± 0.13, one-way ANOVA $p=0.04$). The average area of amyloid plaque-associated microglia in the subiculum was increased in light-treated animals compared to sham (65.15 ± 8.71μm^2 vs. 48.99 ± 5.87 μm^2). **CONCLUSIONS** Here we show rescue of hippocampal-dependent memory, reduction of Tau phosphorylation in the hippocampus, and enhanced microglial reactivity to amyloid plaques in 3xTg-AD mice using a non-invasive treatment with a commercially available 670nm LED light source. Clinical trials have shown short-term improvements in cognition, sleep, and memory performance following PBM therapy in dementia patients. Our data suggest that treatment with 670nm red light could significantly improve cognitive function in AD patients and reduce the propagation of pathological amyloid and tau by stimulating a neuroprotective immune response.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.04

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

Support: FDC Foundation
Medicine by Design

Margaret and Howard Gamble Research Grant
Meredith and Malcolm Silver Scholarship

Title: Focused ultrasound-mediated blood-brain barrier modulation as a therapeutic strategy to enhance oligodendrogenesis

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Abstract: INTRODUCTION: Alzheimer disease (AD) is characterized by the deposition of β-amyloid peptides (Aβ) and tau-containing neurofibrillary tangles, ultimately resulting in neurodegeneration and cognitive impairment. Evidence suggests that Aβ leads to impairment of the lipid-rich myelin sheath and oligodendrocyte (OL) degeneration in the AD brains of humans and transgenic mouse models. Pharmacological strategies enhancing oligodendrogenesis have been shown to promote cognition in mouse models of amyloidosis. However, these approaches do not impact other pathologies of AD (e.g. tau, Aβ, neuroinflammation). Blood-brain barrier (BBB) modulation by magnetic resonance image-guided focused ultrasound in the presence of intravenous microbubbles (MRIgFUS) has been shown to promote regeneration by increasing the plasticity of neurons, astrocytes, and microglia. To date, the effects of MRIgFUS on OLs and their precursors remains unknown, and this knowledge is essential for the establishment of MRIgFUS as a multi-modal therapy to address the complex pathologies of AD. We hypothesise that MRIgFUS-BBB modulation enhances oligodendrogenesis and myelination.
METHODS: MRIgFUS was targeted to the hippocampus and striatum of adult mice. Using standard immunohistochemical procedures, we investigated the proliferation of oligodendrocyte progenitor cells (OPCs) and their differentiation into mature OLs. The activation of glial cells, including astrocytes, is known to influence OPCs and OLs through the secretion of cytokines, chemokines, and signalling molecules. Thus, we evaluated astrocytic activation following MRIgFUS with a machine learning workflow, MORPHIOUS.
RESULTS: At 30-days post-MRIgFUS, oligodendrogenesis, defined by newly dividing OPCs maturing into myelinating OLs was promoted by 5- and 6-fold in the hippocampus and striatum, respectively. FUS-induced proliferation of OPCs in the hippocampus peaked to 5- and 2-fold at 1 and 4-days post-MRIgFUS, respectively, and there was a 5-fold increase in total number of OPCs generated between 1 and 7 days post-FUS in the striatum. Finally, we explored the relationship between astrocytic activation and OPC cell proliferation to identify multifactorial effects induced by MRIgFUS-BBB modulation.
CONCLUSION: Our results support the hypothesis that MRIgFUS-induced BBB modulation can serve as a non-invasive strategy to promote oligodendrogenesis. The non-invasive nature of MRIgFUS, combined with its established effects reducing Aβ pathology and increasing hippocampal neurogenesis, positions MRIgFUS as a multi-modal approach addressing multiple aspects of AD in a single treatment.

Disclosures: K. Noseworthy: None. **J. Silburt:** None. **R.H. Kofoed:** None. **K. Hynynen:** None. **I. Aubert:** None.

Poster

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

Support: CIHR
CGS-M, CIHR
Scace Fellowship in Alzheimer's Research
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FDC Foundation

Title: Dysregulation of nerve growth factor homeostasis in a mouse model of amyloidosis

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Abstract: Alzheimer disease (AD) is the most common form of dementia worldwide and disease modifying therapeutics are currently lacking. The degeneration of basal forebrain cholinergic neurons (BFCNs) underlies many debilitating AD symptoms. BFCNs rely on the retrograde axonal transport of cortical and hippocampal mature nerve growth factor (mNGF) and its precursor (proNGF) for their survival and function. The adequate regulation of the levels of mNGF, proNGF, and their associated receptors dictates whether pro-survival or degenerative pathways are engaged. In human AD pathology, proNGF levels are increased, and signaling dynamics shift from neurotrophic to degenerative. Therefore, establishing rodent models that present with proNGF/mNGF dysregulation will assist and accelerate the development of therapies targeting degenerative processes in central cholinergic systems. This study aimed to characterize a mouse model of amyloidosis with trophic factor receptor imbalances (Xhima et al., 2020) for potential preclinical development of treatments targeting proNGF/mNGF homeostatic dysregulation. Transgenic (Tg) mice expressing a pathologic variant of human amyloid precursor protein (APP) and non-Tg (nTg) controls were aged to 4, 6, 8, and 12 months (mo) to reflect stages of disease progression. Two BFCN nuclei: the nucleus basalis of Meynert (nbM) and the medial septum; and their respective projection targets: the cortex and the hippocampal formation, were collected. mNGF and proNGF ELISAs were used to determine protein levels in these regions. Relative to age-matched nTgs, Tgs had increased proNGF levels in both the medial septum and hippocampal formation at 12 mo. mNGF levels, on the other hand, were increased in these regions only at 8 mo. No significant differences in mNGF or proNGF were detected in the nbM, while cortical mNGF levels were elevated in 8 mo Tgs. The data show region-specific differences in proNGF/mNGF dysregulation in this mouse model. In the septohippocampal pathway of Tg mice, an increase in proNGF at 12 mo concurrent with an increase in mNGF at 8 mo could indicate age-related overproduction of the precursor, changes in post-translational processing, and/or alterations in axonal transport. By contrast, the increase of cortical mNGF levels alone at 8 mo in the basalocortical pathway may indicate a compensatory

response in protein processing or production that is affected by worsening pathology. Such an increase could also indicate dysregulated axonal transport beginning at the 8 mo time point. Our findings support the use of this amyloidosis mouse model for future development of therapies targeting proNGF/mNGF dysregulation.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.06

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

Support: NIH 1R41NS092221-0181
NIH 1R01 NS088766
R21NS096044
Curesanfilippo Foundation

Title: Genetically corrected iPSC-derived Neural Stem Cell Grafts deliver NAGLU-IGFII fusion protein to affect CNS disease in Sanfilippo B Mice

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Abstract: Sanfilippo syndrome type B (Mucopolysaccharidosis type IIIB, MPS IIIB) is a recessive genetic disorder that severely affects the brain and is caused by a deficiency in the enzyme α-N-acetylglucosaminidase (NAGLU), leading to intralysosomal accumulation of partially degraded heparan sulfate. There are no effective treatments for this disorder. We carried out ex vivo, lentiviral correction of neural stem cells derived from Naglu−/− mice (iNSCs) using a modified NAGLU in which the enzyme is fused to an IGFII receptor binding peptide in order to improve NAGLU cross-correction. Following the long-term effect of brain transplantation of these corrected iNSCs into Naglu−/− mice, we detected NAGLU-IGFII activity in all engrafted animals. Treated Naglu−/− mice showed rescue of NAGLU activity, a 1.7 fold decrease in storage material accumulation ($p = 0.0080$), a 2.2 fold reduction in astrocytosis ($p = 0.0007$) and a *1.84-fold* reduction of microglial activation ($p = 0.0193$), throughout the transplanted brain compared with non-treated Naglu−/− brains. We also identified novel neuropathological phenotypes in untreated mutant mice involving a decrease in MAP2 protein and the accumulation of synaptophysin-positive aggregates. Following transplantation, MAP2 expression was restored in Naglu−/− mice ($p =0.0017$) and a significant reduction of synaptophysin-positive aggregate formation were observed (average ± SD vs average ± SD, in

non-treated animals, p = 0.0146). Our results demonstrate the feasibility and long-term benefit of virally-corrected iNSCs engraftment in the brain and effective cross-correcting disease-associated pathology in Naglu−/− mice. Our findings suggest that genetically engineered iNSCs can be used to effectively deliver active cues and treat Sanfilippo type B-associated neuropathology.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Topic: B.09. Glial Mechanisms

Support: NIH Grant 1R01NS126415-01
Weatherhead Foundation
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Title: Mutant KRAS in endothelial cells drive inflammatory responses in a mouse model of brain arteriovenous malformation

Authors: M. LEE[1], S. M. WILFUR[1], S. HUANG[2], J. KÖRBELIN[4], J. P. J. SAVARRAJ[1], H. A. CHOI[1], S. P. MARRELLI[3], J. A. ARONOWSKI[3], P. R. CHEN[1], E. KIM[1], ***E. S. PARK**[1]; [1]Dept. of Neurosurg., [2]Dept. of Diagnos. and Interventional Imaging, [3]Dept. of Neurol., McGovern Med. School, The Univ. of Texas Hlth. Sci. Ctr. at Houston, Houston, TX; [4]II. Dept. of Intrnl. Med., Ctr. of Oncology, Univ. Med. Ctr. Hamburg-Eppendorf, Hamburg, Germany

Abstract: Brain arteriovenous malformations (bAVMs) are tangled blood vessels formed by direct connection of arteries and veins. Although bAVMs cause intracerebral hemorrhage (ICH), the inciting pathophysiology leading to ICH is unknown. Studies have shown that the presence of a bAVM is highly correlated with local inflammation of intranidal and surrounding brain parenchyma. Immunohistochemistry studies have identified dense concentrations of brain resident microglia and infiltrated macrophages surrounding bAVMs, suggesting microglia/macrophages (MΦ) are the prime drivers of bAVM-related inflammation. However, the underlying mechanism of MΦ-mediated inflammation in bAVM has not been studied. To interrogate the mechanisms of bAVM-associated inflammation, we established a pre-clinical bAVM mouse model based on the clinical observation of somatic *KRAS* mutations in human bAVM. We overexpressed the *KRAS* mutant (p.Gly12Val, $KRAS^{G12V}$) in brain endothelial cells (ECs) ($KRAS^{G12V/bEC}$ mice) using an EC-specific adeno-associated virus (AAV-BR1, Retro-orbital venus sinus injection). Our mouse model recapitulates key human bAVM pathology, including tangled/snarled vasculature and the occurrence of spontaneous ICH whereas AAV-

BR1-eGFP shows no bAVM/ICH. Using this model, we have observed that inflammation underlies mechanisms of bAVM-associated ICH. First, brain regions around unruptured bAVMs show a high number of Iba1+ MΦ compared to the intact (non-bAVM) in $KRAS^{G12V/bEC}$ mice at 4 and 8 weeks after AAV-BR1-$KRAS^{G12V}$ and unruptured human bAVMs. Second, in the resected bAVMs from $KRAS^{G12V/bEC}$ mice and in cultured EC carrying mutant KRAS, mRNA levels of pro-inflammatory IL-6/IL-1β/TNF-α and proteolytic enzymes (MMP-2/9) are robustly increased. Moreover, *in vitro* studies show that normal microglia cultured with conditioned medium from $KRAS^{G12V}$-transfected ECs enhance the expression of markers for microglial activation states (IL-6, IL-1β, MMP-9, and Csf1r). Subsequently, normal ECs cultured with conditioned medium from activated microglia show decreased mRNA levels of adherens junction (Cdh5), a marker of blood-brain barrier integrity. Therefore, *in vivo* and *in vitro* studies suggest that mutant KRAS in ECs attracts the MΦ around bAVMs in the $KRAS^{G12V/bEC}$ mouse and substantiate the influence of MΦ in local inflammation that promotes bAVM rupture and ICH. The study will impact the development of new targeted pharmacological therapies to prevent inflammation-associated bAVM rupture in bAVM patients.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.08

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

Support: FRQS
CIHR
HBHL

Title: Alterations in the brain structure of the triple-transgenic (3xTgAD) mouse model of Alzheimer’s disease on a high-fat diet and the effect of two intervention strategies: Exercise and a low-fat diet

Authors: C. L. GARCÍA[1], C. ANASTASSIADIS[1], M. PARK[2], M. UROSEVIC[3], D. R. GALLINO[2], G. A. DEVENYI[2], M. CHAKRAVARTY[2,4];
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Abstract: Modifiable cardiovascular risk factors, such as hypertension, obesity, and cardiovascular diseases increase the risk of developing Alzheimer’s disease (AD). Conversely, lifestyle habits that include frequent exercise and a healthy diet are considered to decreased AD-

risk (Mayeux, R., & Stern, Y., 2012). Given the absence of pharmacological studies for AD-treatment, there is an urgent need to determine how lifestyle interventions can be used to decrease AD-prevalence. . Here we examine the impact of diet and exercise on the brain anatomy of the triple-transgenic (3xTgAD) mouse model of AD fed with a high-fat diet.All mice were fed on a control diet (12% fat) for the first two months of age. At two months of age, a group of mice were assigned to the low-fat diet group (LFD, 10% fat); the rest of the mice started to be fed with a high-fat (60% fat) diet until four months of age. From four to six months of age, mice on a high-fat diet were randomly assigned to one of the following groups: Exercise (HFD+EX), Diet (HFD+D), or no-intervention (HFD). The mice in the HFD+D group were fed a low-fat diet, the mice in the HFD+EX group were given free access to a wheel in their cage without a change in their diet, and mice in the HFD group continued on the high-fat diet. Structural T1-weighted Magnetic Resonance Imaging protocols were used to obtain brain scans at two, four, and six months of age. To study voxel-wise trajectories, Deformation Based Morphometry data were analyzed with Linear Mixed-Effects models, followed by False Discovery Rate correction.We characterized the impact of the genotype by comparing 3xTgAD mice against their wild-type counterpart, we found significant differences in the olfactory bulb, hippocampus, thalamus, and cerebellum. We found that mice in the HFD had a significant volumetric decrease in the striatum, cortex, entorhinal area, brain stem, and cerebellum, compared to LFD mice. HFD+EX had a significant volumetric increase in the striatum, and cerebellum, compared to the HFD mice. HFD+D mice had a significant volumetric increase in olfactory bulbs, cortex, brain stem, entorhinal area, and cerebellum, compared to the HFD mice. These results suggest that interventions positively impact several brain structures, further studies are needed to assess the cellular basis of these changes.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.09

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

Support: ICMR Grant 45/7/2019-PHY/BMS

Title: Effect of low intensity, short duration magnetic field exposure on Alzheimer’s disease progression in Streptozotocin induced rat model

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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disease and begins long before any symptoms become apparent. Various non-invasive therapies propose a promising alternative approach to traditional pharmacological interventions for AD. The present study aims to unravel the effect of low intensity, short duration magnetic field stimulation on AD disease progression in STZ rat model. Adult Male Wistar rats were taken as study model and divided into three groups: Sham, AD and AD+MF. For development of AD, STZ was injected intracerebroventricularly, at a dose of 3mg/kg body weight stereotaxically. AD+MF animals were exposed to low intensity, whole body magnetic field from 8^{th} day of STZ treatment till 15^{th} day. Passive Avoidance Task and novel object recognition task were used for cognitive assessment and lesion volume was assessed by cresyl violet staining. BrdU immunohistochemistry was done to detect the differentiation and proliferation of neuronal stem cells present in hippocampal tissue. MF exposure had beneficial effect on memory retention as evident by increase in step down latency (94±14) in comparison to AD rats (45±23). Moreover, when the novel object was tested 24 hrs. after training, we found that AD animals spent significantly less time exploring the novel object ($p = 0.005$) and more time exploring the familiar object ($p< 0.001$). Histological analysis revealed increase in the number of healthy, viable neurons after MF treatment in CA3 and CA1 sub-regions of hippocampus (P value < 0.001; P value < 0.01 respectively). An overall reduction in cell proliferation and neurogenesis ($P<0.001$) was observed after streptozotocin administration in AD animals in comparison to Sham group. We observed a significant increase ($p<0.01$) in the expression of proliferating neuronal cells (BrdU+ cells) following MF exposure in subgranular zone of dentate gyrus as compared to AD group. The brief exposure duration of 7 days worked as a physical stimulus for enhancing adult neurogenesis. Electrocorticogram from frontal and occipital lobes showed significantly reduced delta power of EEG waves in AD rats ($p<0.001$) in comparison to sham. While MF exposure for short duration of 8 days only, increased the wave power in comparison to AD ($p<0.01$). Present findings suggest that magnetic field stimulation has the ability to ameliorate the cognitive deficits in STZ rat model of AD by attenuating neurodegeneration or neuronal cell loss which is also reflected in altered cortical activity. Magnetic field stimulation is non-invasive strategy and has potential therapeutic value in treating progression of Alzheimer's disease as earlier as symptomatic disease occurrence.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.10

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

Support: JSPS KAKENHI Grant Number 17H05080

Title: Chronic dural port method for a large-volume CSF collection in behaving mice: a novel platform for preclinical biomarker research.

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Abstract: Cerebrospinal fluid (CSF) reflects the pathophysiological changes in the central nervous system (CNS) and has been used for biomarker research for CNS disorders. The CSF obtained from relevant mouse models can be an important resource for successful biomarker and drug development. However, the limited volume of CSF that can be collected from the tiny intrathecal space has been bottlenecks that have hindered the detailed analysis of CSF in mouse models. We developed a uniquechronic dural port (CDP) method in which intrathecal cannulation is not required for CSF collection. This method allows easy and repeated access to the intrathecal space in a behaving, unanesthetized mouse, thereby enabling continuous long-term CSF collection with minimal tissue damage and providing a large volume of high-quality CSF from a single mouse. The CDP method allows real-time monitoring of the dynamic changes in neurochemicals in the CSF at a one-second temporal resolution when combined with high-sensitive chemical biosensors. The CDP provides a direct access point for intrathecal injection of CSF tracers and drugs. Direct intrathecal delivery of drugs via CDP enabled real-time behavioral assessment in free-moving mice. The CDP method, combined with relevant animal models, provides a valuable platform for biomarker research.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.11

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

Support: NIH Grant R01AG068215
pilot funds from the UK Department of Neuroscience

Title: Effect of Thermoneutral temperature exposure in mouse models of Alzheimer’s Disease

Authors: *J. WANG[1], D. IRADUKUNDA[1], D. HUFFMAN[1], C. MCLOUTH[2], M. MURPHY[3], B. F. O’HARA[4], A. BACHSTETTER[5], M. J. DUNCAN[5], S. SUNDERAM[1];

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Abstract: There is growing evidence that disordered sleep, which is known to be associated with Alzheimer's disease (AD), may accelerate neuropathology, thus promoting a vicious cycle. Here we investigate the hypothesis that improving sleep through ambient temperature regulation may slow disease progression in two mouse models of AD. First, female 3xTg-AD mice (~13-14 m.o.; n=16) were instrumented for EEG/EMG monitoring to score sleep. The mice were exposed to a photoperiod of 12 h light:12 h dark. After a week-long baseline recording, one half of the mice (EXPT) were exposed every day to a diurnal cycle of ambient cage temperature (Ta), in which the Ta underwent stepwise increases of 1 °C each hour beginning at lights-on from 22 to 30°C (thermoneutral for mice) followed by stepwise hourly decreases back to 22 °C. The remaining mice constituted the control (CTRL) group which was constantly exposed to 22°C. Vigilance state - i.e., Wake, REM, NREM, and slow wave sleep (SWS) within NREM - was scored in 4-second epochs from the EEG/EMG and sleep metrics computed. SWS was significantly elevated ($p<0.05$) in the light phase for EXPT mice (peak SWS increased to 15% compared to 7% in CTRL) while the total sleep time, NREM, REM, and wake were unchanged. After four weeks of treatment, the animals were euthanized, and the brains removed to assay amyloid-beta (Aβ) levels by ELISA. We found that both Aβ40 and Aβ42 were significantly reduced in the hippocampus(135 in CTRL, 90 in EXPT), but not in the cortex, for the EXPT group. These data imply that thermoneutral warming might have some regional specificity in its effects, with implications for the cognitive and neuropathologic changes found in AD. In a second (ongoing) study we further seek to test whether the effect of thermoneutral warming on amyloid pathology is mediated by sleep changes rather than other non-specific physiologic effects of temperature. Through these experiments, we aim to establish a model for sleep enhancement in order to investigate its effects on AD-related neuropathology.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.12

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

Title: The neuropathological effects of zinc and copper supplementation on a dual transgenic (hAPP/hTau) mouse model of Alzheimer's disease

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Abstract: An imbalance of metals in the brain has been implicated in Alzheimer's disease (AD) neuropathology and behavioral deficits in both mice and humans. Previous studies have shown that excess zinc supplementation exacerbated AD neuropathology and behavioral deficits. Elevated zinc leads to a copper deficiency, which may play a role in the impairments. This study investigated the neuropathological effect of zinc+copper supplementation on a dual transgenic (hAPP/hTau) mouse model of AD. Starting at 8 weeks of age, transgenic AD and wild-type (WT) mice were provided either standard lixit, zinc, or zinc+copper water *ad libitum*. The relative density of AD-related proteins (total tau, phosphorylated tau, and glial fibrillary acidic protein (GFAP)) in the brain were semi-quantified via western blotting. Free zinc, amyloid plaques, and tau tangles per brain region were assessed through the histological stains Zinpyr-1, Congo Red, and Thioflavin-S, respectively. Western blotting suggested that AD mice had higher levels of the inflammatory marker GFAP in the brain than WT mice ($p < .001$). Zinpyr-1 staining revealed that AD mice showed reduced levels of free zinc in the brain compared to WT mice ($p = .041$). Congo Red staining showed that AD mice had significantly greater numbers of amyloid plaques in cortical regions (cortex above the hippocampus and infralimbic cortex) compared to hippocampal regions DG ($p = .038$), CA3 ($p = .020$), and CA1 ($p = .007$). Thioflavin-S staining showed that AD mice also had significantly greater numbers of tau tangles in cortical regions (cortices above and lateral to the hippocampus and infralimbic cortex) than hippocampal regions DG ($p < .001$), CA3 ($p = .032$), and CA1 ($p < .001$). AD mice provided zinc+copper water showed fewer amyloid plaques than those on standard lixit water in hippocampal regions (CA3, $p = .030$, and CA1, $p = .027$) and cortices surrounding the hippocampus (cortex above, $p = .045$, and lateral cortex, $p = .040$). AD mice on zinc+copper water also had significantly less plaques than those on zinc water in the cortex lateral to the hippocampus ($p = .042$) and infralimbic cortex ($p = .037$). Paradoxically, AD mice on zinc+copper water showed greater tau tangles than those on standard lixit ($p = .019$) and zinc ($p = .025$) water in the dentate gyrus. This finding was paralleled with AD mice also showing greater tangles than those on standard lixit ($p = .022$) and zinc ($p = .033$) water in the cortex above the hippocampus. These findings suggest that zinc+copper supplementation may ameliorate amyloid pathology but exacerbate tau pathology in AD mice.

Disclosures: R. Hoyos Justiniano: None. **T.N. Gervase:** None. **A.B. Booth:** None. **R.E. Barkey:** None. **E.Q. Murdoch:** None. **D. Park:** None. **S. Neff:** None. **J.M. Flinn:** None.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.13

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

Support: NIH Grant R01 AG057767
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Dale and Deborah Smith Center for Alzheimer's Research and Treatment
Kenneth Stark Endowment

Title: Chronic Hypothermic Environmental Temperature does not Ameliorate Cognitive Decline in APP/PS1 Mice

Authors: S. A. MCFADDEN[1], M. R. PECK[1], C. A. FINDLEY[1], K. QUINN[1], Y. FANG[1], A. BARTKE[2], E. R. HASCUP[1], ***K. N. HASCUP**[1];
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Abstract: Metabolism declines with age causing glucose dysregulation and insulin insensitivity that impairs cognition and contributes to Alzheimer's disease (AD) progression. We have shown that amyloidogenic APP/PS1 mice develop impairments to insulin sensitivity and glucose homeostasis that contribute to their cognitive deficits at 12 months of age. Mechanisms shown to extend healthy aging may ameliorate or delay cognitive decline. Exposure to a hypothermic environmental temperature (eT) leads to adaptive responses to improve metabolism, but is also detrimental to working, short-term, and long-term memory. We hypothesized that chronic exposure to mild hypothermia would improve the metabolic profile of APP/PS1 mice, but not cognition. Starting at 6 months of age, male and female APP/PS1 and sex-matched littermate C57BL/6 mice were chronically housed in a 16°C eT for 6 months. At 12 months, their metabolic profile was assessed using an insulin and glucose tolerance test followed by cognitive performance on the Morris water maze spatial navigation task. Plasma, hippocampal, and peripheral (adipose and hepatic) samples were procured postmortem and tissue-specific markers of amyloid accumulation and metabolism were assayed. Although a hypothermic eT normalized insulin sensitivity, glucose tolerance and cognition were still impaired in both sexes of AD mice. Plasma FGF21 levels were decreased in both sexes, but additional sexually dimorphic mechanisms contributed to the impaired glucose homeostasis in AD mice. Females had higher plasma glucagon-like peptide 1 and hepatic glucose transporter 2 expression. In males, hepatic insulin receptor as well as visceral adipose tissue adiponectin receptor 1 and PGC-1α expression were all reduced. These indicate reduced insulin signaling mechanisms and lower thermogenic responses to hypothermic eT, respectively. Soluble hippocampal amyloid-β_{42} levels were unchanged. Improving peripheral insulin sensitivity is insufficient to ameliorate or delay the progression of cognitive decline in AD. Additional methods to modulate blood glucose levels may be warranted. Furthermore, this data suggests a dichotomy between mechanisms to improve metabolic function and cognitive health that may be further impaired in AD.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.14

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

Support: discretionary funds of the President of the University of Toyama

Title: Hemopexin, newly identified as unbeneficial myokine, mediates skeletal muscle atrophy-induced cognitive impairment

Authors: *T. IKI[1], C. TOHDA[2];
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Abstract: Physical inactivity is one of risk factors for Alzheimer's disease (AD). Performing physical exercise is difficult at old age, and thus, decline in physical movement may be a cause of age-associated lowering of the brain function. This study aimed to elucidate the onset of the skeletal muscle atrophy-induced acceleration of AD and its molecular mechanism.Pre-symptomatic young AD model mice (5XFAD) or non-transgenic wildtype mice were used. The bilateral hindlimbs were immobilized by cast-attachment for 14 days. Two weeks after the cast immobilization, wet weight of tibialis anterior and triceps surae were significantly lower in cast-attached 5XFAD mice than those in non-cast mice. At the same time, object recognition memory in the cast-attached 5XFAD mice was impaired although in age-matched wildtype and non-cast 5XFAD mice showed normal memory function. The hindlimb muscles were isolated for organ culture. Conditioned media (CM) of each muscle was separated by 2D-PAGE and analyzed by MALDI-TOF MS. Eighty-eight spots were differentially expressed in muscle CM. The most increased spot in the cast-attached 5XFAD muscle CM was hemopexin. Hemopexin levels in the skeletal muscle, plasma, and hippocampus were increased in cast-attached 5XFAD mice. Continuous i.c.v. infusion of hemopexin for 2 weeks induced memory deficits in young 5XFAD mice without casting. Gene microarray analysis of the hippocampus was performed to investigate the molecules involved in the accelerated memory deficit. Lipocalin-2 (Lcn2) mRNA, neuroinflammation-associated factor, was increased in the hippocampus in hemopexin-infused 5XFAD mice compared to in control mice. LCN2 protein in the hippocampus was localized in the neurons, but not glial cells. Lcn2 mRNA levels in the hippocampus were also increased by cast-immobilization of the hindlimbs.These findings provide new evidence indicating that skeletal muscle atrophy has an unbeneficial impact on the occurrence of memory impairment in young 5XFAD mice, which is mediated by skeletal muscle atrophy-driven hemopexin.

Disclosures: T. Iki: None. **C. Tohda:** None.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 040.15

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

Support: NIH Grant UG3NS115608
NIH Grant 5P20GM121310

Title: Functional evaluation of AAV intracerebroventricular delivery of the CTR repressor in adult mice lacking TDP-43 in forebrain neurons

Authors: *Y. LI[1], **R. THAPA**[1], T. CAO[2], R. LIU[1], G. LEE[2], P. WONG[2];
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Abstract: TAR DNA binding protein 43 kDa (TDP-43) is a conserved DNA/RNA binding nuclear protein whose major function is splicing repression of cryptic exon during RNA processing. Mislocalization of TDP-43 is commonly found in Alzheimer's Disease Related Dementia (ADRD) such as frontotemporal dementia (FTD) and mixed etiology dementia such as Alzheimer's disease (AD) with TDP-43 pathology. Multiple lines of evidence support the notion that loss of TDP-43 splicing repression contributes to neuron loss and cognitive deficits in FTD and AD with TDP-43 pathology. A chimeric repressor, CTR that fused the N-terminal fragment of TDP-43 with a well-characterized splicing repressor domain to replace the C-terminal fragment of TDP-43, has been found to mimic splicing repression function of TDP-43 to prevent the incorporation of cryptic exons in the transcripts of cultured cells. In addition, perinatal delivery of AAV9-CTR rescued the pathophysiology and motor deficits in mice lacking TDP-43 in motor neurons. In our present study, we intracerebroventricularly (ICV) delivered AAVPhP.eB-CTR in adult mice lacking TDP-43 in forebrain, to evaluate the rescue efficacy of CTR at neuronal activity and behavioral levels. We performed miniscope *in vivo* calcium imaging to monitor the neural calcium activity in awake, behaving mice and demonstrated that AAVPhP.eB-CTR delivered at 6-month-old age, efficiently rescued the aberrant calcium activity in the forebrain neurons lacking TDP-43. We also demonstrated that while TDP-43 forebrain depleted mice displayed remarkable cognitive behavior deficits as accessed by social behavior test and novel object recognition tests, CTR treatment efficiently rescued these behavioral deficits in TDP-43 forebrain depleted mice. These observations support utilizing AAVPhP.eB-CTR delivery system as an effective therapeutic strategy for the treatment of TDP-43 pathology linked AD and ADRD.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.16

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

Title: Neurometabolite profile by MR spectroscopy in aged rodents: a study in Sprague-Dawley and Wistar rats, and C57Bl/6J mice

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Abstract: Cognitive decline and Alzheimer's disease (AD) are closely related to aging and the need for translational biomarkers is of crucial importance. We have previously shown age-related 1H-MRS phenotype in female Fisher rats (22-24 months; Neurobiol Aging 2014: 2134-46) with reduced N-acetyl aspartate (NAA), GABA and glutamate, and increased myo-inositol together with other AD-like biomarkers (increased CSF levels of AbPP and Tau, defect in Morris Water Maze). The aim of this study was to explore if similar 1H-MRS phenotype is present in other strains of aged rats and mice to allow easier access to cohorts of aged rodents and enable utilization of different strains to model pathology.
Eighteen male Sprague-Dawley rats (n=10/8, 4/24 months), 24 male Wistar rats (n=12/12, 3.25 /21.75 months) and 23 male C57Bl/6J mice (n=12/11, 4.75/23 months) were used. Hippocampal MRS was performed at 11.7T magnet using PRESS sequence (TE/TR = 10/2000 ms, 512 averages) and metabolites were analyzed using LCModel (Provencher, S. (2009) LCModel and LCMgui User's Manual, http://s-provencher.com/lcmodel.shtml).
Although the evaluation was done at slightly different ages, all three models shared a similar phenotype in hippocampal metabolites, i.e. decreased GABA and glutamate, and increased glutamine and myo-inositol. NAA was significantly reduced in aged Wistar rats and C57Bl/6J mice whereas the SD rats showed only non-significant trend of decrease. In a systematic meta-analysis of human data (63 studies, 3271 subjects; Frontiers Aging Neurosci, 2021, article 738971) it was found that increased myo-inositol/NAA ratio could serve as a biomarker for progression from MCI to AD, and severity of AD. We did observe this particular ratio increasing in aged rodents studied here, but also in separate studies with APPSwDI(+)/(+)mNos2(-/-), 5xFAD and Tg4510 AD mice.
Based on the current data, the MRS phenotype is remarkably repeatable in aged rats (SD, Wistar, Fisher) and in aged C57Bl/6J mice. Furthermore, the pattern of increased myo-inositol with decreased levels of glutamate and NAA is descriptive of human AD. Aged rodents can therefore be useful natural models to assess the novel therapies in age-related cognitive decline and AD.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.17

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

Support: National Institute on Aging Grant (R01AG061447)
Eagle UPENN collaborative fund #578808

Title: Effects of intranasal dantrolene on cognitive function in P301S (line PS19) tau pathology mice

Authors: R. VERA[1], N. HONG[1], G. LIANG[1], V. BROWNE[2], V. CHELLARAJ[2], N. DUFF-DESANTIS[2], D. GISEWHITE[2], M. GREENBERG[2], S. RANJAN[2], G. ZHU[2], *H. WEI[1];
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Abstract: This study investigates whether intranasal administration of Eagle Research Formulation of Ryanodex (ERFR), a dantrolene formulation, is neuroprotective in tau pathology mice (P301S, Line PS19). First, we compared the bioavailability of intranasal ERFR in young adult (2 month) and aged (9 months) C57BL/6J male mice. Brain to plasma concentration ratios were studied as a measure of ERFR's potential for CNS therapeutic efficacy and unwanted side effects in relation to the effects of aging. Mice received a single intranasal dose of ERFR equivalent to 5 mg/kg of dantrolene. The concentration of dantrolene in the plasma, obtained from either the left ventricle or chest cavity, and brain were measured via High Performance Liquid Chromatography at 20 minutes following intranasal administration. Compared to young adult mice (N=10), a Two-Way ANOVA revealed no significant differences in brain concentrations, but a significantly lower plasma concentration in aged mice (N = 9) (232 ng/g aged vs. 476 ng/g young, $P<0.01$). The brain to plasma dantrolene concentration ratio trended comparatively higher in aged mice. These results suggest a potential for ERFR to have therapeutic effects but fewer side effects if used chronically for the treatment of AD, a disease most common in an aged population. To study the effect of intranasal ERFR on cognitive function, WT or PS19 mice were tested at either 6 or 11 months of age on the Fear Conditioning and Y-Maze tasks in order to assess contextual and working memory. Four separate cohorts of mice were tested and compared to WT counterparts (M:F=1:1). There were no significant differences on any measure of memory between PS19 (N=44) and WT mice (N = 39) in either age group. Intranasal ERFR did not cause significant system side effects on motor or smell function. Acrolein, a metabolite abnormally increased in the brains of AD patients, also did not worsen cognitive function in WT or PS19 mice in these tests. Our results suggest that PS19 mice may not be a reliable AD animal model to test therapeutic efficacy of new or repurposed dantrolene drug products. In the future, intranasal dantrolene drug products will be tested in a suitable animal model with significant sporadic AD (SAD) risk factors.

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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.; Eagle Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intranasal Administration of Dantrolene for Treatment of Alzheimer's Disease Serial number 62/868,820.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.18

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

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We thank the Milwaukee Institute of Drug Discovery and Shimadzu Laboratory for Advanced and Applied Analytical Chemistry, as well as NSF grant CHE-1625735 for HR spectroscopy

Title: Aberrant dopamine neuron activity in a rodent model of Alzheimer's Disease is reversed by allosteric modulation of the alpha 5 subunit of the $GABA_A$ receptor: relevance to comorbid psychosis

Authors: *N. EASSA[1,2], S. PEREZ[1,2], A. BOLEY[1,2], H. ELAM[1,2], D. SHARMIN[3], M. MIAN[3], J. COOK[3], D. LODGE[1,2];
[1]Dept. of Pharmacol. and Ctr. for Biomed. Neurosci., Univ. of Texas Hlth. Sci. Center, San Antonio, San Antonio, TX; [2]Audie L. Murphy Div., South Texas Veterans Hlth. Care Syst., San Antonio, TX; [3]Dept. of Chem. and Biochem., Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: Alzheimer's Disease (AD) is the most common neurodegenerative disease, affecting 35 million people worldwide, and annually accrues a healthcare burden of $305 billion in the United States alone. Available therapeutics target the hallmark symptom of the disease, cognitive dysfunction; however, up to half of all AD patients also suffer with comorbid psychosis, characterized by hallucinations and delusions, for which there is no viable treatment. Dopamine dysregulation that underlies psychosis is effectively targeted by antipsychotics, but they are contraindicated in elderly populations because of the risk of premature death. Further, the lack of

primary pathology in the dopamine system in and of itself suggests better therapeutic targets may be found in upstream brain regions that regulate dopamine system function, such as the hippocampus. The hippocampus is known to be hyperactive in patients with AD and in psychosis. In rodent models of psychosis, our laboratory has elucidated a multi-synaptic circuit by which hyperactivity of the ventral hippocampus (vHipp) is upstream of increased dopamine neuron population activity in the Ventral Tegmental Area (VTA), and that targeting vHipp hyperactivity reverses dopamine system dysfunction and psychosis-like behaviors. In this study, two human genes causative for AD pathology--the Swedish mutation for Amyloid Precursor Protein (hAPP) and Presenilin 1 lacking exon 9 (hPSEN1)--were expressed by a viral vector in the vHipp of male adult Sprague Dawley rats. After reaching 8 months of age, rats with vHipp AD pathology displayed augmented dopamine neuron population activity and hypersensitivity to the psychomotor stimulant, MK-801, in the locomotor activity assay, which correlated with a 26% decrease in parvalbumin-positive (PV+) inhibitory interneurons in the vHipp compared to eGFP controls. Furthermore, this aberrant dopamine neuron population activity and psychosis-like behavior was reversed by the systemic administration of a positive allosteric modulator of the hippocampus-specific alpha 5 subunit of the $GABA_A$ receptor (alpha 5 PAM), MP-III-022. These data provide a potential circuit-based mechanism underlying comorbid psychosis in AD and indicate that alpha 5 PAMs may be a novel therapeutic option.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 040.19

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

Support: Alzheimer Society
CIHR/IRSC
CRSNG

Title: Impact of ketogenic interventions on peripheral energy metabolism in the 3xTg mouse model of Alzheimer's disease

Authors: *P. MBRA[1,2,3], L. HAMILTON[3], F. PRATESI[1], A. AUMONT[1], K. FERNANDES[1,2]; [1]Neurologie, Univ. de Sherbrooke, Sherbrooke, QC, Canada; [2]Gérontologie, Ctr. de recherche sur le vieillissement, Sherbrooke, QC, Canada; [3]Neurosciences, Univ. de Montréal, Montréal, QC, Canada

Abstract: Metabolic abnormalities involving both glucose and lipids are key features of Alzheimer's Disease (AD). Since ketones are an alternative substrate for cellular energy, and lifestyle factors such as diet substantially influence AD development, we investigated the impact of dietary ketogenic interventions on the metabolic responses of the 3xTg-AD mouse model. Young adult 3xTg-AD and B6;129 strain controls were administered a carbohydrate-rich control diet (CD, 70% carbohydrate, 20% fat, 10% protein), a similar CD that was supplemented with a medium-chain triglycerides (MCT, a ketogenic substrate), or an extreme carbohydrate-free ketogenic diet (KD). After 6 months on these diets, mice were submitted to a variety of longitudinal (blood sampling, EchoMRI, metabolic cages,) and terminal (tissue analyses, gene expression) measures of peripheral energy metabolism. We found that 3xTg and control mice on the CD exhibited baseline differences in body weight evolution, food intake, lean/fat mass body composition, blood glucose and ketone levels, and responses in glucose and insulin tolerance tests. Peripheral metabolic parameters were differentially affected by the MCT and KD diets: while mice on the more extreme, carbohydrate-free KD underwent a global shift towards lipid energy metabolism and displayed a far stronger increase in circulating ketones, only mice on the MCT diet showed evidence of improved peripheral energy metabolism. Interestingly, ongoing analyses suggest that 3xTg and control differ significantly in their metabolic responses to such dietary challenges. Our findings suggest that AD can trigger defects in peripheral energy metabolism that alter the response to dietary challenges, implying the need for precisely targeted dietary interventions.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.20

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

Support: AG057768
AG059195
AG056901

Title: Perinatal choline supplementation ameliorates cognitive deficits, reduces brain amyloid pathology, and modulates hippocampal and cerebral cortical gene expression in APP^{NL-G-F} Alzheimer's disease model mice

Authors: *T. A. BELLIO, M. S. CAMPION, J. CHOU, J. Y. LAGUNA-TORRES, S. YEE, S. DEVARAJ, J. K. BLUSZTAJN, T. J. MELLOTT;
Boston Univ. Sch. of Med., Boston, MA

Abstract: Alzheimer's Disease (AD) is one of biomedical science's greatest challenges. Treatment options are limited and do little to alter the devasting outcomes of the disease. Prevention of AD has thus become a uniquely important field. Studies have shown that diets containing high amounts of the essential nutrient choline consumed either during perinatal development or throughout life, ameliorates amyloidosis, prevents cholinergic deficits, reduces gliosis, and increases neurogenesis in APP.PS1 AD model mice. Here, we have evaluated the behavioral, pathological, and gene expression effects of perinatal choline supplementation in wildtype and in APP^{NL-G-F} AD model mice of both sexes. Pregnant and lactating mice were given an AIN76A diet containing either 1.1 g/kg (control) or 5 g/kg (supplemented) of choline chloride until weaning. Subsequently, all offspring received the control diet throughout their life. Mouse behavior was evaluated in a cross-sectional manner at 3, 6, 9, or 12 months of age using Open Field, Elevated Plus Maze, Barnes Maze, and contextual fear conditioning paradigms. Following euthanasia, the right hemisphere was collected for immunohistochemical analysis, while the left hemisphere was used to dissect the cerebral cortex and the hippocampus. RNA was extracted from these brain regions and sequenced. As compared to wild-type mice, APP^{NL-G-F} mice consuming the control diet exhibited abnormal anxiety-related behavior and choline supplementation alleviated some of these abnormalities. Additionally, APP^{NL-G-F} mice consuming the control diet were characterized by learning and memory deficits and perinatal choline supplementation ameliorated some of these deficits. Perinatal choline supplementation significantly reduced the amounts of Aβ42 deposits in the hippocampus, cortex, and amygdala at 9 and 12 months of age in APP^{NL-G-F} mice as compared to that observed in APP^{NL-G-F} mice consuming the control diet. RNA-sequencing revealed that perinatal choline supplementation dramatically alters the transcriptome of both wildtype and APP^{NL-G-F} mice. Perinatal choline supplementation showed regional effects in the brain as the expression of a different set of genes was found to be modulated in the hippocampus compared to the cortex. Together, these results add to the growing evidence that choline supplementation—either during gestation or throughout life—can modulate progression of AD-like behavior, pathology, and gene expression. Future work should focus on discovering mechanisms through which choline acts to serve these functions and could potentially lead to better informed prevention strategies and novel therapeutics.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.21

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

Title: Exploratory proteomic analysis of a transgenic Alzheimer's disease mouse model treated with young blood plasma

Authors: *A. PETRIE[1], K. PEDEMONTE[1,3], W. ZHOU[2], J. M. FLINN[1];
[1]Psychology, George Mason Univ., Fairfax, VA; [2]Ctr. for Applied Proteomics and Mol. Med., George Mason Univ., Manassas, VA; [3]Surgery, Uniformed Services Univ. of the Hlth. Sci., Bethesda, MD

Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of amyloid plaques and tau tangles. Studies suggest that treatment using young blood plasma in transgenic AD mice is associated with enhanced synaptic activity, increases in neuronal proteins, and behavioral improvements. This exploratory proteomic study used liquid chromatography–mass spectrometry (LC-MS) to analyze the composition of blood plasma for the identification of proteins that may contribute to synaptic and behavioral changes. Plasma samples were collected from AD-type (rTg4510 h-Tau P301/CaMKII) and wild-type (WT) mice treated with saline or young blood plasma. A bottom-up approach was used to identify proteins by assessing fragmentation output from LC-MS and cross-referencing values with validated mouse protein databases. Preliminary results indicated that several proteins involved in the complement cascade of the immune system were differentially expressed between saline- and plasma-treated mice. In AD mice treated with saline, complement component proteins were upregulated significantly more than saline-treated WT mice. To contrast, young blood plasma treatment downregulated complement component proteins in AD mice, to abundances comparable with healthy control mice. These findings are consistent with studies that observe chronic overactive immune systems in AD and other neurological disorders. In addition to complement cascade proteins, several other proteins involved in immune function were elevated in AD mice treated with saline. AD-saline mice exhibited a two-fold upregulation of alpha-1-antitrypsin protein when compared to WT-saline mice. Similarly, treatment with young blood plasma resulted in a two-fold downregulation of alpha-1-antitrypsin protein abundance for AD mice when compared to those treated with saline. Young blood plasma also downregulated cathepsin S isoform 1 preprotein in plasma-treated mice, when compared to both WT and AD mice treated with saline. These results suggest that chronic immune system activation may coincide with AD progression through the involvement of specific proteins and pathways, such as the complement component cascade. By targeting these specific proteins of the immune system, future studies may gain a better understanding of the molecular mechanisms that underlie AD. A further investigation on such proteins following young blood plasma treatment may illuminate the relationship between immune system function and improved outcomes in an AD mouse model.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.22

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

Support: Dissertation Completion Award

Title: The Effects of Young Blood Plasma Transfusions on Older hTau Mice Modeling Alzheimer's Disease

Authors: *K. PEDEMONTE[1,2], A. N. PERLBERG[2], R. E. TAPP[2], R. E. BARKEY[2], J. M. FLINN[2];
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Abstract: Alzheimer's disease (AD) is the most common form of dementia. The biochemical hallmarks of AD include amyloid plaques and tau tangles; symptoms include declines in spatial and episodic memory as well as deficits in activities of daily living. In this study 2-month-old and 4-month-old h-Tau P301/CaMKII (4510) mice received seven repeated 150µL intravenous tail injections of either plasma or saline over 3 weeks, taken from young 4510 noncarrier mice. One week after injections, both groups of mice were evaluated in the Morris water maze (MWM) paradigm to measure spatial memory, nest building to measure activities of daily living and circadian activity. Brain tissue was evaluated using western blot to semi-quantify hyperphosphorylated tau protein and thioflavin-S staining to measure quantity and size of tau tangles. In the mice injected at 4 months, plasma treated AD mice built significantly better nests than the AD mice injected with saline. In MWM, the 24-hour probe showed that the 4-month-old AD mice injected with plasma found the escape platform significantly faster than those injected with saline; however, there was no significant difference in latency across the days. There were no significant findings on circadian activity. Western blot analysis showed that there was significantly less phosphorylated tau in plasma treated AD mice compared to those treated with saline but were no significant differences in total tau between the animals. In contrast, the mice injected at 2 months, there were trending improvements in nesting of the plasma-transfused mice. These behavioral results were in contrast to the Hernandez (2019) findings with similar injections in 8-month-old Tau P301/CaMKII mice that showed no behavioral difference but significant decreases in phosphorylated tau. These findings indicate that the timing of plasma injections is important in reducing behavioral deficits. Future studies will include mass spectrometry to identify differences in proteins between plasma and saline treated groups. This study provides support for young, healthy plasma as a possible treatment for AD.

Disclosures: K. Pedemonte: None. **A.N. Perlberg:** None. **R.E. Tapp:** None. **R.E. Barkey:** None. **J.M. Flinn:** None.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.23

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

Support: Grant support to JMF from CHSS, GMU
Grant support to ANP and RET from OSCAR, GMU
Grant support to REB from Sigma Xi

Title: Effects of repeated young blood plasma injections on nesting behavior in an hTau mouse model

Authors: *A. N. PERLBERG[1], K. A. PEDEMONTE[1,2], R. E. BARKEY[1], R. E. TAPP[1], E. Q. MURDOCH[1], J. M. FLINN[1];
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Abstract: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by tau tangles and amyloid plaques. AD symptoms include cognitive/behavioral impairments and a decreased ability to perform activities of daily living (ADL). Nesting is an innate and measurable ADL in rodents, and nesting abilities are often impaired in AD-modeling mice. To reverse these nesting deficits, transgenic AD-type hTau (rTg4510 P301/CaMKII) and wild-type (Wt) mice were treated with 150 μL of either plasma or saline injections at both 2 months and 4 months. Plasma was collected from healthy 2-month-old donor mice and injected into experimental mice over the course of seven treatments, which were repeated at both 2- and 4-months of age (i.e., repeated injections). To determine if the repeated plasma treatments were effective in reducing behavioral deficits in AD mice, nest-building was assessed at 3- and 5-months of age. Nest quality was blindly scored on a scale of 1-5. Preliminary analysis determined that, at 5 months, nesting behavior did not differ between plasma- and saline-treated mice nor between AD and Wt mice. However, when compared to the nests of 5-month-old mice from a previous study that injected plasma or saline at only four months of age (i.e., singular injections), repeated injections in both AD and Wt mice were associated with significantly better nests after a 2-hour measurement than the nests of mice that received singular injections (p = .003); AD mice with repeated injections of either plasma or saline also trended toward building better nests than the repeated injection Wt mice and singular injection mice after a 2-hour measurement (p = .068). At a 12-hour measurement, Wt mice with singular injections built significantly better nests than AD mice did (p = .002). When compared to saline injections, mice with singular plasma injections built higher-rated nests (p = .010). While repeated injections were correlated with superior nest-building for AD mice after 2 hours, it had no significant effects after 12 hours. Plasma appeared to only affect nesting behavior in the Wt mice given singular injections, suggesting that short-term young blood plasma treatment improves nesting ability in healthy mice, but does not have the same effect in Wt or AD mice given long-term treatment. However, as the AD mice given repeated injections did trend toward building better nests than the singular injection mice, additional behavioral tests and biochemical analyses of the brain will be conducted in a separate cohort that will undergo seven injections at both 2- and 4-months of age in an effort to further investigate the effects of repeated young blood plasma injections in AD mice.

Disclosures: A.N. Perlberg: None. **K.A. Pedemonte:** None. **R.E. Barkey:** None. **R.E. Tapp:** None. **E.Q. Murdoch:** None. **J.M. Flinn:** None.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.24

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

Support: Arvinas

Title: Clearance of Pathologic Proteins in Neurodegeneration by oral PROTAC® Degrader Molecules

Authors: *A. CACACE, J. MEREDITH, D. BRYCE, S. SPARKS, L. KIMMEL, K. KELLY, M. FENNELL, J. GREGORY, M. MATCHETT, D. NICKISCHER, A. HENDRICSON, G. NAUMANN, R. KYNE, R. WILSON, J. CORRADI, L. SOTO, Y. JEONG, J. PIZZANO, G. CADELINA, D. REVELL, L. SNYDER, M. BERLIN;
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Abstract: Proteins prone to aggregation (e.g. tau, alpha-synuclein, and mutant huntingtin) cause neurodegeneration and are the defining pathologic feature of proteinopathies. Our objective is to specifically target pathologic proteins genetically implicated in neurodegeneration for elimination using PROteolysis TArgeting Chimera (PROTAC®) technology. PROTAC® molecules simultaneously bind an E3-ubiquitin ligase and the target protein, thus leading to ubiquitination and targeted degradation of pathologic proteins. Previously, we and others reported tau PROTAC® molecules that degrade pathologic tau species (Silva et al., eLIFE, 2019). In these studies, tau PROTAC® molecules eliminate pathologic aggregates of tau in Frontal Lobar Temporal Dementia models that contain mutations in tau (e.g. P301L). Here, we present data characterizing these heterobifunctional small molecules that induce clearance of a number of proteins genetically implicated in neurodegeneration and neuromuscular diseases. Data will also be presented that show PROTAC molecules target mutant HTT for degradation, are on mechanism, and spare full length wild-type HTT. Importantly, we have made advances in optimization of structure activity relationships and ADME properties including oral blood brain barrier penetration across species. We show for the first time, following oral administration that PROTAC degrader molecules broadly biodistribute across the primate brain and reduce target proteins in deep brain structures anatomically involved in disease progression. These data suggest that PROTAC® and other bifunctional molecules that highjack other degradation mechanisms within the cell may represent future novel efficacious therapeutics for the treatment of neurodegenerative diseases.

Disclosures: **A. Cacace:** A. Employment/Salary (full or part-time):; Arvinas. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Arvinas, Fulcrum Therapeutics. **J. Meredith:** A. Employment/Salary (full or part-time):; Arvinas. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Arvinas. **D. Bryce:** None. **S. Sparks:** A. Employment/Salary (full or part-time):; Arvinas. **L. Kimmel:** A. Employment/Salary (full or part-time):; Arvinas. **K. Kelly:** A. Employment/Salary

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.25

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

Support: National Research Foundation of Korea (2017R1A5A1014708)
National Research Foundation of Korea (2018 R1A2B6002804)
Joint Research Project of Institutes of Science and Technology (2022)
GIST Research Institute(GRI) IIBR (2022)

Title: Vitamin D as a transcriptional regulator of amyloidopathy and gliopathy in Alzheimer's disease

Authors: *J. KANG, M. PARK, J. JUNG, T. KIM;
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Abstract: Backgroud: Alzheimer’s disease (AD) is characterized by amyloid-beta (Aβ) accumulation and memory impairment. Epidemiological studies suggest an association between lower serum vitamin D levels and increased risk of AD. Vitamin D regulates gene expression in conjunction with the vitamin D receptor, a nuclear ligand-dependent transcription factor. However, the underlying mechanism of the increased risk and the rescuing effects of vitamin D supplementation on AD remain unknown. We hypothesize that vitamin D may regulate the expression of genes related to AD pathology. **Methods:** We performed the behavioral test, Aβ ELISA, and qRT-PCR to assess the progression of AD in 5xFAD AD model mice. **Results:** First, we induced vitamin D deficiency in 5XFAD mice by providing a vitamin-D-deficient diet for 6 weeks or more. The mice showed increased Aβ load in the brain and the mRNA of genes for producing Aβ increased, and those for degradation decreased. They demonstrated suppressed expression of genes related to microglial Aβ phagocytosis. In addition, vitamin D deficiency in

the early stage of AD resulted in earlier impairment of spatial memory. Second, we administered vitamin D intraperitoneally to 5XFAD mice with a normal diet. We found lower Aβ levels and suppressed expression of genes for Aβ generation in the vitamin D supplemented mice. We also observed improved memory function, potentially associated with reduced MAO-B expression.**Conclusion:** These findings suggest that vitamin D might be a crucial disease-modifying factor that could modulate amyloid pathology. Further investigation of vitamin D as a potential preventive and therapeutic nutritional agent for AD is warranted.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

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Program #/Poster #: 040.26

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

Support: DST INSPIRE Fellowship IF180507

Title: Therapeutic effects of Hippo signaling inhibition in sporadic Alzheimer's disease: insights from in-vitro and in-vivo studies

Authors: *M. SAHU, A. MONDAL;
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Abstract: Alzheimer's disease (AD) is a form of dementia and a progressive neurodegenerative disorder with no effective therapeutics. The Hippo signaling pathway has recently emerged as a critical pathway influencing cell survival, proliferation, and apoptotic-related pathophysiological conditions. Reportedly, a hyper-activated Hippo pathway drives various neurodegenerative disorders, including AD. In this study, we aim to investigate the therapeutic potential of Hippo signaling inhibition via a chemical inhibitor, Xmu-mp-1, in streptozotocin (STZ) induced *in-vitro* and *in-vivo* models of AD. For in-vitro studies, we have cultured human neuroblastoma cell line SH-SY5Y and differentiated them into mature neuronal cells using retinoic acid treatment. Assessment of cell viability by MTT assay reveals that Xmu-mp-1 treatment reduces cytotoxicity triggered by STZ. Xmu-mp-1 treatment rescues STZ-induced neuronal apoptosis, as evident through acridine orange and ethidium bromide double staining. Further, Xmu-mp-1 also reduces reactive oxygen species generation and mitigates mitochondrial membrane depolarization under an AD background, suggesting that Hippo signaling inhibition protects against AD by reducing oxidative stress. For in-vivo studies, we have randomly distributed 16 adult male Wistar rats weighing 220-280 grams into 4 groups, viz., sham control, AD, AD+Xmu-mp-1, and AD+Donepezil (positive control), with 4 rats per group. AD was established via stereotactic injection of STZ in the brain's lateral ventricles at a dose of 3 mg/kg body weight. To negate the effects of surgical procedures, rats in the Sham control group received the same volume of saline following the same method. 7 days post-surgery, Xmu-mp-1 was intraperitoneally administered

at a dose of 0.5mg/kg body weight, given every 2 days, and lasted for 2 weeks. Assessment of spatial learning and memory deficits via ANY-Maze software analyzed Morris water maze, and novel object recognition tests at the end of the treatment period revealed that Xmu-mp-1 ameliorates STZ-induced cognitive impairments. Additionally, blinded histological examination of the hippocampus and cortex regions of the brain tissue demonstrated that Xmu-mp-1 reduces STZ-induced neurodegeneration and phospho-tau expression. The immunoblotting assessment also revealed a reduction in STZ-induced apoptotic gene expression by Xmu-mp-1. These results suggest that Hippo signaling inhibition holds therapeutic potential against AD pathogenesis, which needs further investigation. Our findings can aid in identifying this novel Hippo signaling inhibitor as a potential intervention for managing and treating AD.

Disclosures: **M. Sahu:** A. Employment/Salary (full or part-time):; DST-INSPIRE Fellowship. **A. Mondal:** None.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.27

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

Support: NRF_2020R1A2C2008480
KMDF_PR_20200901_0103
NRF-2019R1I1A3A01043477

Title: Focused ultrasound restored long term potentiation of hippocampal neuron in transgenic mouse of Alzheimer’s disease

Authors: *C. KONG[1], J. AHN[2], S. KIM[2], J. PARK[1], Y. NA[3], J. CHANG[1], S. CHUNG[2], W. CHANG[1];
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Abstract: Introduction: Focused ultrasound (FUS) combined with microbubble can temporary induces blood brain barrier (BBB) opening. FUS was studied for the main purpose of drug delivery through the control of the BBB, but recently, studies have been reported that FUS improves cognitive function. Here, we investigated the effects of FUS mediated BBB opening on hippocampal long-term potentiation (LTP) and cognitive function in a 5xFAD mouse model of Alzheimer’s disease.Materials and Methods: We applied focused ultrasound with microbubble to the hippocampus and long term potentiation was measured 6 weeks after BBB opening using FUS. Field recordings were made with a concentric bipolar electrode positioned in the CA1 region using an extracellular glass pipette filled with artificial cerebrospinal fluid. Morris water maze was performed to test cognitive function.Results: Long term potentiation induction at

Schaffer collateral - CA1 circuit was significantly suppressed in the 5xFAD group when compared with littermate group and this impairment was almost restored in FUS treated 5xFAD mouse. The increased field excitatory postsynaptic potential rates are as follows: Naïve 40.5 ± 2.304, sham 40.24 ± 5.156, sham with FUS 41 ± 5.949, 5xFAD 12.24 ± 2.598, 5xFAD with FUS 37.87 ±5.689. As a result of Morris water maze test, it was confirmed that cognitive function was recovered in the Tg+FUS group (probe test: crossings, 3.5 ± 0.92; quadrant zone, 23.22 ± 3.73; platform zone, 1.19 ± 0.21) compared to the Tg group (probe test: crossings, 0.33 ± 0.21; quadrant zone, 5.78 ± 1.60; platform zone, 0.05 ± 0.04).Conclusion: Our results demonstrated that FUS mediated BBB opening has a significant impact on increasing long term potentiation at Schaffer collateral - CA1 synapses and rescues cognitive dysfunction and working memory in the 5xFAD mouse model. Therefore, it could be a promising treatment for neurodegenerative diseases in that it remarkably increased long term potentiation thereby improving working memory.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.28

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

Title: A novel Alzheimer’s disease mouse model with earlier Aβ accumulation and later onset of cognitive impairments

Authors: H. QI[1], D. JIA[1], F. LIU[1], H. TONG[1], ***Z. LI**[2], C. JU[1], J. ZHAO[1], X. GAO[1]; [1]GemPharmatech, Nanjing, China; [2]GemPharmatech, GemPharmatech, Wilmington, DE

Abstract: Alzheimer’s disease (AD) is the most diagnosed neurodegenerative disease. Up to date, only six treatments have received FDA approval, while more than 450 have failed. Fuller understanding of the disease mechanisms and novel therapeutic strategies are urgently needed. Appropriate animal models provide indispensable support for both efforts. To enhance the preclinical toolset for AD research, we generated a novel mouse model, FAD4T, in which APP Swe/Indiana and PSEN1 M146L/L286V are inserted into the C57BL/6 mouse genome via two co-injected transgenes. In FAD4T mice, Aβ deposition was detected in both cortex and hippocampus at as early as 1.5 months of age, which gradually increased with age. In addition, gliosis was detected at as early as 2.5 months of age, with glial cells surrounding Aβ plaques. These phenotypes are earlier onset than those in 5xFAD mice, while the severity of both phenotypes is comparable to that observed among 5xFAD mice. Interestingly and different from 5xFAD mice, FAD4T mice exhibited significantly delayed onset of cognitive impairments when comparable levels of Aβ deposition were present. These pathophysiological features suggest that

FAD4T mice not only provide a new model for studying the disease mechanisms of AD, but also a new tool for developing improved and more effective treatments of the disease.

Disclosures: **H. Qi:** A. Employment/Salary (full or part-time):; GemPharmatech. **D. Jia:** A. Employment/Salary (full or part-time):; GemPharmatech. **F. Liu:** A. Employment/Salary (full or part-time):; GemPharmatech. **H. Tong:** A. Employment/Salary (full or part-time):; GemPharmatech. **Z. Li:** A. Employment/Salary (full or part-time):; GemPharmatech. **C. Ju:** A. Employment/Salary (full or part-time):; GemPharmatech. **J. Zhao:** A. Employment/Salary (full or part-time):; GemPharmatech. **X. Gao:** A. Employment/Salary (full or part-time):; GemPharmatech.

Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

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

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Gerald and Carla Connor

Title: Neuron-specific gene immunotherapy against amyloid-beta peptides in a mouse model of Alzheimer Disease

Authors: *Z. NOROOZIAN[1], M. PASTERNAK[2], J. SILBURT[1], C. DIBIA[1,4], D. DAVANI[6], M.-E. PAQUET[7,8], J. MCLAURIN[1,4], K. HYNYNEN[3,5], S. KÜGLER[9], I. AUBERT[1,4];
[1]Biol. Sciences, Hurvitz Brain Sci., [2]Evaluative Clin. Sci., [3]Physical Sci., Sunnybrook Res. Inst., Toronto, ON, Canada; [4]Lab. Med. and Pathobiology, Temerty Fac. of Med., [5]Med. Biophysics, Temerty Fac. of Med., Univ. of Toronto, Toronto, ON, Canada; [6]Abbott, Ottawa, ON, Canada; [7]CERVO Brain Res. Ctr., [8]Biochemistry, Microbiology and Bioinformatics, Laval Univ., Québec, QC, Canada; [9]Neurol., Univ. Med. Göttingen, Göttingen, Germany

Abstract: Aggregation of amyloid-beta peptides (Aβ) in the brain is the hallmark of Alzheimer disease (AD). Treatment efficacy is impacted by the challenging delivery of anti-Aβ therapeutics to the brain due to the blood-brain barrier (BBB) and the course of the disease which can last for decades. A promising approach for long-term production of therapeutics in the brain is gene immunotherapy. Furthermore, magnetic resonance image-guided focused ultrasound in presence of intravenous microbubbles (MRIgFUS) is a technology that can non-invasively increase the

permeability of BBB in a controlled manner, locally and temporarily. MRIgFUS has been used in AD patients, and the preclinical advances in the delivery of gene therapy vectors to the brain are promising for future translational applications. We hypothesized that the delivery of a gene encoding for an anti-Aβ antibody to the brain will lead to the sustained neuronal production and secretion of the therapeutic transgene to improve treatment efficacy. This hypothesis will be tested in a mouse model of amyloidosis using a small antibody that binds to Aβ. Target engagement of the antibody and its lack of toxicity were confirmed *in vitro*. An adeno-associated virus 6 (AAV6) encoding for neuronal anti-Aβ antibody was packaged and delivered to frontal cortex in neonates. The sustained neuronal expression of the antibody lasted for up to 14 months following gene delivery. Significant prevention of Aβ aggregation was found at early and late stages of Aβ pathology, specifically at 3 and 6 months of age. At 6 months post-gene delivery, gliosis and neuritic dystrophies associated with Aβ plaques were significantly reduced. An AAV9 serotype was then selected and evaluated for the delivery of the anti-Aβ antibody-encoded gene with intravenous injections alone, and combined with MRIgFUS for targeted delivery to selected regions of the brain. In conclusion, we have designed a therapeutic that is expressed by neurons, the cells that are highly affected by Aβ pathology. Long-term neuronal production of the therapeutic resulted in preventing Aβ accumulation, protecting both neurons and glia from plaque-associated pathologies. Combined with MRIgFUS this immunotherapy approach could fulfill the unmet need of non-invasive and efficient gene therapy to the brain.

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Poster

040. Preclinical Strategies and Alzheimer's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 040.30

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

Support: KIST Intramural Grant #1711124254

Title: Dysfunction of striatal MeCP2 is associated with cognitive decline in a mouse model of Alzheimer’s disease

Authors: S. LEE, ***H.-I. IM**;
Korea Inst. of Sci. & Technol., Seoul, Korea, Republic of

Abstract: Cerebral Methyl-CpG binding Protein 2 (MeCP2) is involved in several psychiatric disorders that are concomitant with cognitive dysfunction. However, the regulatory function of striatal MeCP2 and its association with Alzheimer's disease (AD) has been largely neglected due to the absence of amyloid plaque accumulation in the striatal region until the later stages of AD progression. Considerable evidence indicates that neuropsychiatric symptoms related to

cognitive decline are involved with striatal dysfunction. To this respect, we investigated the epigenetic function of striatal MeCP2 paralleling the pathogenesis of AD. We investigated the brain from amyloid precursor protein (APP)/presenilin1 (PS1) transgenic mice and postmortem brain samples from normal subjects and AD patients. The molecular changes in the brain, particularly in the striatal regions, were analyzed with thioflavin S staining, immunohistochemistry, immunoblotting, and MeCP2 chromatin immunoprecipitation sequencing (ChIP-seq). The cognitive function of APP/PS1 mice was assessed via three behavioral tests: 3-chamber test (3CT), Y-maze test (YMT), and passive avoidance test (PA). A multi-electrode array (MEA) was performed to analyze the neuronal activity of the striatum in APP/PS1 mice. Striatal MeCP2 expression was increased in the younger (6 months) and older (10 months) ages of APP/PS1 mice, and the genome-wide occupancy of MeCP2 in the younger APP/PS1 showed dysregulated binding patterns in the striatum. Additionally, we confirmed that APP/PS1 mice showed behavioral deficits in multiple cognitive behaviors. Notably, defective cognitive phenotypes and abnormal neuronal activity in old APP/PS1 mice were rescued through the knock-down of striatal MeCP2. We found that the MeCP2-mediated dysregulation of the epigenome in the striatum is linked to the defects in cognitive behavior and neuronal activity in the AD animal model, and that this alteration is initiated even in the very early stages of AD pathogenesis. Together, our data indicates that MeCP2 may be a potential target for the diagnosis and treatment of AD at asymptomatic and symptomatic stages.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 041.01

Topic: C.03. Parkinson’s Disease

Support: HBHL
CIHR

Title: Examining the role of dopamine- and noradrenaline-modulated cognitive deficits in prodromal and established Parkinson's disease

Authors: *S. SUN, V. MADGE, R. B. POSTUMA, L. COLLINS, M. E. SHARP;
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Abstract: The degeneration of dopaminergic (DAergic) substantia nigra (SN) and noradrenergic (NAergic) locus coeruleus (LC) is thought to begin in the prodromal stage of Parkinson’s disease (PD), characterized by the diagnosis of REM sleep behavior disorder (RBD). Currently it is unknown whether cognitive processes reliant on these neurotransmitter systems, such as reward learning and attentional control, are affected in the prodromal stage. Modifiable drugs targeting the DAergic and NAergic systems already exist, so understanding the contribution of early loss

of these systems can reveal whether these existing drugs are suitable for treating cognitive decline.We tested 88 PD patients, 20 RBD patients, and 39 age-matched controls on cognitive tasks measuring DAergic and NAergic function using a biased signal-detection task and a visual oddball task, respectively. Linear and logistic mixed-effects regressions controlling for age and sex were used to examine response time and accuracy on these tasks. Results from the biased signal detection task comparing performance for richly rewarded stimuli compared to leanly rewarded stimuli revealed that PD patients were not impaired in accuracy (β; = -.06, p = .38), but experienced a slowing in responding (β; = .21, p < .001) compared to controls, whereas RBD patients were impaired in accuracy (β; = -.44, p < .001), but not in response time (β; = -.09, p = .23) compared to controls. Surprisingly, results from the visual oddball task revealed that neither PD patients nor RBD patients were impaired in accuracy on infrequent trials relative to frequent trials compared to controls (β; = -.04, p = .79; β; = .42, p = .07), and the same was true for response time (β; = -.0001, p = .98; β; = -.004, p = .29). Participants also underwent MRI of the SN and LC. Preliminary analyses in a subset of 35 PD patients with processed neuromelanin scores indicated that SN structure integrity was not correlated with accuracy nor response time in the biased signal detection task (r(33) = -.08, p = .47; r(33) = -.06, p = .62) and that LC structure integrity was not correlated with accuracy nor response time in the visual oddball task (r(32) = .04, p = .82; r(32) = .02, p = .26). These findings indicate that reward learning impairments can be detected as early as the prodromal stage of disease, but attentional control is preserved. However, on-going research including all neuromelanin scores and diffusion MRI measures of microstructural integrity aims to elucidate the pathology driving these cognitive deficits. This will provide further insight on the neurobiological targets for early intervention of cognitive decline in PD.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 041.02

Topic: C.03. Parkinson’s Disease

Title: Subthalamic nucleus deep brain stimulation modulates dopaminergic and subthalamic contributions to effort-based decision making

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Abstract: Parkinson’s disease (PD) is a common neurodegenerative disease affecting 1% of the elderly population. High frequency deep brain stimulation (DBS) in the subthalamic nucleus (STN) improves motor symptoms, but its precise mechanism of action is still unclear. In cognitive tasks, DBS can lower the 'decision threshold' and increase impulsivity by disrupting

the STN theta-band modulation in response to cognitive conflict. This mechanism has been interpreted in terms of an informational lesion in the STN induced by DBS. The effects of DBS contrast with the effects of dopaminergic medication (DA) which alter the weighting of benefits vs. costs of decisions though both mechanisms (DBS and DA) could account for increased impulsive behavior. Here we evaluate whether low vs high frequency (4 hz vs. 130 hz) stimulation in different STN contact locations (dorsal vs ventral) can have differential effects on decision threshold and/or cost-benefit decision making in a physical effort-discounting task using a within-subject design. We present behavioral data and a computational characterization of DBS' effects on cost/benefit decision making collected from STN DBS PD patients (DA ON and DA OFF) and age matched controls.

Disclosures: G.J. Pagnier: None. **W.F. Asaad:** None. **M.J. Frank:** None.

Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 041.03

Topic: C.03. Parkinson's Disease

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Title: Loss of O-GlcNAcylation is fatal for survival and physiological functions of dopamine neurons

Authors: *B. LEE, H. KIM, J.-I. KIM;
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Abstract: The dopamine system in the midbrain is important for volitional movement, action selection, and reward-related behaviors. Although the dopamine system mediates versatile functions, it contains only a small set of neurons in the midbrain. Moreover, these dopamine neurons are highly susceptible to Parkinson's disease (PD), and prematurely degenerate during disease progression. O-GlcNAcylation is one of the post-translational modifications modulating many fundamental cellular processes, including transcription, translation, and signal transduction. It attaches O-linked N-acetylglucosamine (O-GlcNAc) moieties to serine, threonine residues of cytoplasmic, nuclear, and mitochondrial proteins. Interestingly, O-GlcNAc modification and two enzymes governing O-GlcNAcylation, O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA), are abundant in the mammalian brain, while our understanding of the physiological role of O-GlcNAcylation remains rudimentary in the brain. In this study, we aim to elucidate the role of O-GlcNAcylation in dopamine neurons by utilizing whole-cell patch clamp recording, optogenetics, fast-scanning cyclic voltammetry (FSCV), and immunohistochemistry.

Notably, dopamine neuron-specific OGT conditional knockout (cKO) mice exhibited markedly reduced survival and body weight. Furthermore, down-regulation of O-GlcNAc in dopamine neurons caused severe cell death and dysfunction, implying the critical role of O-GlcNAcylation in dopamine neurons. Juvenile OGT cKO mice showed alterations in physiological functions including reduced input resistance, increased action potential firing, attenuated Ih current, and enhanced inhibitory synaptic transmission in dopamine neurons. Pharmacological down-regulation of O-GlcNAcylation using an OGT inhibitor was also able to change the synaptic transmission of dopamine neurons. Importantly, adeno-associated virus-mediated knockout of OGT in adult midbrain dopamine neurons led to significant neuronal death, potentially excluding the developmental impact of OGT in dopamine neurons. Moreover, 2 weeks of knockout induction by AAV was enough to alter the intrinsic properties of dopamine neurons, further emphasizing the importance of O-GlcNAcylation in the maintenance of dopamine neurons. Together, our data clearly demonstrate that O-GlcNAcylation is vital for survival, maintenance, and physiological functions of dopamine neurons.

Disclosures: B. Lee: None. **H. Kim:** None. **J. Kim:** None.

Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 041.04

Topic: C.03. Parkinson’s Disease

Support: DoD Grant W81XWH-19-0757

Title: Norepinephrine as a pathological index of parkinson’s disease in the prefrontal cortex

Authors: *K. K. DOSHIER, I. SOTO, R. B. MCMANUS, W. R. NAVARRETE-BARAHONA, E. A. KASANGA, M. F. SALVATORE, V. A. NEJTEK;
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Abstract: Parkinson’s disease (PD) is a neurodegenerative disease marked by a substantial loss of motor function. Recently, subtle cognitive problems are gaining attention as a prodromal feature of PD that precedes the onset of motor decline up to 10-years. Due to the role of the prefrontal cortex (PFC) in goal-directed behaviors, decision making, and memory, degradation of catecholamine function in the PFC could underlie progressive cognition decline in prodromal PD. Therefore, early-stage PD pathology in the PFC may include loss of dopamine (DA) and norepinephrine (NE) innervation. Newly identified DA degeneration in the ventral tegmental area (VTA) in PD with its VTA and PFC connection, it is conceivable that DA loss would likewise extend to the PFC. We used the PD neurotoxin rat models (6-OHDA) as an established method to address DA and NE tissue levels in the PFC. Utilizing high-performance liquid chromatography (HPLC), preliminary data taken from two separate rat PD model studies found a significant difference in NE loss between lesioned groups. The first study controlled for sex as a

biological factor in 12 unilaterally lesioned rats (6 male and 6 female). Although there was no sex interaction effect, there was a significant decrease in NE between lesioned vs contralateral sides (F (1,19) = 74.28, p < .001). The second study examining exercise impact on lesioned rats also found a significant loss of NE, and tyrosine hydroxylase protein, without effect of exercise. These results are consistent with behavioral data suggesting significant losses in executive function and recollection memory across both cohorts. This novel evidence suggests that DA loss in PD rat models does indeed extend into the PFC. Moreover, NE/DA dysfunction in the PFC can be quantified, thus, providing insight into severity of PD disease and intervention efficacy.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 041.05

Topic: C.03. Parkinson’s Disease

Support: CIHR Grant

Title: Using structural magnetic resonance imaging to identify features of early-stage Parkinson's disease and rapid eye movement sleep behaviour disorder

Authors: *E. ALUSHAJ[1,2], D. HEMACHANDRA[3,4], N. HANDFIELD-JONES[1], A. KUURSTRA[4], R. S. MENON[6], A. M. OWEN[5], A. R. KHAN[3,4], P. A. MACDONALD[7,2]; [1]Neurosci., [2]Brain and Mind Inst., [3]Med. Biophysics, [4]Robarts Res. Inst., [5]Physiol. and Pharmacol., Western Univ., London, ON, Canada; [6]Imaging Res. Labs, Robarts Res. Inst., London, ON, Canada; [7]Clin. Neurolog. Sci., Univ. Hosp., London, ON, Canada

Abstract: The midbrain dopaminergic system plays a major role in the pathophysiology of Parkinson’s disease (PD). Excessive iron accumulation in the substantia nigra pars compacta (SNc) is thought to cause degeneration in the nigrostriatal pathway leading to motor symptoms. Rapid eye movement sleep behaviour disorder (RBD) is a preclinical form of PD and presents with similar structural features. Magnetic resonance imaging (MRI) can localize and quantify iron in the brain based on its magnetic susceptibility. Currently, there are no validated imaging diagnostic or preclinical biomarkers of PD, but MRI has great potential for their discovery. Early-stage PD patients (n = 22), RBD patients (n = 10) and age-matched healthy controls (n = 23) were scanned using 3T MRI. T1-weighted anatomicals were used for segmenting the midbrain nuclei and striatum subregions based on the CIT168 probabilistic subcortical atlas (2018). Then using quantitative susceptibility mapping (QSM) images registered to these anatomicals, we segmented the regions of interest to analyze average susceptibility. Repeated measures analysis of variance of average susceptibility values from QSM revealed significantly higher SNc iron content in early-stage PD patients compared to healthy controls. RBD patients

did not display significant iron elevation, but data collection is on-going for this cohort. No significant group differences in iron content were found in the SNr, VTA, or striatum. Findings from receiver operating characteristic curves with repeated 5-fold cross validation suggest that QSM in the SNc could be a diagnostic biomarker of PD following validation, given its great diagnostic accuracy (AUC = 0.83) at the single-subject level.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 041.06

Topic: C.03. Parkinson’s Disease

Support: CIHR PhD Scholarship

Title: Beta amyloid deposition and cognitive decline in Parkinson's disease: a study of the PPMI cohort

Authors: *A. MIHAESCU[1,2], M. VALLI[2,1], C. URIBE[2], M. DIEZ-CIRARDA[2], M. MASELLIS[3], A. GRAFF-GUERRERO[2], A. STRAFELLA[2,1];
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Abstract: The accumulation of beta amyloid in the brain has a complex and poorly understood impact on the progression of Parkinson's disease cognitive decline sequelae. Some studies have found increased beta amyloid burden in the brain is associated with worsening cognitive impairment in Parkinson's disease, especially in cases where dementia occurs, while other studies failed to replicate this finding. Furthermore, most studies quantify beta amyloid with a binary positive/negative metric if the global burden is above or below a cut-off value. To this end, we focused instead on a regional analysis of beta amyloid burden in specific brain regions to elucidate its role in cognitive decline. We examined a cohort of 25 idiopathic Parkinson's disease patients and 30 healthy controls from the Parkinson's Progression Marker Initiative database. These participants underwent [^{18}F]Florbetaben positron emission tomography scans to quantify the amount of beta amyloid deposition in 20 cortical regions. We then analyzed this beta amyloid data alongside the longitudinal Montreal Cognitive Assessment scores across three years (at baseline and up to two years followup) to see how participant’s baseline beta amyloid levels affected their cognitive scores prospectively. The first analysis we performed with these data was a hierarchical cluster analysis to help identify brain regions that shared similarity in their beta amyloid deposition patterns. We found that beta amyloid clusters differently in Parkinson’s disease patients compared to healthy controls. In the Parkinson’s disease group, increased beta

amyloid burden in cluster 2 was associated with worse cognitive ability, compared to deposition in either clusters 1 or 3. We also performed a step-wise linear regression where we found a model with an adjusted R^2 of 0.495 explaining 49.5% of the variance in the Parkinson's disease group's Montreal Cognitive Assessment score one-year post-scan. This model included the left gyrus rectus, the left anterior cingulate cortex, and the right parietal cortex. Taken together, these results suggest regional beta amyloid deposition alone has a moderate effect on future cognitive decline in Parkinson's disease patients. The patchwork effect of beta amyloid deposition on cognitive ability may be part of what separates cognitive impairment from cognitive sparing in Parkinson's disease. Rather than assess global amyloidopathy through a binary positive/negative metric, we suggest it would be more beneficial to measure beta amyloid in specific brain regions and use this as a prognostic tool to determine which Parkinson's disease patients are most at risk for future cognitive decline.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 041.07

Topic: C.03. Parkinson's Disease

Support: DFG, SFB 1506

Title: Identification of a novel, DAT-negative subpopulation of dopaminergic neurons in the lateral Substantia nigra, using tailored deep learning-based high-throughput image analysis.

Authors: *M. HÄUSLER[1], S. ROY[1], N. BURKERT[1], D. WUTTKE[2], S. MÜLLER[1], J. WIEMER[1], H. HOLLMANN[1], J. BENKERT[1], C. PÖTSCHKE[1], J. DUDA[1], M. MÜNCHMEYER[2,3], R. PARLATO[1,4], B. LISS[1,5];
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Abstract: Dopaminergic (DA) midbrain neurons are mainly located in the Substantia nigra pars compacta (SN) and the ventral tegmental area (VTA). Their dysfunction can cause diseases like Parkinson's (PD) or Schizophrenia. In PD, mesostriatal SN DA neurons are particularly prone to degeneration, while VTA DA neurons remain largely intact. The reason for this differential vulnerability is still unclear, and a better understanding is required for the development of more specific therapies. Cellspecific gene-expression analysis with spatial localization and preserved tissue context, in health and disease, is an essential tool for identifying molecular determinants underlying differential neuronal function and pathophysiology. However, manual image-

analysis, for cell count, anatomical mapping, and quantification of gene expression is very time consuming and prone to human error. To overcome these limitations, we developed an automated, deep learning-based analysis platform, to quantify neuronal numbers in defined areas, as well as fluorescence signals, derived from RNAscope-probes or immuno-histochemistry. We have optimized our algorithms to detect, count, and analyse individual DA midbrain neurons in mouse and human sections, stained for tyrosine hydroxylase (TH), the key enzyme for dopamine-synthesis. However, our approach can be easily adapted to other cell types and target areas.
Here, we analysed more than 20.000 TH-immuno-positive SN neurons in PFA-fixed brain-sections from adult mice. By co-expression analysis of the plasmalemma dopamine transporter (DAT), and by generation of anatomical expression-maps of TH-positive cells, according to their 3D-coordinates, we identified a novel, small subpopulation of neurons (~5% of all TH-positive SN neurons), mainly located in the lateral SN, that was immunofluorescence-negative for DAT. DAT is an electrogenic symporter, crucial for dopamine re-uptake, and thus dopamine homeostasis. Changes in DAT expression were reported in PD. However, absence of DAT-protein has been described only for mesocortical VTA DA neurons. Our finding of a DAT-negative subpopulation of SN neurons is not only of physiological relevance, but it has additional implications, as DAT is commonly used as marker for SN DA neurons, and for their specific targeting, e.g. to generate DA neuron specific conditional knock out mice, expressing Cre-recombinase under the control of the DAT-promoter. We are currently further characterising this novel group of DAT-negative SN neurons by analysing the co-expression of D2-autoreceptors and of calbindin-d28k, additional markers for distinct subpopulations of DA midbrain neurons.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Program #/Poster #: 041.08

Topic: C.03. Parkinson’s Disease

Support: Horizon 2020 (H2020)

Title: Serotonergic and dopaminergic neurons in Dorsal Raphe Nucleus: from physiology to Parkinson’s Disease pathology.

Authors: *L. BOI[1], Y. JOHANSSON[2], G. SILBERBERG[1], G. FISONE[1];
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Abstract: The Dorsal Raphe Nucleus (DRN) contains different populations of serotonergic ($DRN_{5\text{-}HT}$) and dopaminergic (DRN_{DA}) neurons. Because of their anatomic and genetic features, attempts to target these populations are difficult and have often generated contrasting results. Parkinson's Disease (PD) is a neurodegenerative disorder associated with motor and non-motor symptoms. While the motor symptoms are mostly related to the loss of dopaminergic nigrostriatal neurons, the non-motor symptoms may involve other systems, including the $DRN_{5\text{-}HT}$ and DRN_{DA}. The DRN receives an excitatory noradrenergic input from the Locus Coeruleus (LC), which is also affected by PD. To test the possible involvement of $DRN_{5\text{-}HT}$ and DRN_{DA} and their connection with the noradrenergic system in PD, we first investigated the electrophysiological and morphological properties of $DRN_{5\text{-}HT}$ and DRN_{DA} in physiological conditions and in a mouse model of PD, in presence and absence of noradrenergic damage. Wild-type and DAT-tdTomato mice were bilaterally injected in the dorsal striatum with vehicle or 6-hydroxydopamine (6-OHDA). One group was pre-treated with desipramine prior to 6-OHDA in order to protect the noradrenergic system. Three weeks later, midbrain slices were prepared for whole-cell patch-clamp recordings of DRN neurons. Recorded neurons were stained and classified as $DRN_{5\text{-}HT}$ or DRN_{DA} using immunohistochemistry. We found that in control mice, $DRN_{5\text{-}HT}$ and DRN_{DA} neurons differ in many of their electrophysiological and morphological profiles. In 6-OHDA lesioned mice, $DRN_{5\text{-}HT}$ showed changes in the excitability and smaller and more circular cell bodies. DRN_{DA} neurons in lesioned mice had altered action potential properties as well as morphological changes such as swelling of cell bodies and increased dendritic branching. Treatment with desipramine partially protected from the changes observed in the parkinsonian condition. Our study identifies several electrophysiological properties that can be utilized to differentiate $DRN_{5\text{-}HT}$ and DRN_{DA} neurons. Moreover, we show that changes caused by the 6-OHDA lesion are partially rescued by desipramine. Taken together, these results provide useful information to determine the relative contribution of $DRN_{5\text{-}HT}$, DRN_{DA} and their noradrenergic afferents to non-motor comorbidities in PD, including affective and sleep disorders.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson's Disease

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Title: Single cell expression profiling reveals a novel dopamine neuron subtype with distinct circuitry and anatomical distribution

Authors: *Z. GAERTNER[1], M. AZCORRA[2], C. HAYES[1], R. AWATRAMANI[1];
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Abstract: In recent years, several studies have performed single cell profiling of gene expression in midbrain dopamine (DA) neurons, revealing many molecularly distinct subpopulations. Utilizing intersectional genetic methods to explore the circuitry, function, or vulnerability of these subtypes in isolation has revealed highly distinct properties within traditionally homogenous nuclei including the substantia nigra pars compacta (SNc), and adjacent ventral tegmental area (VTA). Here, we utilized single-nucleus RNA sequencing and generated the largest transcriptomic dataset to date of DA neurons in mice, which has revealed an additional level of heterogeneity within a known subtype of DA neurons expressing Aldh1a1. Based upon this, we developed a novel transgenic mouse line to genetically access this subtype and thereby uncovered highly specific anatomical distributions and circuitry. Based upon these patterns, we believe this novel subtype may be vital in driving the pathophysiology of Parkinson's disease.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Topic: C.03. Parkinson’s Disease

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Title: The progression of bradykinesia in people with Parkinson's disease with and without REM sleep without atonia

Authors: *S. L. AMUNDSEN-HUFFMASTER[1], J. CHUNG[1], R. SUMMERS[1], A. GROTHE[1], A. VIDENOVIC[2], M. HOWELL[1], P. J. TUITE[1], C. D. MACKINNON[1];
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Abstract: The co-morbidity of rapid eye movement (REM) sleep without atonia (RSWA) and Parkinson’s disease (PD) may influence PD symptom progression due to involvement of brainstem areas contributing to movement control. We hypothesized that the slowing of arm

movement (bradykinesia) would progress faster in PD with RSWA (PD+RSWA) compared to people with PD without RSWA (PD-RSWA) and healthy older adults.
Fifty-four people (PD: 18 PD+RSWA, 18 PD-RSWA, mean time since diagnosis = 2.4 ± 1.8 years; Controls: 18, age- and sex-matched to PD) completed an overnight polysomnography test and a ballistic elbow flexion task (72 deg. ROM) at baseline and 36 ± 7 months. The primary outcomes measures were the percentage of REM sleep with phasic and tonic chin electromyography and elbow flexion peak velocity and movement distance. Linear mixed effects models (RStudio) were used to investigate the effects of group, visit, arm (more vs. less affected, MA vs. LA), and their interactions. Significance was set to $p < 0.05$.
Peak velocity showed a significant visit x arm x group interaction effect, with sex, age, and movement distance as significant covariates. At baseline, both arms of the PD+RSWA participants were significantly slower (mean MA = 309 deg/s, LA = 298 deg/s) than controls (388 deg/s), but not in the PD-RSWA group. Instead, the PD-RSWA showed significant asymmetry between arms (MA =341 deg/s, LA = 364 deg/s) at baseline. At 3 years, both arms in the PD-RSWA group slowed significantly, but the rate of progression was greater in the LA arm, such that at follow-up, both arms were significantly slower than controls and asymmetry between sides was no longer present. Both arms of the PD+RSWA group remained significantly slower than controls, but there was no significant progression over 3 years. Movement distance also showed a significant group x visit interaction reflecting alterations in the overshoot of the target over time. At baseline, the PD+RSWA group moved a shorter distance (mean 76.7 deg) than the PD-RSWA (80.7 deg) and control (82.6 deg) groups, but at 3 years, PD-RSWA group now moved a shorter distance (77.5 deg) than controls (81.0 deg) while the PD+RSWA group marginally increased movement distance at follow-up (78.4 deg).
The course of progression of bradykinesia was different between PD+RSWA and PD-RSWA over 3 years. Contrary to our hypothesis, PD-RSWA progressed more rapidly and transitioned from a predominantly asymmetric to a symmetric presentation of bradykinesia, such that the two PD groups were similar at follow-up. The increased rate of progression in PD-RSWA group, particularly on the LA side, may reflect accelerated nigrostriatal dopamine depletion over the 3-year period.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

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Program #/Poster #: 041.11

Topic: C.03. Parkinson’s Disease

Support: Parkinson Canada

Title: The role of glutamate co-transmission by serotonin neurons of the dorsal raphe nucleus in L-Dopa-induced dyskinesia

Authors: *L. SAIDI[1], S. RAJAN[2], S. POZZI[1], E. METZAKOPIAN[2], C. PROULX[1], M. PARENT[1];
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Abstract: OBJECTIVE: Parkinson's disease is mainly characterized by the progressive loss of midbrain dopaminergic neurons that innervate the striatum. The dopamine precursor L-3,4-dihydroxyphenylalanine (L-Dopa) is the most effective pharmacotherapy but its chronic use is hampered by side effects such as abnormal involuntary movements (AIMs), also termed L-Dopa-induced dyskinesia (LID). Studies have shown the crucial role of serotonin (5-HT) neurons in the conversion of exogenous L-Dopa and LID expression. Through this study, we specifically addressed the functional role of glutamate co-transmission by 5-HT neurons of the dorsal raphe nucleus (DRN) in the regulation of motor behavior and LID expression. **METHODS**: We used CRIPSR-Cas9 technology and viral injections to knock-out or overexpress the atypical vesicular glutamate transporter 3 (VGluT3), specifically in the DRN 5-HT neurons of adult mice. Two weeks later, mice were injected with 6-OHDA in the medial forebrain bundle to selectively damage dopaminergic axons, and then treated with L-Dopa to induce AIMs. **RESULTS**: *Post-mortem* analysis confirmed the depletion or overexpression of VGluT3 in AAV-infected 5-HT neurons of the DRN as well as a severe dopamine denervation. After dopamine lesion and L-Dopa administration, VGluT3-depleted mice show exacerbated AIMs following the administration of a low dose of L-Dopa (1mg/kg), compared to controls and transgenic mice overexpressing VGluT3. At higher L-Dopa doses (3, 6, 12 mg/kg), mice overexpressing VGluT3 show higher severity of the orofacial AIMs subtype. **CONCLUSIONS**: Glutamate that is co-released by 5-HT neurons of the DRN appears to be involved in the expression of LID.

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Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

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Program #/Poster #: 041.12

Topic: C.03. Parkinson's Disease

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Title: Early synaptic alterations and reduced brain connectivity in a PD-like mouse model with depressive phenotype upon human α-synuclein overexpression in serotonin neurons

Authors: L. MIQUEL-RIO[1,2,3], M. TORRES-LÓPEZ[1,2], V. PAZ[1,2,3], C. CASAL[1], E. MUÑOZ-MORENO[4], X. LÓPEZ-GIL[4], *A. BORTOLOZZI[1,2,3];
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Abstract: Introduction: Anxiety and depression are common comorbid conditions in Parkinson's disease (PD) and are major contributing factors to poor quality of life. Dysfunction of the serotonergic (5-HT) system, which regulates mood and emotional circuits, occurs during the premotor phase of PD and contributes to a wide range of non-motor symptoms. Furthermore, α-synuclein (α-Syn) aggregates were identified in raphe nuclei in the early stages of the disease. However, the relationship between α-Syn pathology and the structural and functional changes occurring in the brain is not well understood. We aimed to study whether synaptic plasticity and functional connectivity are affected by α-Syn accumulation in efferent brain regions from 5-HT raphe nuclei in the early stages using a PD-like mouse model with depressive phenotype overexpressing human α-Syn in raphe 5-HT neurons. **Methods:** We used a new mouse model of α-synucleinopathy in the 5-HT system based on AAV5-induced overexpression of wild-type human-α-synuclein (h-α-Syn) in 5-HT neurons of raphe nuclei. Mice were assessed 4 and 8 weeks later. Cytoskeletal motor components (MAP2), synaptic vesicle SV-associated proteins (SV2A), and synaptophysin were examined by confocal microscopy. Brain functional connectivity was analyzed using the resting state (rsfMRI) by BOLD and ICA signals. The cellular activity was measured by Egr-1 mRNA expression in several interconnected brain areas. **Results:** AAV5-induced human α-Syn accumulation in the 5-HT neurons leads to a progressive presynaptic pathology in interconnected brain regions characterized by downregulation of MAP-2 protein and up-regulation of SV2A and synaptophysin proteins, essential components of synaptic structural integrity and function. In parallel, abnormalities in neuronal activity were found in cortical and subcortical brain areas, assessed by Egr-1 mRNA expression. Specific regional differences in resting-state functional activity changes occur in caudate-putamen and hippocampus eight weeks post-injection, prior to neurodegeneration. **Conclusions:** This study provides preliminary evidence for synaptic and fMRI markers linked to Syn pathology in emotional brain circuits and has translational importance for identifying PD patients at risk for depression.

Disclosures: L. Miquel-Rio: None. **M. Torres-López:** None. **V. Paz:** None. **C. Casal:** None. **E. Muñoz-Moreno:** None. **X. López-Gil:** None. **A. Bortolozzi:** None.

Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 041.13

Topic: C.03. Parkinson’s Disease

Support: 240891 Parkinson's UK
NINDS 900023

Title: Causes of Dopaminergic Neuron Dysfunction in Parkinson's Disease: from LRRK2 to Rab10 and beyond

Authors: *N. WANG[1], C. ELLIOT[2], V. BHANDAWAT[1];
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Abstract: A notable observation in Parkinson's disease is the loss of dopaminergic neurons (DANs) in the *substantia nigra*. Inherited mutations in the LRRK2 gene are a significant risk factor which contribute to neurodegeneration in Parkinson's. In particular, a common single amino-acid change in LRRK2, *G2019S*, induces a gain-of-kinase-function, leading to a toxicity cascade involving neuronal excitotoxicity, calcium overload in DANs, and eventually causes cell death. However, due to the limitation in studying the DANs *in vivo*, the critical process underlying the physiological and pathological pathways remains to be illuminated. This study is aimed at overcoming this limitation by investigating DANs in *Drosophila*. We focus on a single DAN called TH-VUM whose role has been examined in a fly model of Parkinson's; LRRK2-G2019S mutation was found to induce Parkinsonism in the context of proboscis extension, a behavior modulated by TH-VUM. We combined genetic tools in *Drosophila* with *in vivo* whole-cell patch-clamp recordings to investigate physiological and pathological changes under different conditions. First, we noticed that TH-VUM is a pace-maker neuron which spikes as a mix of singlets and doublets. By modifying voltage-gated ion channels, we found that 1) calcium is not necessary but contributes to pace-maker. Manipulating Ca^{2+} influx does not abolish pace-making but alters the inter-spike interval and size of the spikes 2) sodium plays a pivotal role in spiking, as neurons with inactivated voltage-gated sodium channels exhibit abnormal action potentials and disappearance of doublets. Second, we observed that LRRK2 mutant caused increase in spike rate which supports the hypothesis that LRRK2 increases Ca^{2+} influx. In previous studies (Steger *et al.* 2017), Rab10 has been shown to be the direct target of LRRK2. Consistent with this idea, the effect of LRRK2 mutant on spiking is reversed in Rab10 knockout; LRRK2 double mutant. However, the morphology of the DAN is also affected in the double mutant. Overall, this study makes an important contribution in bridging the gap between LRRK2's effect on Parkinson's pathology and its effect on the physiology of DANs. Steger, M., Diez, F., Dhekne, H. S., Lis, P., Nirujogi, R. S., Karayel, O., Tonelli, F., Martinez, T. N., Lorentzen, E., Pfeffer, S. R., Alessi, D. R., & Mann, M. (2017). Systematic proteomic analysis of LRRK2-mediated Rab GTPase phosphorylation establishes a connection to ciliogenesis. *eLife*, 6, e31012.

Disclosures: **N. Wang:** None. **C. Elliot:** None. **V. Bhandawat:** None.

Poster

041. Parkinson's Disease: Dopamine and Non-Dopamine Pathways

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 041.14

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

Title: Electromagnetized Graphene Facilitates Direct Lineage Reprogramming into Dopaminergic Neurons

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Abstract: Graphene is a carbon nanomaterial that has unique characteristics, including ballistic conduction, thermal and electrical conductivity, and biocompatibility. It is emerging as a promising tool for controlling various cell behaviors, such as viability, evelopment, and differentiation. Here, it is reported that the magnetized graphene nanosheets facilitate direct lineage reprogramming of induced dopaminergic (iDA) neurons. The graphene nanosheets are exposed to specific intensities and frequencies of electromagnetic fields, which lead to the accumulation of histone acetylation, including H3K27ac and H4K12ac, for the robust direct reprogramming of DA neurons. Remarkably, electromagnetized graphene nanosheet-mediated in vivo reprogramming significantly enhances the generation of iDA neurons in the mouse models of Parkinson's disease (PD), which efficiently ameliorate PD symptoms. Taken together, the results provide evidence that magnetized graphene can be used as a novel therapeutic application for PD which expands the applications of graphene as biomaterials for regenerative therapeutics.

Disclosures: Y. Hwang: None. **J. Kim:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.01

Topic: C.03. Parkinson's Disease

Title: Phenotypic characterization of hA53Ttg mice as Parkinson's disease model

Authors: *R. RABL[1], **T. LOEFFLER**[1], M. MIKUSCH[1,2], R. SCHEYTT[1,3,4], J. NEDDENS[1], M. DAURER[1], L. BREZNIK[1], S. PEINKIHER[1], S. FLUNKERT[1], S. SIDEROMENOS[1], M. PROKESCH[1];
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Abstract: Introduction: Aggregation of α-synuclein (α-syn) plays a crucial role in Parkinson's disease (PD) and other synucleinopathies. Point mutations in α-syn have been identified in rare forms of familial PD and are reported to accelerate α-syn oligomerization and aggregation as well as age of symptom onset. Here, we characterized human α-syn transgenic mice with A53T mutation (hA53Ttg) developed by Sudhof and colleagues for brain pathology and motor deficits. Methods: hA53Ttg mice at an age of 2, 4 and 6 months were tested for motor deficits in the

beam walk test. Afterwards, animals were euthanized, and brain tissue evaluated for human α-syn, pSer129 α-syn, as well as GFAP and Iba1 as marker of neuroinflammation. Plasma of older animals was further evaluated for neurofilament light chain levels as marker of neurodegeneration using a commercially available assay. Tissues were analyzed by immunofluorescent labeling and biochemical methods. All experiments were performed in animals of both sex and compared to age-matched non-transgenic littermates.
Results: Already at the age of 2 months, hA53Ttg mice present severe motor deficits in the beam walk test. Histological and biochemical analyses are currently performed, and first results suggest highly increased human α-syn and pSer129 α-syn levels in the hippocampus but only minor changes in neuroinflammation markers. Neurofilament-light chain (NFL) levels are increased in older animals. Quantitative immunofluorescence of different neuronal populations is currently ongoing (ChAT, Ctip2, TH), and levels of murine α-syn are histologically analyzed, too.
Conclusions: Our analyses revealed a very early motor phenotype in hA53Ttg mice and highly increased human α-syn and pSer129 α-syn levels in the brain. Together with the increased NFL levels our data suggest that this model is well-suited for testing new PD drugs and early interventions of synucleinopathies.

Disclosures: **R. Rabl:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **T. Loeffler:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Mikusch:** None. **R. Scheytt:** None. **J. Neddens:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Daurer:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **L. Breznik:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Peinkiher:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Flunkert:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **S. Sideromenos:** A. Employment/Salary (full or part-time):; QPS Austria GmbH. **M. Prokesch:** A. Employment/Salary (full or part-time):; QPS Austria GmbH.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.02

Topic: C.03. Parkinson’s Disease

Support: Bikaintek (Basque Government)
PUE21-03 (Basque Government)

Title: Prodromic Parkinson´s Disease biomarkers in a rodent model overexpressing alpha-synuclein in the Substantia nigra

Authors: C. DOMÍNGUEZ-FERNÁNDEZ[1,4], L. ARANA[4,2], E. PAREDES[1], E. ASTIGARRAGA[3], C. MIGUÉLEZ[4,5], *G. BARREDA-GÓMEZ[6];
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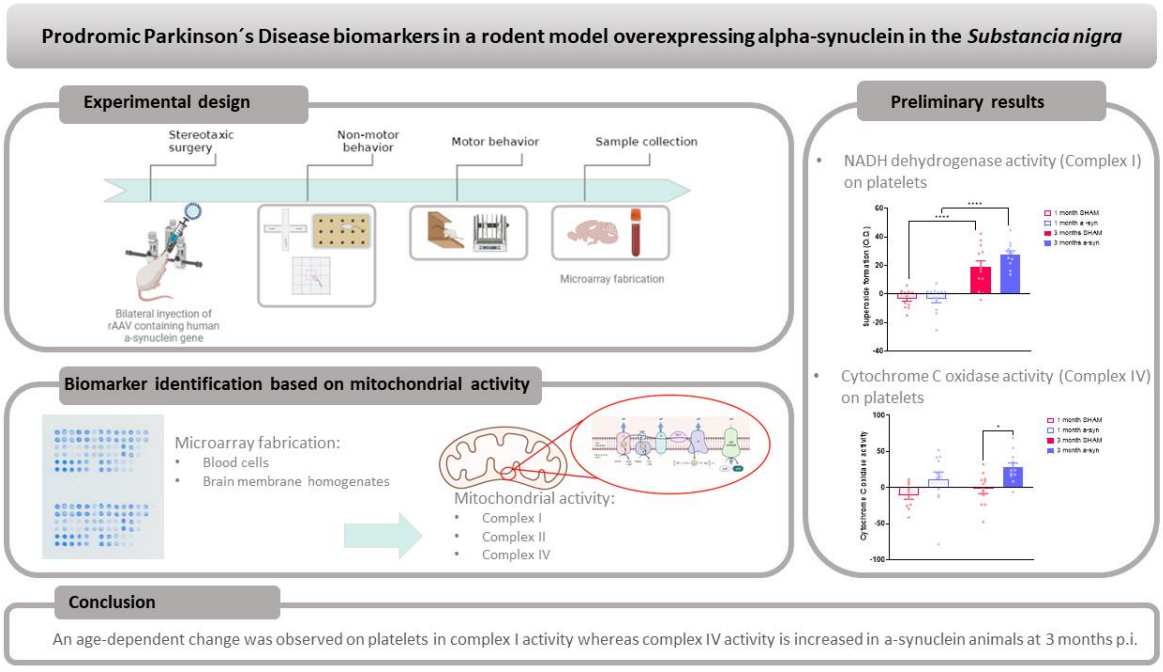
Abstract: Parkinson´s Disease (PD) is a neurodegenerative disorder characterized by neuron loss in different brain areas, especially dopaminergic neurons in the *Substantia Nigra* (SN). The alterations cause motor symptoms such as tremor, rigidity and bradykinesia among others. However, it is not until these symptoms appear that the pathology can be diagnosed, when the neuron loss in SN is over 60%, because there is a lack of early stage biomarkers that allow the diagnosis. The trigger of the degeneration remains unknown but some features have been associated with neuron death, such as the presence of Lewy bodies. These structures are formed by the accumulation of alpha-synuclein and play a key role in neurodegeneration since its presence could disturb the mitochondrial function, leading to the formation of reactive oxygen species (ROS), that induce oxidative stress and cell death.
The aim of this study is to identify PD biomarkers on different kinds of central and peripheric tissue samples. In order to achieve this purpose, we have stablished a rodent model based on the overexpression of alpha-synuclein in the SN. We have performed a behavioral evaluation of our model testing motor and non-motor behavior. We also collected blood and brain samples and fabricated cell membrane microarrays (CMMAs) to analyze the activity of the mitochondrial electron transport chain (ETC). Alterations in blood mitochondrial activity were observed although no significant behavioral changes have yet been detected. Hence, with the current studies we are aiming to identify early stage PD biomarkers that allow a sooner diagnosis of the disease.
This research was conducted in the scope of the Transborder Joint Laboratory (LTC) "non-motor CoMorbidities in Parkinson's Disease (CoMorPD)". No conflict of interest.



Disclosures: C. Domínguez-Fernández: None. **L. Arana:** None. **E. Paredes:** None. **E. Astigarraga:** None. **C. Miguélez:** None. **G. Barreda-Gómez:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.03

Topic: C.03. Parkinson’s Disease

Title: Intestinal model for Parkinson's disease development suggests goblet cell targeting

Authors: *H. N. TEMPLETON[1], L. A. SCHWERDTFEGER[5], C. MCDERMOTT[2], S. ROCHA[3], R. B. TJALKENS[2], J. A. MORENO[4], S. A. TOBET[4];
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Abstract: Accumulating evidence suggests that Parkinson’s disease (PD) pathology can arise in the gut. A hallmark of PD is the neuronal accumulation of misfolded α-synuclein (α-syn) proteins. The enteric nervous system (ENS) facilitates bidirectional communication between the brain and the gut. Insults to the ENS have been shown to trigger the formation and progression of α-syn pathology from ENS to central nervous system. Rotenone is a pesticide known to induce α-syn aggregation providing a model for PD-like pathology in mice. The goal of this study was to gain insight into how rotenone contributes to accumulation of α-syn aggregate pathologies in the gut and identify cellular mechanisms of aggregate formation and uptake in an organotypic slice model. Mice received intraperitoneal injections of rotenone once daily for 14 days. For immunohistochemistry, 50 μm thick section were cut from sections of ileum and colon. Cell counts in ileum were analyzed via the crypt-villus axis and colon analyzed from crypt to lumen. Rotenone treated tissue showed striking alterations in Goblet cells and their vesicles characterized by their mucopolysaccharides identified by the fluorescently labeled lectin Ulex Europaeus Agglutinin I (UEA) that recognizes terminal α-linked fucose residues. Subjective ratings of Goblet cell vesicles ranged from 4 (fluorescent vesicles filling over 90% of Goblet cell cytoplasm) to 1 (vesicles not visible). Goblet cells with vivid and dim vesicles covering 50-90% of the cell were assigned a rating of 3 and Goblet cells with fewer, but detectable fluorescent vesicles received a rating of 2. Rotenone treated tissue had fewer Goblet cells in the top half of the crypts that were more fluorescent. Control colon had an average rating of 2.88+/-0.12 for bottom half crypt Goblet cells and somewhat less at 2.31+/-0.18 for the upper half. In rotenone treated tissue, the crypt Goblet cells were 2.58+/0.12, but the upper half Goblet cells were 3.67+/0.17. Goblet cells secrete mucus to help prevent pathogen infiltration. These results suggest that rotenone reduces Goblet cell number and perhaps prevents mucopolysaccharide secretion. Rotenone also appeared to decrease immunoreactive collagen I and the tight junction protein, occludin, indicating potential impairment of the intestinal epithelial structure. Ongoing studies are examining changes in immune cells (e.g., ACK2 immunoreactive Mast cells). Together, these results indicate the intestines as a possible origin of PD pathogenesis. Advancing understanding of aggregate formation and uptake in the gut will help identify causal relationships between their dysregulation and the development of PD pathologies.

Disclosures: **H.N. Templeton:** None. **L.A. Schwerdtfeger:** None. **C. McDermott:** None. **S. Rocha:** None. **R.B. Tjalkens:** None. **J.A. Moreno:** None. **S.A. Tobet:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.04

Topic: C.03. Parkinson's Disease

Support: Aligning Science Across Parkinson's: ASAP (ASAP-000592)
The Parkinson's Foundation (PF-FBS-1894)
The Van Andel Research Institute

Title: Adult-onset knockout of Atp13a2 in the mouse ventral midbrain disrupts lysosomal biology and causes progressive degeneration of substantia nigra dopamine neurons

Authors: *M. ERB, N. LEVINE, K. SIPPLE, D. MOORE;
Van Andel Res. Inst., Grand Rapids, MI

Abstract: Although most cases of Parkinson's disease (PD) have no known cause, mutations in over 15 genes cause heritable PD. A surprising number of familial PD genes and PD risk genes are involved in intracellular trafficking and protein degradation. For example, loss-of-function mutations in ATP13A2, a lysosomal type 5 P-type ATPase, and polyamine export protein, have been implicated in early-onset familial PD. Interestingly, *ATP13A2* mutations have also been linked to a variety of other neurodegenerative diseases including Kufor-Rakeb syndrome (KRS), hereditary spastic paraplegias (HSPs) and amyotrophic lateral sclerosis (ALS). Given the severe effects of *ATP13A2* mutations in humans, it is surprising that *ATP13A2* knockout (KO) mice do not exhibit measurable neurodegeneration even at advanced ages. Although KO mice exhibit robust lysosomal pathology and moderate motor deficits that progress with age, their lack of neurodegeneration makes it challenging to study the neuropathological effects of Atp13a2 loss *in vivo*. We suspect germline deletion of *ATP13A2* in rodents may trigger upregulation of compensatory pathways during early development that prevent the neurotoxic effects of Atp13a2 loss in the brain. Since fully developed adult brains are less adaptable than embryonic brains, knocking out *ATP13A2* in the adult brain could enhance the neurotoxic effects of losing this critical gene. To knockout *ATP13A2* in the substantia nigra of young adult mice, we performed unilateral intranigral injections of AAV2/5-Cre-GFP or AAV2/5-GFP in *ATP13A2*$^{flox/flox}$ conditional KO mice. We assessed these mice at 3 or 10 months after Cre injection and found loss of dopaminergic terminals in the striatum at both time points, with more severe loss after 10 months. Cre-injected mice also experience significant dopaminergic neuron degeneration in the substantia nigra over 10 months. Additionally, Cre-injected animals have increased LAMP2 levels in dopaminergic neurons and enlarged LAMP2-positive lysosomes in surrounding tissue. Together, these results suggest that adult-onset *ATP13A2* deletion causes a disruption in lysosome biology that likely contributes to neurodegeneration in this model. We did not observe

phosphorylated α-synuclein, pathologic tau (AT8), or axonal pathology in Cre-injected animals 10 months after surgery. This is the first demonstrated rodent model of *ATP13A2* loss-of-function that leads to frank neurodegeneration. As such, it will provide a useful system for studying neurotoxicity in ATP13A2-related neurodegenerative diseases.

Disclosures: M. Erb: None. **N. Levine:** None. **K. Sipple:** None. **D. Moore:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.05

Topic: C.03. Parkinson’s Disease

Title: CRISPR/Cas9-parkinR275W: a new mouse model of Autosomal Recessive Juvenile Parkinsonism (ARJP)

Authors: *L. ZANETTI[1], M. REGONI[2], M. SEVEGNANI[3], C. DOMENICALE[4], F. ALBANESE[5], F. PISCHEDDA[6], N. MOHAMMADI[2], E. MONZANI[2], A. CIAMMOLA[7], F. VALTORTA[8], S. TAVERNA[2], M. MORARI[9], G. PICCOLI[6], J. SASSONE[2];
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Abstract: Mutations in the *PARK2* gene encoding PARKIN protein cause autosomal recessive juvenile parkinsonism (ARJP), a neurodegenerative disease characterized by early and severe neuronal loss in substantia nigra pars compacta (SNc). No therapy is currently available to prevent the disease onset or slow down its progression. Animal models that can mimic key pathological hallmarks in the patient brains are important for studying ARJP disease mechanism and for developing effective therapeutic strategies. With reference to this, several PARKIN models were generated in the past, but none of them was found to efficiently recapitulate both the genetics and the pathology of the disease. Aiming at filling in this gap, we created a new, innovative knock-in mouse model expressing PARKINR275W missense mutation, which is associated with the highest allelic frequency in *PARK2* patients. We inserted this mutation by CRISPR/Cas9 genome editing; this allowed the mutant parkin expression under the endogenous promoter. Characterization of this model showed early SNc DA neurons dysfunction, severe mitochondrial morphological abnormalities as well as progressive nigral DA neuron loss at 6 months (-23%), 12 months (-30%) and 18 months (-40%). When tested, mice also showed movement and coordination deficits, accounting for later stages motor symptoms of the disease.In conclusion, ParkinR275W model displays major ARJP hallmarks, standing as the first

transgenic mouse model faithfully recapitulating *PARK2*-related ARJP both genetically and phenotypically.

Disclosures: L. Zanetti: None. **M. Regoni:** None. **M. Sevegnani:** None. **C. Domenicale:** None. **F. Albanese:** None. **F. Pischedda:** None. **N. Mohammadi:** None. **E. Monzani:** None. **A. ciammola:** None. **F. Valtorta:** None. **S. Taverna:** None. **M. Morari:** None. **G. Piccoli:** None. **J. Sassone:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.06

Topic: C.03. Parkinson’s Disease

Title: Attenuated dendritic spiking in tuft dendrites of M1 pyramidal tract neurons in a mouse model of Parkinson's disease.

Authors: *M. KURTAM[1], Y. SCHILLER[1,2], J. SCHILLER[1];
[1]Dept. of Neurosci., The Rappaport Fac. of Medicine, Technion - Israel Inst. of Technol., Haifa, Israel; [2]Dept. of Neurol., Rambam Med. Ctr., Haifa, Israel

Abstract: Parkinson’s disease (PD) is a neurodegenerative disorder primarily characterized by motor dysfunction, associated with the loss of dopaminergic innervation to the basal ganglia (BG) system. The primary motor cortex (M1) is the chief cortical brain region responsible for the learning and execution of skilled movements; M1 integrates and processes complex inputs from several cortical and subcortical brain regions and carries out motor commands to lower brainstem and spinal cord execution centers through its Layer 5 (L5) pyramidal tract neurons. Pyramidal tract neurons feature elaborate dendritic tufts, which are electrically active structures that provide these cells with much of their computational power, shaping their output and eventually affecting motor behavior. Importantly, PT tuft dendrites found in cortical Layer 1 (L1) are the primary recipient of BG information relayed through the thalamic motor nuclei. Still, despite the critical position of M1 in linking BG output with motor performance, little is known about its contribution to PD motor features. In this project, we study the involvement of pyramidal tract neurons in the development of PD motor symptoms. Specifically, we investigate the activity of PT tuft dendrites in L1 at single dendrite resolution, looking into their dynamics, their representation of motor information, and the correlation between their activity and somatic responses under control and PD conditions. To do so, we conduct two-photon calcium imaging during the execution of a lever-pull task in head-fixed control and 6-hydroxydopamine-induced PD mice. In addition, we use optogenetic activation of thalamocortical axons in M1 of Parkinsonian mice and examine its effect on the activity of PT tuft dendrites, somas, and motor performance. In control animals, task performance results in large motor-related calcium signals mediated by dendritic spikes in many tuft dendrites. After PD induction, behaviorally, the performance of the lever-pull task is impaired. Two-photon calcium imaging reveals that PD

markedly attenuates dendritic spiking activity in pyramidal tract neurons' tuft dendrites, as well as the somatic firing. These findings suggest PD disrupts thalamocortical induced dendritic spiking in tuft dendrites of pyramidal tract neurons, resulting in weakened output motor commands from M1.

Disclosures: **M. Kurtam:** None. **Y. Schiller:** None. **J. Schiller:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.07

Topic: C.03. Parkinson's Disease

Title: Copper effect administration in a Parkinson's disease experimental model induced by intraneuronal accumulations of iron generated by the administration of SiO_2-Fe in the SNpc of rat.

Authors: *A. PONCE-JUÁREZ[1,2], B. RIVERO-CELAYA[1], C. RUBIO[4], L. TRISTÁN-LÓPEZ[1], F. MISSIRLIS[5], G. ROLDÁN-ROLDÁN[3], J. MORALES-MONTOR[6], C. RÍOS[1], M. RUBIO-OSORNIO[1];
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Abstract: Parkinson's disease (PD) is characterized by degeneration of dopaminergic neurons of the Substantia Nigra pars compacta (SNpc). Among the most important neurochemical damage markers in PD are the increase in iron (Fe) content, decreased activity in the mitochondrial electron transport chain, decreased GSH content and oxidative stress in the SNpc. Copper (Cu) is a trace metal that participates as a prosthetic group of proteins involved in the antioxidant response and iron metabolism, among others. In the present study, male Wistar rats were used, subjected to a unilateral stereotaxic intraigral lesion of 20 μg of SiO_2-Fe in 2 μL of PBS, and 24 hours later they were administered intraperitoneally with 10 μmol/kg of $CuSO_4$. Circling behavior results show significant damage in the groups treated with $FeSO_4$ and SiO_2-Fe in relation to the control group, showing at the same time a significant recovery with the copper post-treatment in both groups. Significant intraneuronal iron deposits, up to 71.51% (± 3.64) and 87.8% (±8.84) where characterized with Perls Staining 3 and 7 days respectively after SiO_2-Fe administration. Groups treated independently with PBS and SiO_2 with a post-treatment of s.s. did not show positive iron staining. Aditionally, preliminary results indicate an increase in the formation of fluorescent lipid products, a reduction in the content of GSH and a decrease in the expression of tyrosine hydroxylase in the groups treated with $FeSO_4$ and SiO_2-Fe, and a partial

recovery due to the post-treatment of $CuSO_4$. The administration of SiO_2-Fe it´s a good model of PD by intraneuronal accumulation of Fe in the SNpc, and the possibility of a therapeutic alternative with $CuSO_4$.

Disclosures: **A. Ponce-Juárez:** None. **B. Rivero-Celaya:** None. **C. Rubio:** None. **L. Tristán-López:** None. **F. Missirlis:** None. **G. Roldán-Roldán:** None. **J. Morales-Montor:** None. **C. Ríos:** None. **M. Rubio-Osornio:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.08

Topic: C.03. Parkinson’s Disease

Title: Novel EAAT2 activator promotes glutamate homeostasis and improves cognition without producing impulsive behaviors in a rodent model of Parkinson's disease.

Authors: *S. DAS, K. MCCLOSKEY, S. KORTAGERE;
Drexel Univ., Philadelphia, PA

Abstract: Novel EAAT2 activator promotes glutamate homeostasis and improves cognition without producing impulsive behaviors in a rodent model of Parkinson’s disease. Glutamate induced excitotoxicity has been shown to be one of the causal factors in several neurodegenerative diseases including Parkinson’s disease (PD). The excitatory neurotransmitter glutamate is tightly regulated by a network of receptors and transporters in the brain. One such transporter is Excitatory Amino Acid Transporter 2 (EAAT2) which is predominantly localized to astrocytes and is responsible for clearing ~90% of the glutamate in the synapse. Under conditions of PD, studies have shown that EAAT2 is downregulated, and aberrant activation of presynaptic glutamatergic neurons leads to excitotoxicity and subsequent death of dopaminergic neurons. Dysregulation of dopamine and glutamate neurotransmission are not only implicated in motor and cognitive impairment in PD but also in promoting compulsive and impulsive behaviors. Therefore, we hypothesized that small molecule activators of EAAT2 that can effectively reduce excitotoxicity will be beneficial to treat motor and cognitive impairment without promoting impulsive behaviors. We tested GTS467 - a novel small molecule activator of EAAT2 that was recently developed in our laboratory in a unilaterally lesioned rodent model of PD. Results from the study confirm that GTS467 significantly improved performance in a 5-choice serial reaction time task with reduced premature impulsive responses and omissions in comparison to vehicle treated PD animals. Ex vivo biochemical analysis of the tissue from prefrontal cortex and striatum from these treated animals showed increase in EAAT2 and a reduction in post synaptic glutamate receptor protein expression with a normalization of signaling functions. These results suggest that GTS467 can be developed as a novel therapeutic to treat excitotoxicity in neurodegenerative diseases.

Disclosures: **S. Das:** None. **K. Mccloskey:** None. **S. Kortagere:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.09

Topic: C.03. Parkinson's Disease

Support: NIH Grant R01AG059721
NIH Grant R01AG067741
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Title: Pathological characterization of a novel mouse model expressing the PD-linked CHCHD2-T61I mutation

Authors: *T. R. KEE[1,2], J. L. WEHINGER[2], P. ESPINOZA GONZALEZ[2], E. NGUYEN[2], K. C. MCGILL PERCY[1], S. A. KHAN[1], D. CHAPUT[3], X. WANG[1], T. LIU[1], D. E. KANG[1,4], J.-A. A. WOO[1];
[1]Pathology, Case Western Reserve Univ., Cleveland, OH; [2]Mol. Med., [3]Cell Biology, Microbiology, and Mol. Biol., Univ. of South Florida, Tampa, FL; [4]Louis Stokes Cleveland VA Med. Ctr., Cleveland, OH

Abstract: Coiled-coil-helix-coiled-coil-helix domain containing 2 (CHCHD2) is a mitochondrial protein that plays important roles in cristae structure, oxidative phosphorylation, and apoptosis. Multiple mutations in CHCHD2 have been associated with Lewy body disorders (LBDs), such as Parkinson's disease (PD) and dementia with Lewy bodies (DLB), with the CHCHD2-T61I mutation being the most widely studied. However, at present, only CHCHD2 knockout or CHCHD2/CHCHD10 double knockout mouse models have been investigated. They do not recapitulate the pathology seen in patients with CHCHD2 mutations. We generated the first transgenic mouse model expressing the human PD-linked CHCHD2-T61I mutation driven by the mPrP promoter. We show that CHCHD2-T61I Tg mice exhibit perinuclear mitochondrial aggregates, neuroinflammation, and have impaired long-term synaptic plasticity associated with synaptic dysfunction. Dopaminergic neurodegeneration, a hallmark of PD, is also observed along with α-synuclein pathology. Significant motor dysfunction is seen with no changes in learning and memory at one year of age. A minor proportion of the CHCHD2-T61I Tg mice (~10%) show a severe motor phenotype consistent with human Pisa Syndrome, an atypical PD phenotype. Unbiased proteomics analysis reveals surprising increases in many insoluble proteins predominantly originating from mitochondria and perturbing multiple canonical biological pathways as assessed by Ingenuity Pathway Analysis, including neurodegenerative disease-associated proteins such as tau, cofilin, SOD1, and DJ-1. Overall, CHCHD2-T61I Tg mice exhibit pathological and motor changes associated with LBDs, indicating that this model

successfully captures phenotypes seen in human LBD patients with CHCHD2 mutations, and demonstrates changes in neurodegenerative disease-associated proteins, which delineates relevant pathological pathways for further investigation.

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Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.10

Topic: C.03. Parkinson's Disease

Support: NCN grant Opus 13 2017/25/B/NZ7/02406

Title: Phenotypic alterations observed in mice genetically depleted of Dbh-expressing cells in the locus coeruleus associated with the prodromal symptoms of Parkinson's disease

Authors: *K. RAFA-ZABLOCKA, J. BARUT, G. KREINER;
Dept. of Brain Biochem., Maj Inst. of Pharmacology, PAS, Krakow, Poland

Abstract: Background: Parkinson's Disease (PD) is characterized by the degeneration of not only dopaminergic (DA) neurons of the substantia nigra (SN), but also noradrenergic (NA) neurons in the locus coeruleus (LC). Loss of NA cells may be associated with nonmotor symptoms of PD i.e. depression or anxiety. Previously we proposed a transgenic mouse model of progressive degeneration of NA neurons induced by deletion of the gene encoding TIF-IA in cells harboring dopamine β-hydroxylase (TIF-IA Dbh^{Cre}). Loss of TIF-IA in NA cells did not result in DA cell death in SN at 3 months, however, increased markers of inflammation and gliosis in SN were observed. Due to the short lifespan of these animals caused by targeting peripheral tissues, further observations were not possible. Therefore, we created a new model combining CRISPR/Cas9 and Cre/loxP system to restrict the genetic modification only to LC.
Aim: Evaluation of a transgenic mouse model of progressive noradrenergic degeneration restricted to LC as a tool to study presymptomatic PD.
Methods: Mice were injected with LVV in LC region (A/P: -5.35; M/L: -0.90; D/V: -3.75). The efficacy of mutation was confirmed by 1) immunostaining of tyrosine hydroxylase in the region of LC, and 2) HPLC measurement of NA content in the striatum. Behavioral testing was performed at two time points: 4 and 6 months after stereotaxic delivery of LVV. Following tests were performed: open field, light-dark box, tail suspension (TST), rotarod, and multiple static rods. Male and female mice were analyzed separately. The statistical significance was confirmed using the t-student test; $p<0.05$ was considered significant.
Results: Immunostaining on LC sections confirmed loss of 30-50% of NA cells 6 months after stereotaxic surgery. HPLC studies revealed reduced striatal content of NA by 40% ($p=0.02$) in

male mutant mice. Behavioral studies showed no changes in locomotor activity and anxiety in mutants. However, after 6 months mutant mice manifested increased immobility time in TST (by 72% p=0,02 in males and by 20% p=0.003 in females). Surprisingly already 4 months after viral transduction mutant mice presented shorter endurance time on the rotarod (by 32%, p=0.007) and longer transit time on a static rod with the smallest diameter (by 58%, p<0.001) in multiple static rods test, suggesting motor coordination disturbances.**Conclusions:** These results suggest that progressive loss of NA innervation induces depressive behavior and impacts motor coordination in mice even before neurodegenerative changes in SN occur, however, further biochemical and electrophysiological studies are needed to explain this phenomenon.

Disclosures: K. Rafa-Zablocka: None. **J. Barut:** None. **G. Kreiner:** None.

Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 042.11

Topic: C.03. Parkinson’s Disease

Support: John and Maurine Cox Endowed Chair

Title: A novel rat model of vascular parkinsonism using the vasoconstrictor endothelin-1

Authors: *C. KAUFHOLD[1], L. GRIMALDO[2], R. SRINIVASAN[3], F. SOHRABJI[4];
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Abstract: Vascular parkinsonism (VP) is a condition in which symptoms similar to idiopathic Parkinson’s disease are produced by one or more small strokes in the basal ganglia. Patients with vascular parkinsonism usually are unaware of the individual strokes that occur, until progressive motor symptoms gradually appear. The diagnosis is suggested by predominant involvement of the legs (“lower-body parkinsonism”), CT or MRI brain scans showing multiple minute or more extensive strokes, and a poor or lack of response to levodopa (as opposed to Parkinson’s disease). There is currently no clinically effective treatment for this condition and no rodent model established in the field to study VP. To stimulate the onset of VP, middle-aged, male Sprague Dawley rats (10-12 mos) were injected with the vasoconstrictor endothelin-1 (ET-1), stereotaxically guided into the dorsolateral striatum (DLS) or sham operation. We tested sensory-motor function using the adhesive-tape removal test and observed increased latency to remove the tape in ET-1 injected animals at 2 days, but not 5 days post ischemia. Immunohistochemistry for tyrosine hydroxylase indicated a reduction of tyrosine hydroxylase-positive cells in the lesioned hemisphere at 21- and 28-days post. These data suggest that ET-1-mediated ischemia into the DLS is a promising model for vascular parkinsonism and will be used as a model to study the brain-gut axis in parkinsonism.

Disclosures: **C. Kaufhold:** None. **L. Grimaldo:** None. **R. Srinivasan:** None. **F. Sohrabji:** None.

Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.12

Topic: C.03. Parkinson's Disease

Support: NIH Grant ZIA-AA000421
Karolinska Institutet/NIH Doctoral program
Swedish Research Council (2019-01170)

Title: Dopamine dysregulation syndrome in a mouse model of Parkinson's disease

Authors: *C. PLEWNIA[1], R. BOCK[2], D. MASINI[3], V. A. ALVAREZ[2], G. FISONE[1];
[1]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden; [2]Lab. on Neurobio. of Compulsive Behaviors, Natl. Inst. on Alcohol Abuse and Alcoholism, Bethesda, MD; [3]Dept. of Biochem. and Biophysics, Stockholm Univ., Stockholm, Sweden

Abstract: Treatment of Parkinson's disease (PD) is based on the use of dopaminergic drugs, such as L-Dopa and dopamine receptor agonists. These substances compensate for the lack of dopamine and counteract motor symptoms, but long-term treatment is accompanied by motor and non-motor complications. Among these latter conditions a neurobehavioral disorder similar to drug abuse, known as dopamine dysregulation syndrome (DDS), is attracting increasing interest because of its profound negative impact on the patients' quality of life. We used a PD mouse model based on a bilateral injection of 6-hydroxydopamine into the dorsal striatum to test whether it reproduces features of DDS. Using the conditioned place preference paradigm, we found that L-Dopa induces conditioned place preference in PD mice, but not in control mice, suggesting that this drug acquires rewarding properties following dopamine depletion. We are currently extending this analysis by examining the reinforcing properties of L-Dopa on a self-administration operant task. In this standard operant test of drug-seeking and drug-taking behavior, PD and control mice lever-press to earn an intravenous infusion of L-Dopa under fixed ratio and progressive ratio reinforcement schedules. Once these tests are complete, we also test for extinction of drug seeking behavior during sessions in which lever presses will result in saline infusions. This procedure will further assess the reinforcing effects of L-Dopa in the PD mouse model. Chronic treatment with L-Dopa in our PD model is accompanied by abnormal signaling in dopamine D1 receptor (D1R) striatal projection neurons, which was measured as hyperactivation of the cAMP signaling cascade and accumulation of the transcription factor ΔFosB in these neurons. Pharmacological inactivation of D1Rs abolished these effects and prevented the development of conditioned place preference for L-Dopa. These results suggest that D1-like antagonism can counteract the psychostimulant effect of L-Dopa. Interestingly, blockade of dopamine D2 receptors was also able to reduce the accumulation of ΔFosB and

abolish conditioned place preference. We are currently investigating the impact of dopamine D3 receptors on the development of DDS and abnormal signaling in D1R striatal projection neurons. Overall our results suggest that, in PD, combined activation of dopamine D1 and D2 receptors by L-Dopa results in DDS, and that this effect may be linked to abnormal accumulation of ΔFosB in striatal neurons.

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Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.13

Topic: C.03. Parkinson's Disease

Support: Contera Pharma A/S

Title: Microglia activation in Substantia Nigra Pars Compacta of mice results in a similar Parkinson's Disease like Phenotype as α-Synuclein overexpression

Authors: *S. L. FRANDSEN[1], K. V. CHRISTENSEN[2], M. M. NIELSEN[1], B. C. LUZON[1], S. RASMUSSEN[2], A. A. JENSEN[1], A. M. KLAWONN[1];
[1]Univ. of Copenhagen, Univ. of Copenhagen, Copenhagen, Denmark; [2]Contera Pharma, Servier, Horsholm, Denmark

Abstract: Clinical evidence points towards a critical role of neuroinflammation in the pathology of Parkinson's Disease (PD). Activated microglia are found in brains of PD patients and are believed to be involved in the development and progression of the disease. Considering the progressive loss of dopaminergic neurons within the Substantia Nigra Pars Compacta (SNc) in PD, the microglia-population in this brain area may be important for disease progression. We aim to implement the first microglia-specific neuroinflammatory PD model and elucidate if affective and motor symptoms are separate and distinct phenomena. For this purpose, we used an AAV-DREADDs-based approach to activate microglia in the SNc of male transgene Cx3cr1Cre mice, 8-12 weeks old. We screened for changes in behaviors over 10 days of consecutive 2 mg/kg CNO i.p. injections and sampled behavior on day 1, 4, 7 and 10 of microglia activation. As the human α-synuclein (α-syn) overexpression mouse model is a well-integrated PD model, that recapitulates progressive dopaminergic neuronal loss, we chose to compare this model to the neuroinflammatory PD model. Mice were injected bilateral in the SNc with an AAV9 leading to overexpressing of the human α-syn, 4 weeks later they were tested in motoric and affective behavioral assays.This work shows selected data where behavioral results from the two models are alike. The results are part of a larger study on acute microglia activation - as presented on the poster 'Chemogenetic activation of microglia in the Substantia Nigra Pars Compacta causes Parkinson's Disease like symptoms'. Mice with SNc microglia activation and overexpression of

α-syn have impaired balance and motor coordination in the Pole test. Microglia activation in the SNc decreases locomotor activity acutely; however after 4 days of continuous microglia activation, locomotion is increased which may be the result of dopaminergic compensatory mechanisms due to neuron loss. Similarly, overexpression of human α-syn induces hyperlocomotion, which indicates a state of denervation hypersensitivity. None of the disease-models causes anhedonic behaviors, as seen in the Orofacial reactivity assay and sucrose preference. As normal exploratory behavior was observed in the OFT and Novel object interaction test, none of the models display an anxiety phenotype. These results suggest that acute SNc microglia activation, in the same manner as α-syn overexpression, result in PD like motor symptoms without influencing affective state. The similarity between PD-phenotypes arising from acute and continuous microglia activation (day 4) and α-syn overexpression may indicate a shared disease mechanism.

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Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.14

Topic: C.03. Parkinson’s Disease

Support: COMECyT FICDTEM-2021-066
PAPIIT-DGAPAIN216821
FESI-PAPCA2021-2022-32

Title: Defective mitochondrial activity and motor alterations in a Parkinson disease model by manganese inhalation

Authors: *C. GARCIA-CABALLERO[1], J. ORDOÑEZ-LIBRADO[1], C. DORADO-MARTÍNEZ[1], A. GUTIÉRREZ-VALDEZ[1], E. MONTIEL-FLORES, Sr.[1], J. PEDRAZA-CHAVERRI[3], O. APARICIO-TREJO[4], L. REYNOSO-ERAZO[2], M. AVILA-COSTA[1]; [1]Neuromorphology Lab., [2]Hlth. Educ. Project, FES Iztacala, UNAM, Estado de México, Mexico; [3]Biol., Facultad de Química, UNAM, CDMX, Mexico; [4]Cardio-Renal Pathophysiology, Inst. Nacional de Cardiología, CDMX, Mexico

Abstract: The increasing life span of the global population has brought as consequence the rising incidence of Parkinson disease (PD) around the world. This represents a health problem that is growing worldwide. Despite this, the precise mechanisms underlying neuronal injury are not yet fully elucidated, whereby it is necessary to develop animal models that reproduce most features of this disorder. In our laboratory has been developed a murine model by manganese chloride ($MnCl_2$) and manganese acetate ($Mn(OAc)_3$) which has shown a depletion of substantia nigra compacta dopaminergic neurons, the exposed animals show tremor, rigidity, and

bradykinesia, these changes were gradual and bilateral consistent with what was reported in PD patients. The aim of this work was to determine mitochondrial I and IV complexes activity in substantia nigra, striatum and globus pallidus. Furthermore, we evaluate motor performance through beam walking and reaching task tests. To achieve this, we used male Wistar rats with an initial weight of 180-200 g. They were randomly divided into two groups; a) rats which inhaled deionized water (n=20) and b) rats which inhaled 0.04 M of $MnCl_2$ and 0.02 M of $Mn(OAc)_3$ (n=20), one hour, three times a week during three and six months. Motor behavior was evaluated weekly. Mitochondrial activity was determined after three and six months of inhalation. All data were analyzed using two-way analysis of variance. Our results show that Mn mixture inhalation induces motor alterations in both tests, similar to those reported in PD patients. Also, impairs mitochondrial complexes I and IV, in the three analyzed nuclei. In conclusion, the PD model by manganese mixture inhalation is a useful tool for analyzing this pathology. The exposed rats showed motor alterations and bilateral and progressive mitochondrial dysfunction such as patients.

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Poster

042. Mouse Models of Parkinson's Disease

Location: SDCC Halls B-H

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Program #/Poster #: 042.15

Topic: C.03. Parkinson’s Disease

Title: A new Parkinson’s disease model - Intracolonic rotenone causes alterations of gut microbiota and induces α-synuclein aggregation in the brain

Authors: *M. SONG, Y.-S. KIM;
Rutgers-Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: Parkinson’s disease (PD) is a progressive neurodegenerative disease characterized by the loss of dopaminergic neurons in the substantia nigra (SN) and is linked to α-synuclein (α-Syn) misfolding and aggregation. The Braak hypothesis states that abnormal α-Syn can spread from the gut to the brain. Rotenone, a mitochondrial complex I inhibitor, has been used to model PD in animals by oral gavage or intraperitoneal injection. Based on this, we hypothesize that intracolonic gavage of rotenone could induce α-Syn pathology in the gut, which propagates to the SN and cause PD pathology. We developed a PD model by rotenone administration into the proximal colon for 6 weeks in C57BL6J mice. 28 weeks after treatment, we investigated α-Syn pathology in the gut led to dopaminergic neuronal death in the SN. Rotenone-treated mice showed increased α-Syn expression in the gut mucosal layer and increased pS129 expression in the myenteric plexus. Proteinase K-resistant staining in the colon showed increased aggregate staining patterns in the submucosal plexus area. In the brain, pS129 expression was increased in

the SN of rotenone-treated mice, similar to the findings in the colon. Tyrosine Hydroxylase (TH)-positive dopaminergic neurons in the SN were significantly reduced in rotenone-treated mice compared to vehicle-treated mice. We assessed motor dysfunction with the rotarod test, and the fall latency time was significantly reduced in rotenone-treated mice. Next, we investigated changes in gut microbiomes by 16S rRNA sequence analysis. Intriguingly, changes in microbiome persist even 22 weeks after rotenone administration. Firmicutes/Bacteroidetes ratio was significantly increased in the rotenone treatment group, and we found a strong negative correlation between *Lactobacillus* abundance and the number of dopaminergic neurons. Our study indicate that abnormal α-Syn may originate in the colon and propagate to the brain, leading to dopaminergic neuronal loss, and gut microbiota might contribute to this process.

Disclosures: M. Song: None. **Y. Kim:** None.

Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.16

Topic: C.03. Parkinson’s Disease

Support: DFG GRK/RGT1789/2
SFB 1506

Title: The role of aging for α-synuclein oligomer accumulation in a mouse model for Parkinson’s disease

Authors: *V. BOPP[1], J. KÜHLWEIN[1], B. MÖHRLE[2], H. GEIGER[2], K. M. DANZER[1,3]; [1]Dept. of Neurol., Universitätsklinikum Ulm, Ulm, Germany; [2]Inst. for Mol. Med., Univ. of Ulm, Ulm, Germany; [3]DZNE, Ulm, Germany

Abstract: Aggregation and Oligomerization of α-synuclein (asyn) plays a central role in the development of Parkinson’s disease (PD). Recently, we identified an age dependent accumulation of asyn oligomers accompanied by neuronal cell loss and diminished motoric abilities (Kiechle et al, 2019) using a PD mouse model based on human asyn protein complementation expressing asyn split Gaussia luciferase (called S1/S2 model). The underlying mechanisms responsible for an accumulation of asyn oligomers during aging are not clear to date. The aim of this study is to understand whether neurons at a certain age are most vulnerable for asyn oligomerization or whether the exposure time to those oligomers is responsible for the detrimental effects on neurons. We have therefore induced asyn oligomer expression for four months (M) at different ages (short expression) in our S1/S2 model and compared them with mice expressing asyn oligomers from birth regarding their motor abilities using Rotarod analysis. While mice expressing asyn for a short time (Syn ON 12-16 M) at the age of 16 month do not significantly differ from control mice (Syn OFF) short expressing mice (Syn ON 20-24 M) at the age of 24 month show a clear decline in motoric abilities compared to control mice similar to

mice expressing asyn from birth on (Syn ON 0-24M). To determine whether motoric alterations correlate with asyn oligomer load, we performed size exclusion chromatography (SEC) with subsequent luciferase activity measurement in each fraction. Short expressing mice at the age of 16 M (Syn ON 12-16) revealed a similar heterogeneous profile of asyn oligomers as asyn expressing mice from birth (Syn ON 0-16 M). In contrast, 24 M old mice differed in their oligomer profile depending on whether asyn oligomers were present since birth (Syn ON 0-24 M) or only for 4 M (Syn ON 20-24 M). To get further insight into the underlying mechanism we studied protein degradation mechanisms as well as performed single cell transcriptomics. Since Cdc42 activity is increasing with age we further asked whether modulation of Cdc42 activity might rescue the age dependent PD phenotype. We find evidence that Cdc42 modulation rescues motoric disabilities and results in a shift of oligomeric asyn species. These results suggest that a certain age seems critical for asyn aggregation and a concordant age dependent motoric decline. Modulation of Cdc42 activity might therefore be an attractive target for therapeutic intervention in PD.

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Poster

042. Mouse Models of Parkinson's Disease

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Program #/Poster #: 042.17

Topic: C.03. Parkinson’s Disease

Support: UPCST/BSBE 2018002

Title: Effect of chemical pesticides on cellular stress response and their possible role in neurodegeneration

Authors: *P. BHADAURIYA[1], R. PARIHAR[2], S. GANESH[3];
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Abstract: Pesticides are synthesized chemicals used for killing or avoiding any pest. These are nearly ubiquitous in our environment, due to their extreme use in agriculture and household work. These are known to cause toxicity to the targeted/non-targeted organisms. Repeated or occupational exposure to these chemicals may have chronic health hazards and a possible risk factor for neurodegeneration. On exposure to various kinds of adverse environmental conditions and chemicals like pesticides, eukaryotic cells activate stress response pathways that respond to promote cell survivability and cytoprotectivity. In recent years, several cytoplasmic foci/granules that accommodate proteins and RNA have been described. Two of them have been studied in more detail as they are related to mRNA silencing: stress granules (SG) and processing bodies

(PB). Stress granules are non-membranous cytoplasmic foci ranging in size from 0.1 to 2.0 μm, composed of non-translating messenger ribonucleoproteins (mRNPs) that rapidly aggregate in cells exposed to adverse environmental conditions or chemicals. Worldwide 4.6 million tons of chemical pesticides are annually used in the environment. Mostly 99% of chemical pesticides released in the environment affect the non-targeted organism and may be the cause of neurodegeneration that affects millions of people globally. SGs formation has been found to be involved in neurodegenerative disorders like Huntington's, Amyotrophic lateral sclerosis (ALS), and Alzheimer's disease (AD). The connection between pesticides and neurodegeneration has not been much explored. Therefore, we have hypothesized that these chemical pesticides may play an important role in SG biogenesis, and this work is an attempt to understand the triggers of neurodegeneration, with particular attention to mechanisms of SGs clearance through the autophagy pathway. We have studied three commonly used pesticides and their effects on stress-responsive proteins in the neuronal cell line as a model system for our study. Intriguingly three of them cause acute stress to the cells and transiently assembled stress-responsive proteins. We observed the transient global shutdown of translation is typically due to the sparking of the cellular stress response pathway via phosphorylation of translation initiation factor eIF2alpha. We have found impaired autophagy in the pesticide exposed cells that could be a possible cause of impaired SGs clearance that eventually leads to neurodegeneration.

Disclosures: P. Bhadauriya: None. **R. Parihar:** None. **S. Ganesh:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Program #/Poster #: 043.01

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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NIH Grant DA056288
NIH Grant MH106392
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Title: Comorbid cocaine dependence and HIV-1 induce frontal-striatal circuit dysfunction

Authors: *K. A. MCLAURIN, H. LI, C. F. MACTUTUS, S. B. HARROD, R. M. BOOZE; Univ. of South Carolina, Columbia, SC

Abstract: Injection drug use (e.g., cocaine) significantly increases an individuals' risk of acquiring human immunodeficiency virus type 1 (HIV-1). Independently, chronic cocaine use and HIV-1 viral protein exposure induce significant neurocognitive deficits and structural reorganization of the frontal-striatal circuit; how the frontal-striatal circuit responds to HIV-1 infection following chronic drug use, however, has remained elusive. The recent extension of the chimeric HIV (EcoHIV) model of HIV-1 infection to rats may afford a biological system to

investigate these comorbidities. First, male (*n*=19) and female (*n*=19) F344/N rats developed a drug dependent phenotype evidenced by a prominent escalation of cocaine-maintained responding. Second, after establishing a history of both sucrose and cocaine self-administration, a pretest-posttest experimental design was utilized to evaluate preference judgment, a simple form of decision-making dependent upon the integrity of frontal-striatal circuit function. During the pretest assessment, male rats exhibited a clear preference for cocaine (0.33 mg/kg/infusion), whereas female rats preferred sucrose (5% w/v). Rats received bilateral retro-orbital injections of either saline (male, *n*=9, female, *n*=9) or EcoHIV (male, *n*=10, female, *n*=10); inoculations which induced significant EcoHIV infection that was harbored in microglia. Two posttest evaluations (3 Days and 6 Weeks Post Inoculation) of preference judgment revealed that EcoHIV, but not saline, disrupted decision-making and blunted extinction learning in both male and female rats. Critically, alterations in preference judgment occurred in the absence of any genotypic alterations in sensitivity to sucrose or cocaine. Third, approximately eight weeks post-inoculation, pyramidal neurons, and their associated dendritic spines, in the medial prefrontal cortex (mPFC) were evaluated as an index of frontal-striatal circuit function. EcoHIV animals exhibited prominent structural neuroadaptations in pyramidal neurons of the mPFC evidenced by decreased synaptic connectivity and a population shift towards an immature dendritic spine phenotype (i.e., increased head and neck diameter). Collectively, the EcoHIV rat affords a biological system to model functional and structural frontal-striatal circuit responses to HIV-1 infection following chronic drug use. Funded by: DA013137, DA056288, MH106392, NS100624.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Program #/Poster #: 043.02

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Blueprint D-SPAN K00NS118713
NIH/NIMH Pilot Award P30MH075673

Title: Lipid Distribution is Highly Variable in the Virally Suppressed SIV-Infected Macaque Brain

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Abstract: Human immunodeficiency virus infection (HIV) still results in higher indices in neurocognitive impairment, mood disorders, and brain atrophy in the modern era of viral suppression (VS) achieved using antiretroviral therapies (ARTs). Despite VS, HIV infection results in considerable bioenergetic strain to the brain. Brain lipids are vulnerable to HIV-associated energetic strain due to their high abundance and unique composition compared to other tissues. Dysfunctional lipid metabolism has been linked to many neurological disorders. Specifically for HIV, studies show viral proteins increase expression of lipid breakdown genes and decrease the abundance of key structural lipids in brain. However, brain lipid metabolism and hallmarks of HIV brain pathology are region dependent. Regional metabolic changes after HIV infection may impact neurologic function and quality of life. Therefore, it is critical to spatially characterize the impact of HIV infection on the brain lipid profile.
To address this gap, we evaluated brain lipid distribution using matrix laser desorption/ionization imaging mass spectrometry (MALDI-IMS) using brain regions from a VS simian immunodeficiency virus (SIV)-infected rhesus macaque, a well-controlled model of HIV infection. MALDI-IMS was performed on parietal cortex, temporal cortex, thalamus, and midbrain as well as using kidney, a peripheral tissue control with common HIV pathology. Of the broadly imaged metabolites, we focused on lipids indicative of fat breakdown, acylcarnitines (ACs), as well as critical structural lipids, phosphatidylcholine (PC) and phosphatidylethanolamine (PE). Species from these lipid classes contain fatty acids (FAs) with a wide range of chain lengths and degrees of unsaturation that impact physical properties and signaling roles.
We found abundance and distribution of ACs, PCs, and PEs is highly species dependent. Distinct patterns were observed in ACs and PCs, while no clear trend was seen in PEs. ACs with very long-chain, polyunsaturated FAs were much more abundant in brain regions than shorter chain, saturated species of AC. Very interestingly, portions of respective regions would be completely absent of ACs. PCs with shorter chain FAs were more abundant in brain and kidney. However, these common PCs, found in other brain regions, were absent in parietal cortex. Overall, our SIV-infected, ART-treated macaque MALDI-IMS data used an innovative approach to determine variability in the localization and abundance of ACs, PCs, and PEs across brain regions and kidney. We are expanding upon this work to evaluate changes in lipid distribution due to differences in infection status and ART regimen.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Title: Methamphetamine alters the astrocyte K^+ channel activities in the context of HIV

Authors: *L. CHEN, S. CASSODAY, A. DONNER, L. AL-HARTHI, X.-T. HU;
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Abstract: Methamphetamine (Meth) is a highly addictive and widely abused psychostimulant. There is no FDA-approved medicine for treating people with Meth use disorders (MUD). Chronic exposure to Meth decreases neuronal activity in certain brain regions, including the medial prefrontal cortex (mPFC, one of the key regulators of cognition and addiction), which may contribute to the mechanism underlying Meth addiction. The mPFC is profoundly altered by Meth and HIV. However, little is known whether such neuronal dysfunction resulting from the alterations in synaptic/intrinsic excitability of neurons, or dysregulation of the extracellular environment (e.g., glutamate and K^+ levels) mediated by astrocytes, or both. Our previous study revealed that acute exposure (4-6 hr) to Meth decreased functional activity of K^+ channels through the TAAR1-mediated signaling pathway in human fetal astrocytes. To determine the effects of Meth and/or neuroHIV on extracellular K^+ homeostasis mediated by astrocytes, we assessed functional activity of various K^+ channels in astrocytes using brain slices containing the mPFC from adolescent HIV-1 transgenic (HIV-1 Tg) rats at the age of 5~7-week (wk). Whole-cell patch-clamping approaches were used to assess mPFC astrocyte dysfunction in the context of Meth abuse and/or neuroHIV. Age-matched F344 non-Tg rats were used as control. First, we accessed the effects of acute Meth on the functional activity of mPFC astrocytes from HIV-1 Tg and non-Tg rats. We found that mPFC astrocytes displayed large, outflowing voltage-gated K^+ currents (I_{Kv}, characteristic of astrocytes) and moderate inwardly-flowing K^+ currents (I_{Kir}) in both HIV-Tg and non-Tg rats. We also identified that acute exposure (>10 min) to Meth in bath significantly reduced the activity of K_{2P} channels in both HIV-Tg and non-Tg rats, leading to depolarization of the resting membrane potential. Further, we will evaluate the effects of chronic Meth exposure on the activity of other subtypes of K^+ (e.g., K_v and K_{ir}) channels in mPFC astrocytes. Meanwhile, we will treat adolescent non-Tg and HIV-1 Tg rats with daily repetitive s.c. injection of Meth (5 mg/kg/day) for 5 days followed by a 3-day withdrawal. Inhibition of the trace amine-associated receptor type 1 (TAAR1), PKC, or PKA-like activity will also be applied to these rats in the context of Meth abuse and neuroHIV, respectively or combinedly, to determine astrocytic K^+ channel dysfunction through TAAR1-mediated PKA and/or PKC signaling pathway.

Disclosures: L. Chen: None. **S. Cassoday:** None. **A. Donner:** None. **L. Al-Harthi:** None. **X. Hu:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.04

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant DA046258
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Title: Involvement of TRPM7 in alcohol-induced damage of the blood-brain barrier in the presence of HIV viral proteins

Authors: M. L. MACK[1,2], W. HUANG[1,2], M. BISHIR[1,2], *S. CHANG[1,2];
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Abstract: Transient receptor potential melastatin 7 channels (TRPM7) play an essential role in maintaining cellular homeostasis of divalent ions, including Mg^{2+} and Ca^{2+}. We have shown that TRPM7 is highly expressed in primary cultures of rat brain microvascular endothelial cells (rBMVECs), the major cellular component of the blood-brain barrier (BBB). The HIV-1 transgenic (HIV-1Tg) rat contains a gag- and pol-deleted HIV-1 viral genome, expressing 7 of 9 HIV-1 viral proteins. This model mimics HIV-infected patients on combination anti-retroviral therapy (cART) and has been used to study the effects of ethanol (EtOH) in the presence of viral proteins. EtOH exerts its effects through many target proteins, including TRPM7; however, the mechanisms underlying involvement of TRPM7 in EtOH modulation on various biological functions, particularly on the BBB integrity, are not fully understood. We analyzed rBMVECs by flow cytometry and demonstrated TRPM7 expression is lower in HIV-1Tg rats at 3-4 weeks compared to F344 control animals, however both F344 and HIV-1Tg rats expressed similar levels at 9 weeks, indicating persistent presence of HIV-1 viral proteins delays TRPM7 expression. Binge EtOH decreased TRPM7 expression in F344 rBMVECs in a concentration dependent manner, and completely abolished TRPM7 expression in HIV-1Tg rats. Using an *in vitro* BBB model, we demonstrated the involvement of TRPM7 in EtOH-mediated changes of BBB permeability. Treatment with 52.2 mM EtOH down-regulated TRPM7 expression and increased the BBB permeability. We then analyzed TRPM7 expression using human BMVECs (hBMVECs). TRPM7 expression was downregulated after 24-hour treatment with EtOH (61%), HIV-1 viral protein gp120 (57%), and in combination (24%). Next, we constructed a hBMVEC *in vitro* BBB model and found that TRPM7 antagonists, NS 8593, CyPPA or SKA-31, enhanced EtOH-mediated changes in the integrity of the BBB. Our data have shown that the combination of TRPM7 antagonists, EtOH and HIV viral proteins work in a synergistic manner to further disrupt BBB permeability. This study suggests that alcohol decreases TRPM7 expression, whereby TRPM7 could be involved in the mechanisms underlying alcohol-induced damage at the BBB of the HIV-1 patients on cART treatment.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Phase II SBIR grant from NIH/NIMH R44MH119621

Title: Development of the hCNS-HIV/ARV platform for pre-clinical testing of anti-retrovirals on iPSC-derived models of the developing human brain.

Authors: *C. G. RINES[1], K. L. GORDON[1], D. RODRIGUEZ[2], A. V. TERSKIKH[2], K. L. JORDAN-SCIUTTO[3], J. H. PRICE[1], P. M. MCDONOUGH[1];
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Abstract: Anti-retroviral (ARV) therapy (ART) is the main treatment for HIV-1 infected people. Although ART has extended the life expectancy and reduced the incidence of HIV-associated dementia (HAD), other forms of HIV-associated neurocognitive disorders (HAND) remain at high levels despite reduced HIV load. ART may contribute to HAND, especially ART with increased brain penetrance. HIV-positive pregnant women are given ART during pregnancy, and infants are given ART prophylactically after birth. HIV-positive children are recommended ART therapy that will continue for their lifetime. ART may thus impact the neurocognitive development of infants and children.
In this project, we developed assays to investigate the effects of ARVs on the developing CNS. We utilized induced pluripotent stem cell (iPSC)-derived Neural Progenitor Cells (NPCs) to investigate the effect of ARVs on viability, self-renewal, senescence, and epigenetic markers. We seeded NPCs (Elixirgen Scientific) on 384-well plates and exposed them to four ARVs (either alone or in combinations) for 3 days (10µM, N=6). We used the Click-iT EdU assay (Thermo Fisher) to identify proliferating cells. We co-stained nuclei with Hoechst and used Vala Sciences' CyteSeer automated image analysis software to count total and proliferating cells (EdU positive nuclei). We also performed immunofluorescence for Sox2 (marker of self-renewal), p16 (marker of senescence) and H3K27ac (marker for epigenetic changes). Elvitegravir and Dolutegravir, two integrase strand inhibitors, reduced live cell count alone and in combination with Tenofovir and Emtricitabine, two nucleoside reverse transcriptase inhibitors. Interestingly, Tenofovir increased the percentage of proliferating cells in a dose dependent manner alone and in combination with other ARVs. The ARV treatments also caused epigenetic changes as analyzed using the Microscopy Imaging Epigenetic Landscape (MIEL) methodology. The MIEL analysis of the H3K27ac labeling demonstrated changes in chromatin features after treatment with ARV. Changes were most pronounced in NPCs treated with Elvitegravir alone and with the combination therapies. These results suggest that ART can affect neural progenitor cell function in vitro and may also affect the developing CNS.

Disclosures: C.G. Rines: None. **K.L. Gordon:** None. **D. Rodriguez:** None. **A.V. Terskikh:** None. **K.L. Jordan-Sciutto:** None. **J.H. Price:** None. **P.M. McDonough:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA048882

Title: Central nervous system (CNS) transcriptomic correlates of brain human immunodeficiency virus (HIV) RNA load in HIV-infected individuals

Authors: *V. R. CANONIGO[1], Y. FU[1,2], E. MASLIAH[3], C. LEFEBVRE[4], P. SANNA[1]; [1]Immunol. and Microbiology, The Scripps Res. Inst., La Jolla, CA; [2]European Bioinformatics Inst. (EMBL-EBI), Hinxton, United Kingdom; [3]Neurosci. and Lab. of Neurogenetics, Natl. Inst. on Aging, Bethesda, MD; [4]INSERM Unit U981, Gustave Roussy Inst., Villejuif, France

Abstract: To generate new mechanistic hypotheses on the pathogenesis and disease progression of neuroHIV and identify novel therapeutic targets to improve neuropsychological function in people with HIV, we investigated host genes and pathway dysregulations associated with brain HIV RNA load in gene expression profiles of the frontal cortex, basal ganglia, and white matter of HIV+ patients. Pathway analyses showed that host genes correlated with HIV expression in all three brain regions were predominantly related to inflammation, neurodegeneration, and bioenergetics. HIV RNA load directly correlated particularly with inflammation genesets representative of cytokine signaling, and this was more prominent in white matter and the basal ganglia. Increases in interferon signaling were correlated with high brain HIV RNA load in the basal ganglia and the white matter although not in the frontal cortex. Brain HIV RNA load was inversely correlated with genesets that are indicative of neuronal and synaptic genes, particularly in the cortex, indicative of synaptic injury and neurodegeneration. Brain HIV RNA load was inversely correlated with genesets that are representative of oxidative phosphorylation, electron transfer, and the tricarboxylic acid cycle in all three brain regions. Mitochondrial dysfunction has been implicated in the toxicity of some antiretrovirals, and these results indicate that mitochondrial dysfunction is also associated with productive HIV infection. Genes and pathways correlated with brain HIV RNA load suggest potential therapeutic targets to ameliorate neuropsychological functioning in people living with HIV.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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Title: Escalated (dependent) oxycodone self-administration is associated with cognitive impairment and transcriptional evidence of neurodegeneration in human immunodeficiency virus (HIV) transgenic rats

Authors: Y. FU[1,2], I. LORRAI[1], B. ZORMAN[3], D. MERCATELLI[4], C. SHANKULA[1], J. MARQUEZ GAYTAN[1], C. LEFEBVRE[1,5], G. DE GUGLIELMO[6], H. KIM[3], P. SUMAZIN[3], F. GIORGI[4], V. REPUNTE-CANONIGO[1], ***P. P. SANNA**[1];
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Abstract: Substance use disorder is associated with accelerated disease progression in people with human immunodeficiency virus (HIV; PWH). Problem opioid use, including high-dose opioid therapy, prescription drug misuse, and opioid abuse, is high and increasing in the PWH population. Oxycodone is a broadly prescribed opioid in both the general population and PWH. Here, we allowed HIV transgenic (Tg) rats and wildtype (WT) littermates to intravenously self-administer oxycodone under short-access (ShA) conditions, which led to moderate, stable, “recreational”-like levels of drug intake, or under long-access (LgA) conditions, which led to escalated (dependent) drug intake. HIV Tg rats with histories of oxycodone self-administration under LgA conditions exhibited significant impairment in memory performance in the novel object recognition (NOR) paradigm. RNA-sequencing expression profiling of the medial prefrontal cortex (mPFC) in HIV Tg rats that self-administered oxycodone under ShA conditions exhibited greater transcriptional evidence of inflammation than WT rats that self-administered oxycodone under the same conditions. HIV Tg rats that self-administered oxycodone under LgA conditions exhibited transcriptional evidence of an increase in neuronal injury and neurodegeneration compared with WT rats under the same conditions. Gene expression analysis indicated that glucocorticoid-dependent adaptations contributed to the gene expression effects of oxycodone self-administration. Overall, the present results indicate that a history of opioid intake promotes neuroinflammation and glucocorticoid dysregulation, and excessive opioid intake is associated with neurotoxicity and cognitive impairment in HIV Tg rats.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.08

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: FAPERJ/Proc. n.º 201.551/2021
FAPERJ/E-26/202.740/2019
CNPq/305511/2018-1

Title: Hypothalamic damage caused by Zika virus in adult mice.

Authors: *E. VASCONCELLOS DE LIMA[1], C. O NOGUEIRA[2], M. O L SILVA[2], G. T VENTURIN[3], I. ASSUNÇÃO-MIRANDA[2], C. P. FIGUEIREDO[4], G. F. PASSOS[2], J. R. CLARKE[4];
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Abstract: Zika virus (ZIKV) is highly neurotropic, causing neurological complications after vertical transmission. Considerable evidence suggests that the consequences of ZIKV infection in adult subjects go far beyond the acute phase symptoms classically associated to arbovirus infections. Recent work has shown that the virus affects the function of the hypothalamus in animals exposed to the virus early during development. Previous results from our group have shown that considerable levels of ZIKV RNA reach the hypothalamus when adult mice are infected, but how the virus affects hypothalamic function remains unknown. This work aims to evaluate the effects of ZIKV isolated in Brazil on the hypothalamus, including the physiological control of metabolism and other hypothalamic functions in adult animals. Two- to 3-month-old male *Swiss* mice received an intracerebroventricular (i.c.v.) injection of ZIKV or virus-free supernatant (control - Mock), and had metabolic alterations evaluated through microPET imaging, astrocytic and microglial number and morphology assessed through immunohistochemistry, and expression of genes directly or indirectly regulated by the hypothalamus evaluated using qPCR. At the peak of brain infection (6 days post-infection; dpi) it was possible to observe an increased microglial activation in the hypothalamus and an increase in IL-6 expression. At 6dpi, using microPET imaging, no significant differences were observed between the experimental groups, demonstrating that the viral infection did not change net glucose consumption in this brain structure. We also observed a significant reduction in synaptophysin (presynaptic marker) suggesting that the infection may be leading to a reduction in synapse levels. However, astrocytic activation was only found at later times after infection (30 dpi). As for circadian cycle genes, there was a significant reduction in CLOCK expression in 6dpi compared to the control, and this effect was reversed in 30dpi. We also observed a reduction in the expression of estrogen alpha and beta receptors in the hypothalamus. Together, these data demonstrate that ZIKV infection causes hypothalamic inflammation capable of promoting synaptic loss and disrupts the expression of both estrogen receptors and of

sleep/wake-related genes.Ethical approval: CEUA/CCS/UFRJ protocol no. 126/18, A15/21-126-18.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.09

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Office of the Director award P51-OD011107

Title: Treatment with monoclonal antibodies reduces neuroinflammation in a monkey model of acute SARS-CoV-2 infection

Authors: *A. BONILLAS, D. BECKMAN, G. B. DINIZ, S. OTT, B. A. SCHMIDT, S. R. ELIZALDI, S. IYER, K. K. A. VAN ROMPAY, J. MORRISON;
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Abstract: In the face of the COVID-19 pandemic, development of rapid, effective treatments against SARS-CoV-2 became necessary. With reports stating that over 80% of COVID-19 patients develop neurological or psychiatric symptoms, and findings from animal models demonstrating SARS-CoV-2 infiltration of the central nervous system via the olfactory route, causing neuroinflammation and neuronal damage- it is imperative that treatments are evaluated for efficacy in reducing neuropathogenesis. Treatment with monoclonal antibodies (mAbs) targeting the SARS-CoV-2 spike protein was shown to significantly reduce viral replication and hyperinflammation in the respiratory tracts of both young-healthy (YH) and aged-diabetic (AT2D) rhesus macaques. However, it has yet to be shown whether mAb treatment is effective in reducing viral infiltration, replication, and neuroinflammation in the brain. Here, we aimed to investigate whether treatment with mAbs was effective in reducing signs of neuropathology through immunohistochemistry and confocal microscopy. Rhesus macaques were infected with a large dose ($2.5x10^6$ PFU) of SARS-CoV-2 and treated with a human mAb cocktail (C135-LS + C144-LS) either 3 days prior to infection (AT2D: 18-23y, n=8) or 1-day post-infection (YH: 3-6y, n=8), then humanely euthanized at 7-days post-infection. Our initial qualitative findings suggest prophylactic treatment with mAbs in AT2D animals reduces inflammation in the piriform cortex (primary olfactory cortex), as demonstrated by a reduction in HLA-DR expression in both microglia and vascular endothelial cells. Overall, AT2D animals in the untreated group also displayed a greater proportion of microglia with an ameboid, reactive morphology. Conversely, there did not appear to be a significant difference in HLA-DR expression or microglial morphology between mAb-treated and untreated groups in the YH

animals. Further work to evaluate presence of viral markers and neuronal damage, along with 3d image reconstruction and quantitative analysis of neuroinflammation, neuronal damage, and viral proteins is currently ongoing. Identifying whether mAb treatment reduces acute neuropathogenesis in SARS-CoV-2 infection may be key in finding ways to reduce susceptibility for long-term neurological complications.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Title: Human brain organoids co-cultured with patient-derived monocytes as a model to study HIV-associated neurocognitive disorders.

Authors: *E. M. MEDINA COLÓN, Y. M. CANTRES-ROSARIO, E. RODRIGUEZ, B. DÍAZ, M. MATOS, V. WOJNA MUÑIZ;
Univ. of Puerto Rico, Med. Sci. Campus, Rio Piedras, Puerto Rico

Abstract: Human immunodeficiency virus (HIV) targets immune cells, including T lymphocytes and monocytes. In patients with HIV, monocytes infiltrate the brain, and it is not clear how they contribute to neuroinflammation and neuronal damage. For this project, we developed an *in vitro* 3D model focused on HIV associated neurocognitive disorders (HAND) neuropathogenesis co-culturing monocytes from HAND patients and brain organoids. This model allows us to identify inflammatory and neuronal injury markers associated to cognitive decline by comparing brain organoids exposed to monocytes from normal and cognitive impaired HIV patients. In the brain, type I interferon receptor (IFNAR) signaling regulates microglia activation through neurons and astrocytes. We hypothesize that HIV dysregulates IFNAR signaling in the brain, contributing to HAND neuropathogenesis. To test this hypothesis, we differentiated human inducible pluripotent stem cells (iPSCs) into 3D cultures and grew brain organoids models for 45 days. The brain organoids were then co-cultured with HAND patient-derived monocytes for 24 hours and were afterwards fixed and homogenized. We tested differentiation markers and IFNAR signaling

molecules using Western Blot and immunofluorescence confocal microscopy. By labeling with sex determining region Y-box 2 (SOX2), a marker of pluripotency, Western Blot confirmed that the iPSCs differentiated into organoids. Vesicular glutamate transporter 1 (VGLUT1), the presynaptic vesicle marker Synaptophysin, and microtubule-associated protein 2 (MAP2) markers confirmed the presence of differentiated and functional neurons in organoids. Co-culture with monocytes increased VGLUT1 and phosphorylated interferon regulatory factor 3 (pIRF-3), but decreased IFNAR levels in organoids. Phosphorylation of IRF-3 induces activation of Interferon alpha and beta cytokines promoters. Interestingly, pIRF-3 levels were lower in organoids co-cultured with HIV positive monocytes compared to organoids with HIV negative monocytes, suggesting HIV infection downregulates the Interferon type 1 response. In conclusion, infiltrating HIV-infected monocytes alter IFNAR signaling in the brain, which may contribute to neuronal dysfunction and chronic neuroinflammation in the central nervous system. Our study demonstrates that brain organoids are an effective model to study how monocytes infiltrate the brain and will allow for further study of therapeutic approaches for HAND patients.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.11

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Title: A single m6A methylation event in the SARS-CoV-2 5’ UTR doubles viral protein translation efficiency

Authors: *A. ALY, P. HAGHIGHI;
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Abstract: The coronavirus disease 2019 (COVID19) pandemic yet continues to worsen despite global vaccination efforts thus highlighting the need for alternative therapeutic strategies. Methylation of viral mRNAs is now emerging as a potent regulator of viral infectivity. Interestingly, neurons display the highest levels of mRNA methylation in humans, and are among the cells most affected by COVID19 with neurological sequelae to infection being most prominent among what is now termed long-COVID. Here, we identify a highly conserved methylation event in the 5’ UTR of the severe acute respiratory syndrome coronavirus (SARS-CoV-2). Using a series of luciferase assays, we demonstrate that despite the predicted structural complexity of the SARS-CoV-2 5’ UTR, it does not hinder translation initiation. With a combination of RNA-IP and pharmacological or genetic inhibition of m6A methylation, we discovered that a single m6A methylation event greatly enhances SARS-CoV-2 5’ UTR translation efficiency. We then developed a modified reverse transcription assay to reveal that m6A methylation of the 5’ UTR reduces mRNA secondary structure complexity, presumably

allowing easier scanning of the 5' UTR by the ribosomal pre-initiation complex. Indeed, follow up analysis of ribosome profiles showed reduced loading of heavy polysome fractions by SARS-CoV-2 5' UTR-initiated mRNAs in the absence of m6A methylation. Finally, we extend our results using analyses of publicly available datasets of 5' UTR secondary structure, methylation, and protein translation efficiency to reveal a novel dual-axis regulating translational efficiency wherein the negative correlation of 5' UTR secondary structure with translation efficiency is ameliorated by local m6A site enrichment in regions of heightened complexity. As a proof of concept, we assayed a selection of genes and identified a class of transcripts characterized by high levels of both m6A sites and secondary structure that demonstrate reduced translational efficiency in the absence of m6A. Our results thus provide a mechanistic description of a key regulator of SARS-CoV-2 protein translation, and point toward novel therapeutic designs to counter the replicative potential of SARS-CoV-2.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Title: Pharmacological immunossupression leads to new rounds of Zika virus replication and behavioral deficits in mice

Authors: *C. NOGUEIRA[1], E. VASCONCELLOS DE LIMA[1], M. O L SILVA[2], N. E SILVA[2], I. ASSUNÇÃO-MIRANDA[2], C. P. FIGUEIREDO[3], J. R. CLARKE[3];
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Abstract: Zika Virus (ZIKV) is a neurotropic arbovirus which causes microcephaly and other neurological malformations following in utero exposure. Several studies have shown that ZIKV persists for several weeks or months in different tissues and body fluids. Using a mouse model of early-life ZIKV infection (post-natal day 3), our group has previously shown that the virus persists in the brain until the animals reach adulthood, even though they no longer show any physical changes comparable to the acute phase of infection. The consequences of ZIKV persistence in different tissues after the acute phase of infection are still unknown. Here we evaluated whether immunosuppression in adult mice submitted to neonatal ZIKV infection induces new cycles of viral replication and new rounds of neurological symptoms. For this, 3-day-old Swiss mice were infected with ZIKV (10^6 PFU via s.c.) and, as adults, submitted to a

protocol of immunosuppression by treatment with Dexamethasone (DX; 50 mg/kg/day via i.p.). As a control, Mock animals (virus-free supernatant) treated with either DX or saline were used. Our results show that DX led to reduced number of circulating lymphocytes, and decreased body weight in ZIKV infected mice but not in Mock mice treated with DX. Viral RNA was quantified by qPCR in different tissues (spleen, brain, muscle and testicles) before and after DX treatment. Although DX treatment did not cause new rounds of viral replication in the brain, spleen or skeletal muscle, it caused a significant increase in viral replication in the testicles. Expression of inflammatory mediators and cytokines were increased in the brain and testicles of ZIKV infected mice treated with DX, compared to infected mice that were not submitted to DX treatment. Interestingly, we also observed that immunosuppression altered the expression of steroidogenic enzymes in the testicles, which are important for testosterone synthesis. DX-treated animals were evaluated for locomotor ability in the open field test and sensitivity to induction of seizures by pentylenetetrazole. We observed that immunosuppression in animals infected with ZIKV leads to a worsening of locomotor performance and an increase in the number of seizures induced by pentylenetetrazole. Our results suggest that testes and brains are reservoirs of ZIKV persistence and that in situations of immunosuppression, new behavioral changes can be observed as well as increased inflammation in the brain and testis, alterations in hormone synthesis pathways in the testes, and that new rounds replication can take place in that tissue. Ethical approval: CEUA/CCS/UFRJ protocol no. 093/19.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 043.13

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01-NS104016
R21-MH125716
R01-HL152804
P51-OD11104
U42-OD024282
U42-OD010568

Title: Glial senescence in HIV-associated neurocognitive disorders is not reversed by cannabinoid receptor agonism

Authors: *M. D. HORN[1], A. R. VAN ZANDT[2], E. M. FROST[3], A. G. MACLEAN[3,4,5]; [1]Brain Inst., [2]Biomed. Sci. Doctoral Program, [3]Div. of Comparative Pathology, Tulane Natl. Primate Res. Ctr., [4]Dept. of Microbiology & Immunology, Tulane Univ. Sch. of Med., [5]Tulane Ctr. for Aging, Tulane Univ., New Orleans, LA

Abstract: Over half of people infected with HIV continue to have HIV-associated neurocognitive disorders (HAND) despite successful viral suppression through antiretroviral therapy (ART). The mechanisms underlying this are unknown, though cellular senescence has emerged as a potential mechanism. However, the extent of cellular senescence within the CNS in the context of HIV remains largely unexplored. HIV and Simian immunodeficiency virus (SIV) induce a senescent phenotype *in vitro*. Interestingly, cannabis exposure decreases the risk of neurocognitive impairment in people living with HIV and cannabinoid receptor agonists have been shown to reverse cellular senescence *in vitro.* We therefore hypothesize that HIV/SIV induces astrocyte senescence and premature neurodegeneration which can be prevented or reversed by cannabinoid receptor agonists. Our four experimental groups were: naïve (n = 5), SIV-infected (n = 5), SIV-infected ART-treated (n = 7), and SIV-infected ART- and CRISPR-Cas9 treated (n = 8) rhesus macaques. All subjects were between five and fifteen years of age and both male and female subjects were included. We examined cellular senescence using immunohistochemistry for $p16^{INK4a}$ in astrocytes, microglia, and neurons of the grey and white matter of the frontal lobe. HALO® image analysis of whole slices found significantly increased $p16^{INK4A}$ expression in the CRISPR-Cas9 SIV-infected group compared to the naïve and SIV-infected ART-treated groups. This might be an acute response to the adeno-associated virus used to deliver the CRISPR due to an anti-proliferative effect on cells but warrants further investigation. Next, we tested the effect of WIN-55,212-2, a cannabinoid receptor agonist, on primary mixed glial cultures from acutely SIV-infected rhesus macaques (n = 3). We found no significant differences in cell viability between control media, media + vehicle (dimethyl sulfoxide), or media + 10μM WIN-55,212-2, indicating that WIN-55,212-2 is not capable of reversing SIV-induced senescence *in vitro*. Future studies will analyze other brain regions implicated in HAND, including the hippocampus, basal ganglia, thalamus, and cerebellum. To test for cellular senescence more rigorously, we will also investigate the senescence markers $p21^{CIP1}$ and GL13. Additionally, we will examine the effect of WIN-55,212-2 on primary mixed glial cultures from chronic SIV-infected rhesus macaques at additional concentrations and assess the presence of senescence markers in the CNS of SIV-infected tetrahydrocannabinol-treated macaques.

Disclosures: **M.D. Horn:** None. **A.R. Van Zandt:** None. **E.M. Frost:** None. **A.G. MacLean:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

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MH122195

Title: Select antiretrovirals from multiple classes induce excitatory synapse loss in hippocampal cultures

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Univ. of Minnesota, Twin Cities, Minneapolis, MN

Abstract: Antiretroviral drugs (ARVs) have significantly improved prognoses for HIV+ individuals. However, HIV-associated neurocognitive disorders (HAND) persist despite undetectable viral loads. Some ARVs have been linked to neuropsychiatric effects that may contribute to HAND, although the mechanisms of these effects remain unclear. Synapse loss correlates with cognitive decline in HAND. It is possible that ARV drugs contribute to synaptic deficits that drive their neuropsychiatric effects. Using an automated synapse imaging assay, we assessed the change in synaptic connections between rat hippocampal neurons cultured in 96-well plates following treatment with clinically used ARVs. Cultures were infected with a helper-dependent adenovirus containing PSD-95-eGFP and mCherry constructs under the human synapsin promoter; the mCherry filled neuronal structure and the PSD-95-eGFP labelled glutamatergic synapses. An automated analysis program was used to identify synaptic puncta. Each plate was validated with positive control treatments 2-bromohexadenoic acid (2-BP) and a combination picrotoxin/4-aminopyridine (pic/4-AP) to confirm the basic functionality of the assay and that functional synaptic networks had formed in the culture, respectively. Cell culture platings were included only if puncta loss in untreated wells were less than 10%, and 2-BP and pic/4-AP evoked at least 10% puncta loss. 24 ARV drugs were tested in this assay at a concentration of 10 µM and 5 elicited more than 10% synapse loss during a 24 h exposure. We found that the protease inhibitors nelfinavir and saquinavir; the non-nucleoside reverse transcriptase inhibitors efavirenz and etravirine; and the integrase inhibitor bictegravir produced synaptic toxicity. These results suggest that some ARVs are synaptotoxic, which may underpin their neuropsychiatric effects. This automated assay provides a means to evaluate new drugs targeted to the reservoir of HIV in the brain and to test drug combinations for potential CNS toxicity.

Disclosures: H.M. McMullan: None. **S.A. Thayer:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5T32GM081740
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Title: S-equol: a novel therapeutic for hiv-1 induced dysbiosis

Authors: *M. T. RODRIGUEZ, K. A. MCLAURIN, C. F. MACTUTUS, R. M. BOOZE; Univ. of South Carolina, Columbia, SC

Abstract: HIV-1 infection has been found to be associated with microbial translocation and dysbiosis. These changes are associated with the progression and severity of HIV associated neurocognitive disorder (HAND) symptoms in humans and are exacerbated in the presence of comorbidities such as substance use disorder and apathy. HIV-1 associated dysbiosis is characterized by an increase in the bacteria Prevotella and a reduction in Akkermansia Muciniphila, a combination that may modulate motivational behaviors through dysregulation of the dopaminergic system. Current research has pointed to the gut-brain-microbiota axis as a potential target to treat cognitive and motivational deficits by improving the composition of the gut microbiome. The present study investigated the effect of S-equol (SE) at 0.2mg on cocaine-maintained responding via modulation of the gut microbiome. The study included 42 female ovariectomized rats, 21 HIV-1 Transgenic rats (Tg) and 21 F344 control animals. A discriminant function analysis (DFA) was used to see if Prevotella and Akkermansia Muciniphila strains could be used to differentiate between genotypes. An ANCOVA was then used to determine if the previously mentioned strains covary with the cocaine-maintained responding on a PR schedule of reinforcement. Baseline measures of Prevotella_UCG_001 were found to significantly covary with lever presses for drug ($p \leq 0.001$), after treatment the change in Prevotella_UCG_001 was found to covary based on treatment and genotype ($p \leq 0.035$), suggesting an initial effect on motivation for cocaine that was altered by SE. The DFA of the pre- and post- microbiome composition revealed SE treated animals could be differentiated by Alloprevotella ($p \leq 0.044$), indicating that SE differently modulates Alloprevotella abundance between genotypes. The use of SE as a treatment for HIV associated dysbiosis was partially supported due to Prevotella_UCG_001 being found to covary with motivation for cocaine and SE being shown to reduce the prevalence of Prevotella_UCG_001 in the gut microbiome of HIV-1 Tg rats, leading to reduced cocaine intake. However, the abundance of Akkermansia Muciniphila was not improved by SE, indicating that SE treatment may not attenuate all the microbial alterations associated with HIV infection. Additionally, F344 animals increased cocaine intake when treated with SE, indicating an altered response possibly due to the difference in microbiome composition. Specifically, SE may be useful in modulating motivational behaviors in HIV-1 Tg rats by decreasing Prevotella strains and altering dopaminergic activity. Support/Grant: NIH 5T32GM081740, MH106292, DA013137, NS100624

Disclosures: M.T. Rodriguez: None. **K.A. McLaurin:** None. **C.F. Mactutus:** None. **R.M. Booze:** None.

Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant R01MH108466
NIH Grant F99NS124189

Title: Frontostriatal white matter microstructure alterations in middle-to-advanced aged adults with HIV

Authors: *S. HASSANZADEH-BEHBAHANI[1], K. F. SHATTUCK[1], F. ZHANG[4], R. C. GALLAGHER, Jr.[1], P. KUMAR[2], L. J. O'DONNELL[4], A. S. VANMETER[3], D. J. MOORE[5], R. J. ELLIS[6], X. JIANG[1];
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Abstract: Despite the advent of combination antiretroviral therapy, the prevalence of HIV-associated neurocognitive disorders (HAND) remains high. While the neural mechanisms of HAND are unknown, prior studies point to dysfunction within the frontostriatal pathway in people with HIV (PWH). In this study, we tested the effects of aging and HIV disease on frontostriatal white matter microstructure as measured by diffusion tensor imaging. Diffusion MRI (dMRI) data were acquired with 70 gradient directions from 66 PWH (mean age: 57 years, 27% female, 56% Black) and 20 demographically comparable controls (mean age: 58 years, 25% female, 60% Black) using a 3T Siemens Prisma Fit scanner at Georgetown University. 65 PWH were on antiretroviral therapy and 90% had undetectable HIV viral load (<50 copies/ml). Whole-brain tractography was computed for each participant using the two-tensor unscented Kalman filter (UKF) method, as implemented in the open-source ukftractography package (https://github.com/pnlbwh/ukftractography). We performed tractography analysis and visualization in 3D Slicer (http://www.slicer.org) via the SlicerDMRI project (https://github.com/SlicerDMRI). Using the O'Donnell Research Group Atlas, the left and right frontostriatal tracts were identified in each participant. Three measures of white matter microstructure were calculated from the frontostriatal tracts: fractional anisotropy, mean diffusivity, and number of streamlines. We then entered these three dMRI measures as dependent variables in 6 ANCOVA models examining the impact of HIV serostatus and age on left or right frontostriatal tract microstructure. Education, sex and race were additional covariates. The alpha level was set to 0.05 and *p*-values were corrected for multiple comparisons using the false discovery rate. Increased age was associated with higher left (p=0.003) and right (p=0.003) frontostriatal mean diffusivity and fewer left (p=0.04) and right (p=0.006) frontostriatal streamlines. Compared to controls, PWH had higher mean diffusivity in the left frontostriatal tract (p=0.046) and fewer streamlines in the right frontostriatal tract (p=0.046). Our results support the hypothesis that frontostriatal white matter microstructure is compromised in middle-to-advanced aged PWH compared to controls. Analysis of the main effects of age and HIV serostatus indicates that deterioration in the frontostriatal connections in PWH could be independently impacted by older age. Identification of frontostriatal changes in PWH could aid early diagnosis of HAND.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Training Grant T32GM008076
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Title: HIV antiretroviral drugs trigger stress granule formation via the integrated stress response in differentiating oligodendrocytes

Authors: *E. VON KRUSENSTIERN[1], L. ROTH[4], L. FESTA[2], J. B. GRINSPAN[5], K. L. JORDAN-SCIUTTO[3];
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Abstract: Approximately half of people with HIV (PWH) experience HIV-Associated Neurocognitive Disorder (HAND), a spectrum of neurocognitive impairments. While more than half of PWH in the United States are virally suppressed or undetectable, the overall proportion of PWH with HAND symptoms has remained unchanged. One of the persistent pathologic features of HAND in the era of antiretroviral therapy (ART) is white matter abnormalities. The duration of ART treatment in patients has been shown to correlate with the observed thinning of the corpus callosum, suggesting that ART drugs may be contributing to this pathology. Previous work in our lab has demonstrated that select ART drugs prevent the maturation of oligodendrocytes (OLs) and remyelination. We have shown that treating maturing OLs with select ART drugs, such as Elvitegravir (EVG), activates the Integrated Stress Response (ISR) and that co-treatment with the ISR inhibitor ISRIB rescues differentiation. Here we show that in addition to ISR activation, treatment with these ART drugs during OL differentiation also leads to the formation of cytoplasmic stress granules (SGs), as shown via immunohistochemical staining of G3BP1, an RNA-binding protein that is ubiquitous to SGs. During cellular stress that inhibits global protein translation, SGs sequester proteins, mRNAs and translation machinery critical for cell survival following resolution of the stress; while they have yet to be thoroughly studied in OLs, their chronic presence in neurons may cause acceleration of neurodegeneration in a variety of neurodegenerative diseases. We further demonstrate that when differentiating OLs are co-treated with ART drugs and ISRIB, SGs do not form, indicating that these SGs are formed canonically via the ISR. Finally, we have found that SGs are present in the cortical white matter

of post-mortem tissue from PWH; In a small cohort of PWH with and without HAND, we observed increased percentage of OLs with stress granules and an increase in the number of stress granules/OL in PWH with HAND compared with neurocognitively normal individuals. These findings suggest that formation of SGs in OLs may contribute to persistent white matter pathology in PWH with HAND. These studies are be among the first to study stress granules in OLs, and have the potential to contribute to the improvement of patient outcomes for PWH on ART.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH MH087332
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Title: A chronic, low-dose treatment with methamphetamine modulates genes implicated in neurodegeneration in a transgenic model of NeuroHIV

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Abstract: The advent of modernized medicine has brought the life expectancy of people living with HIV (PLWH) to that approximately of non-infected individuals. Despite the success of combined antiretroviral therapy (cART), up to 50% of PLWH continue to develop HIV-associated neurocognitive disorders (HAND). The neuropathological features of HIV infection of the brain (neuroHIV) are referred to as HIV encephalitis (HIVE). HIVE includes astrogliosis, microgliosis, and synaptic and dendritic loss. Interestingly, increased amyloid-beta (Aβ)1-42 and phosphorylated Tau (pTau) has been observed in the cerebrospinal fluid of HAND patients but are considered hallmarks of Alzheimer's disease (AD). This suggests the possibility of a

common therapeutic target for both HAND and AD, as they share pathways of injury in the central nervous system (CNS). Methamphetamine (METH) is widely known as a strong and addictive psychostimulant that is frequently abused. METH use is highly prevalent in PLWH and the HIV and drug interaction may promote progression of HAND. PLWH who use METH reportedly have increased neuronal injury, cognitive impairment and viral load. However, low concentration of METH has an approved clinical use as a treatment for patients diagnosed with attention-deficit/hyperactivity disorder (ADHD). Recent studies have also reported potential points of neuroprotection via low-dose METH exposure, where improved learning and memory, and decreased neuronal injury were observed. This suggest that the limited dosage of METH plays a key role in determining whether or not a desired therapeutic effect can be achieved. This study explored in vivo the effects of a long-term, low-dose METH regimen (12 weeks) in the HAND animal model with inducible expression of HIV-1 transactivator of transcription (Tat). We observed METH driven decrease in the expression of cytokines and signaling factors such as Grk2, Grk5, IL1B, IgF1, AMPKa, and STAT3. Interestingly, genes involved in the amyloid precursor protein (APP) processing pathway seems to be affected by the presence of METH. A disintegrin and metalloproteinase 10 (ADAM10) expression was increased, whereas expression of Gamma-secretase activating protein (GSAP) was decreased. This suggests that METH affects APP processing, which could change the ratio of amyloidogenic ($A\beta_{42}$) vs non-amyloidogenic amyloid-beta ($A\beta_{40}$). In our observation, low dose of METH has an effect that may ameliorate AD-like pathology and in turn improved HAND. The observations from this study creates the framework for future identification of potential targets for the treatment of neuroHIV/HAND.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.19

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: Phase II SBIR grant from NIH/NIMH R44MH119621

Title: Ipsc-derived co-culture models with neurons, microglia, and astrocytes for development of the hcns-hiv/arv platform for pre-clinical testing of hiv antiretroviral cns toxicity

Authors: *K. L. GORDON[1], C. G. RINES[1], A. S. SMITH[1], A. V. TERSKIKH[2], K. L. JORDAN-SCIUTTO[3], J. H. PRICE[1], P. M. MCDONOUGH[1];
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Abstract: Despite improvements in HIV treatment that reduce the severity of AIDS-defining illnesses, milder forms of HIV-associated neurocognitive disorders (HAND) still affect half of HIV+ individuals. We have previously tested the toxicity of existing antiretrovirals (ARVs) on iPSC-neurons alone. Microglia and astrocytes may also contribute to the development of HAND: HIV replicates in microglia, and microglia and astrocytes could be activated by ARVs leading to neuronal loss. In this study, we are working to develop a tri-culture model of IPSC-derived neurons, microglia, and astrocytes to improve pre-clinical testing of ARVs for neurotoxicity. To begin to establish co-culture systems, we cultured iPSC-derived microglia with iPSC-derived glutamatergic neurons (Fujifilm CDI). We treated these co-cultures with the nucleoside/nucleotide reverse transcriptase inhibitors tenofovir disoproxil fumarate (TDF) or emtricitabine (FTC), or the integrase strand transfer inhibitors elvitegravir (EVG) and dolutegravir (DTG) for 7 days with 10μM of each drug. We also treated co-cultures with currently prescribed ARV combinations. Compared to cultures of neurons alone, the microglia protected the neurons from ARV-induced toxicity. The presence of microglia increased neuron viability and reduced neuron cell death and neurite loss in the most toxic treatments (EVG, EVG-FTC-TDF, and DTG-FTC-TDF). Microglia also protected against a loss of pre-synapses by EVG and EVG-FTC-TDF and a reduction in calcium transient activity after treatment with EVG, EVG-FTC-TDF, and DTG, FTC-TDF. Our results demonstrate that microglia can protect neurons from ARV-induced toxicity. We are currently incorporating iPSC-derived astrocytes into our co-cultures to further improve the predictivity of our pre-clinical assays for HAND.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: 3RF1AG061001-01S3
3RF1AG061001-01S1

Title: Neuroinflammation in a non-human primate model of SIV infection with intermittent ART treatment

Authors: *G. B. DINIZ[1], D. BECKMAN[1], S. OTT[1], A. BONILLAS[1], S. R. ELIZALDI[2], Y. S. LAKSHMANAPPA[2], C. E. HAWES[2], B. A. SCHMIDT[2], A. M. SCOTT[1], J. JEFFERSON[1], L. NOVIK[1], M. G. BAXTER[3], S. IYER[2], J. H. MORRISON[1];
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Abstract: Antiretroviral therapy (ART) has significantly extended the lifespan of HIV+ individuals, with over 50% of the almost 1.1M Americans living with the virus now 50 years or older. Despite recent progress, HIV remains an important public health concern, resulting in lifelong financial and medical burdens to underserved communities. Notably, 50% of aging HIV+ individuals will develop a spectrum of neurological symptoms collectively known as HIV-associated neurological disorders (HAND), even in individuals with suppressed viremia. Considering that the mechanistic interactions between HIV infection and aging leading to cognitive decline in HAND remain poorly understood, there is an urgent need for highly translational animal models that may facilitate the development of effective therapeutic interventions. Towards that goal, we have developed a non-human primate model of HAND by infecting middle-aged Rhesus monkeys (14-18 years old) with a neurotropic strain of SIV (CL757) and intermittently treating them with ART for 2 years. During the study, plasma, serum, and CSF were periodically collected; MRI and PET scans were performed; animals were behaviorally characterized in a delayed non-match-to-sample task; and at the endpoint, brains were harvested, fixed, and then histologically processed for immunohistochemistry and confocal microscopy. Our preliminary results suggest qualitative neuroinflammatory alterations take place in this animal model, including white matter inflammation mediated by increased microglial HLA-DR expression; perivascular cuffing, with substantial enlargement of the perivascular space induced by large numbers of macrophages; and increased numbers of lymphocytes in the white matter, particularly associated the local vasculature. In one case, we also observed substantial neuronal loss in the visual cortex, in a discontinuous pattern that spans multiple cortical layers and involves an almost complete absence of neurons, a profound increase in microglia/macrophage expansion and activation, reactive astrogliosis, and lymphocytic translocation. While only in its initial stages, our preliminary results suggest that prolonged intermittent ART treatment in SIV-infected macaques results in neuroinflammatory alterations that may recapitulate neurodegenerative processes in aging HIV+ individuals. The next steps include quantitating the observed alterations and integrated analyses of behavior, pathology, imaging, and biomarkers. Further characterizing our model may help us understand the neurochemical and immunological landscape contributing to behavioral impairment in this vulnerable population.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 DA045588
R01 DA034231
F32 DA053163

Title: HIV-1 Tat and morphine exposure dynamically shifts striatal monoamine levels and exploratory behaviors over time.

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Abstract: Despite the advent of combination anti-retroviral therapy (cART), 30-50% of people with HIV (PWH) on cART still exhibit HIV associated neurocognitive disorders (HAND). The neurocognitive impairment is worsened with opioid use disorder (OUD). The basal ganglia are particularly vulnerable to HIV-1 and exhibit higher viral loads and more severe pathology the latter of which is exacerbated by co-exposure to opioids. The striatal region of the basal ganglia is critical for integrating behavioral patterns that impact a wide range of motor, reward, social, attentional, and emotion-related outcomes/actions. Both clinical evidence and experimental models suggest that dopaminergic neurotransmission is disrupted by HIV exposure, however, there is little known about how co-exposure to opiates may alter dopamine and other neurotransmitters in the striatum and the extent to which the neurochemical disruptions alter behavior. Therefore, we assayed motor, anxiety, novelty seeking, exploratory and social behavior, and levels of monoamines and their metabolites, after exposure to Tat and/or morphine for 2-weeks and 2-months in doxycycline-inducible GFAP-driven Tat transgenic mice. A 3-way ANOVA (genotype × treatment × time) revealed a main effect of morphine in decreasing dopamine levels, while levels of norepinephrine and the monoamine oxidase metabolites DOPAC, HVA, and 5-HIAA were significantly elevated in morphine-treated mice. Moreover, Tat and morphine co-exposure significantly interacted to alter dopamine and its metabolites DOPAC, HVA, and 3-MT at both 2 weeks and 2 months. Behaviorally, Tat exposure for 2-weeks or 2-months increased the latency to explore a novel environment or interact with conspecifics, indicating that Tat inhibited initial environmental and social exploratory behaviors. By contrast, morphine exposure across both timepoints reduced latency to seek novel food and lengthened the time spent exploring a novel environment or food, but not a conspecific. Finally, Tat and morphine and duration of exposure interacted to affect locomotor activity, while having no effect on grip strength or rotarod performance. Together, these results provide novel insight into the unique HIV-1 Tat and morphine-induced interactive effects on dopamine and monoamine metabolism that in turn may drive dichotomous effects on neurocircuitry involved in motor and motivational behavior.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 043.22

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R01 MH087332
NIH R01 DA052209
NIH P50 DA026306 (P5)

Title: Impaired behavioral performance caused by methamphetamine and NeuroHIV correlates with long-term changes of neurotransmission-related gene expression

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Abstract: Methamphetamine (METH) use is frequent among people living with HIV-1 and aggravates HIV-associated neurocognitive disorders (HAND). Yet, the underlying pathological mechanisms are incompletely understood. Transgenic mice expressing a soluble viral envelope protein gp120 of HIV-1 in the brain (gp120tg) share key neuropathological features with NeuroHIV/AIDS patients. We previously exposed young gp120tg mice to an escalating METH binge regimen for 25 days and analyzed the animals 7 months afterwards. We found that METH-exposed gp120tg animals displayed reduced post-tetanic potentiation, while both gp120 expression and METH treatment diminished long-term potentiation. Moreover, METH and gp120 exposure caused neuronal injury, behavioral impairment and lasting differential expression of neurotransmission-related genes. For this study, we correlated neurotransmission-related gene expression with gp120 and METH exposure and with behavioral performance. Significant correlations were found for gp120 and METH in both cortex and hippocampus, indicating that neurotransmitter receptors, such as Htr7 and Gria 3, as well as transporters, such as Slc7a11, and signaling factors, such as Grk6, are affected by METH, HIV and their combination. However, behavioral outcomes for spatial learning and memory (Barnes Maze, % time in target quadrant, spatial strategy, errors) correlated most significantly with different genes of all three categories, such as receptors Gabra1, Gabrg3, Htr2b, transporter Slc32a1, and signaling factors Akt1, Plcb3, Sncaip. Thus, METH and HIV affect neurotransmission and behavior in a lasting fashion at multiple levels, and our study provides a framework for the future identification of causal gene networks.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Program #/Poster #: 043.23

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: DA034231
DA044939

Title: Short-term CCR5 inhibition improves spatial learning and memory performance in male but not female mice in a non-infectious model of HIV-associated neurocognitive disorder

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Abstract: People with HIV (PWH) continue to experience HIV-associated neurocognitive disorders (HAND), even though combinational antiretroviral therapy (cART) successfully suppresses HIV replication. Although there has been a shift from severe to milder forms, roughly 50% of PWH develop cognitive, motor and mood problems resulting in a lower quality of life. Neuroinflammation plays a large role in HAND, so targeting an inflammatory pathway could be the solution for therapeutic relief. C-C chemokine 5 (CCR5) is a G-protein coupled receptor (GPCR) that can bind multiple ligands (CCL3, CCL4, CCL5) resulting in functional selectivity. Notably, CCR5 acts as a co-receptor to facilitate HIV entry into cells, in addition to its role as a chemoattractant to aid in the migration of cells to areas of infection. Moreover, CCR5 can play a larger role in behavioral deficits beyond HIV infection. For instance, inhibition of CCR5 has been shown to improve cognitive function in both pre-clinical and clinical models unrelated to HIV infection (Joy *et al.* 2019; Zhou *et al.* 2016; Frank *et al.* 2018; Shen *et al*. 2022). Using a non-infectious murine model of HAND, 2–3-month-old mice were placed on a doxycycline diet to induce Tat expression for an 8-week period that was maintained throughout behavioral testing followed by maraviroc (MVC), a CCR5 antagonist, for 10 days. In males (n=11-12), but not females (n=10), we saw Tat-mediated deficits in the Barnes maze test of spatial learning and memory that were reversed by MVC. There were no differences in open-field behaviors or rotarod coordination in any group. Surprisingly, we did not see significant changes in novel object recognition or fear conditioning tasks which also assess elements of cognitive performance (n=15-17). This may indicate variable effects of CCR5 inhibition dependent upon the specific domains of learning and memory being tested and brain regions involved. To further assess CCR5's role in cognition, we generated a novel cross between homozygous, constitutive CCR5 knockout mice and our Tat-transgenic mice. We tested male mice only (n=9-22) in the same experimental paradigm using Barnes maze, open field and rotarod assessments. We found no deficits in any treatment group. While this could reflect a strain difference, we speculate that this more likely reflects the lack of CCR5 in all tissues during development and afterwards versus a short-term CCR5 inhibition by MVC gavage. Although CCL5 is presumed to be the major ligand for CCR5, we are presently investigating the role that alternative ligands (CCL3 and CCL4) may play in mediating cognitive impairments in HAND.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Program #/Poster #: 043.24

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIDA F32 DA053163
NIDA R01 DA018633
NIDA R01 DA045588

Title: Anhedonia induced by HIV-1 Tat and morphine is associated by neuroinflammation and synaptodendritic injury in the prefrontal cortex

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Abstract: The prevalence of opioid use is high in persons infected with HIV (PWH) and can exacerbate cognitive and emotional deficits. In the CNS, the HIV-1 viral protein Tat can be detected in PWH despite antiretroviral therapies. Tat induces neuronal injury either directly by excitotoxic mechanisms or indirectly through neuroinflammation, which can be worsened by opioid co-exposure. Within the prefrontal cortex, neuroinflammation and synaptodendritic injury can lead to depression and anxiety. However, the mechanisms of increased comorbid depression in PWH who use opioids is underexplored. To investigate HIV-1 Tat- and morphine-induced depressive-like behavior, male Tat(+) and Tat(-) (control) mice were given doxycycline chow for 8 weeks to induce expression of the transgene and administered saline or ramping doses of morphine twice daily (s.c.) during the last 2 weeks of HIV-1 Tat exposure. Mice were given a battery of tests for depressive-like behaviors 4 h after morphine administration to limit opioid-induced locomotor effects. In the 2-bottle choice test, morphine decreased sucrose intake in home cages, whereas in the novelty-induced hypophagia test, Tat decreased sucrose intake in a novel environment, indicating that morphine and Tat differentially alter anhedonia depending on environmental context. Mice were also tested in assays of daily living to model apathy. In home cages, Tat also decreased nesting behavior, whereas morphine decreased burrowing. Together, these data suggest that Tat and morphine differentially induce depressive-like behavior. Within the PFC, Tat increased the proinflammatory chemokine CCL3, and Tat and morphine in combination increased anti-inflammatory cytokine IL-10, whereas morphine by itself decreased the proinflammatory cytokine TNFα. Although these data support the theory that chronic Tat exposure induces innate immune tolerance, depressive-like behavior positively correlated with increased PFC expression of proinflammatory chemokines and cytokines. We previously found

that Tat decreases inhibitory synaptic connections within the anterior cingulate (ACC) region of the prefrontal cortex. Tat, but not morphine, decreased dendritic spine density on pyramidal neurons in layer V of the ACC and Tat increases hyperphosphorylated tau in the ACC and primary motor cortex. Together, our findings suggest that HIV-1 Tat and morphine differentially induce anhedonia and apathy and this depends on environmental factors and is associated with synaptic losses and neuroinflammation within the prefrontal cortex.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: HD043680
MH106392
DA013137
NS100624

Title: Microglia-associated synaptic damage in prefrontal cortex of EcoHIV-infected rat

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Abstract: HIV-associated neurocognitive disorders (HAND) is correlated with synaptic loss and dysfunction. Thus, HIV infection of microglia results in abnormal dendritic spines; however, the mechanism of spine alterations remains unknown. To address our understanding of HIV-related synaptic dysfunction, we used a chimeric HIV (EcoHIV)-based infection model in rats. First, 8 weeks after EcoHIV infection of rats, Iba1 and Ki67 immunostaining was performed to identify infected microglia in the medial prefrontal cortex. Second, synaptophysin was used as an immunohistochemical marker of neuronal damage. In addition, pyramidal neurons from layers II-III of the medial prefrontal cortex (mPFC) were assessed for changes in dendritic complexity, synaptic connectivity, and dendritic spine morphology using ballistic labelling techniques. Third, cellular microtubules and neurofilaments were assessed with the end-binding protein 3 (EB3 or MAPRE3) in vivo and ex vivo with electronic microscopy. We found that microglia were 1) the cell type for harboring EcoHIV and 2) increased in both the density (240.16 ± 11.52 vs. 142.74 ± 17.33 in control, n=8) and percentage (80.19% vs.45.18% in control group, n=8) of $Iba1^+$ cells expressing $Ki67^+$ in the medial prefrontal cortex 8 weeks after EcoHIV. Synaptophysin immunostaining revealed an altered amount of synaptophysin in EcoHIV infection group compared to F344/N control rats. Furthermore, the density of neurofilaments was significantly

decreased in EcoHIV, compared to control primary neurons (725.428 μm^2 ± 94.76 vs. 1319.26 μm^2 ± 96.29, n=8). Collectively, these changes are similar to our findings in human HAND brains, and indicate synaptic dysfunction and microtubule dynamics play a key role in HAND.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Program #/Poster #: 043.26

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH Grant 5R01DA052859-02

Title: Endocannabinoid system receptors are differentially expressed across brain regions and unaltered by simian immunodeficiency virus infection or antiretroviral therapy

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Abstract: HIV systemically infects immune cells, resulting in widespread chronic inflammation despite successful antiretroviral therapy (ART). Prolonged exposure to this myeloid cell induced inflammatory environment causes tissue damage, particularly to the brain. Indicators of neuronal damage are associated with poorer daily functioning in up to 50% of infected people, a growing prevalence due to survival benefits of ART. There is a rapidly growing need for new strategies targeting chronically activated myeloid cells to reduce HIV associated inflammation. While activation of the endocannabinoid system (ECS) elicits homeostatic immune modulating effects in many inflammatory disorders, the ability of the ECS to mediate chronic HIV associated neuroinflammation is not yet established. To evaluate the interplay between the ECS and HIV associated neuroinflammation we employed a virally suppressed juvenile male rhesus macaque model (SIV/ART, n=1-5 per group) and used qRT-PCR to characterize region specific expression of ECS receptors in brain tissue (basal ganglia, cerebellum, frontal cortex, hippocampus, midbrain, occipital cortex, parietal cortex, temporal cortex, thalamus). Canonical ECS receptors were differentially expressed across uninfected brain regions. CB1 was highly expressed in all regions, except for the midbrain. CB2 was not expressed in any brain regions and requires further investigation of potential cell specific expression. Additional receptors perform their main function apart from the ECS, but can be induced to perform alternative functions by canonical ECS lipid ligands. The transient receptor protein vanilloid 1 and 2 (TRPV1 and TRPV2) ion channels are nociceptors found on neurons and respond to heat and lipid ligands. The metabolic markers peroxisome proliferator activated receptor alpha and gamma (PPARα and PPARγ), found in adipose and liver, are also lipid sensors. These receptors

and others comprise the extended ECS and were expressed differentially across uninfected brain regions, excepting PPARg with consistent expression across all brain regions assessed. TRPV1 was most highly expressed in cerebellum and thalamus, whereas TRPV2 was expressed in all regions except the midbrain. PPARa was expressed in all regions except the frontal and occipital lobes. Altogether, novel characterization of ECS receptor expression in the context of SIV and ART shows brain region specific expression that is unaltered by SIV infection or ART treatment. These results lay a foundation for assessing the intersection of anti-inflammatory compounds and the endocannabinoid system and their role in mediating HIV associated neuroinflammation.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Support: NIDA Grant: DA052851
R00 DA039791
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the Cole-Eftink Fellowship Program

Title: Minor Cannabinoids and Terpenes Ameliorate HIV Protein-Related Neuroinflammatory Pain and Primary Microglia Activation

Authors: *E. MOSS, M. SALAHUDDIN, F. MAHDI, A. QRAREYA, M. A DE LEON, M. FOSTER, J. MARSHALL, A. WANAS, M. RADWAN, M. ELSOHLY, N. ASHPOLE, J. PARIS;
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Abstract: Introduction: The human immunodeficiency virus type 1 (HIV-1) persists as a global pandemic with approximately 36.7 million individuals living with the disease worldwide. While combined antiretroviral therapies (cART) are peripherally efficacious at attenuating viremia they are poorly retained in the central nervous system, allowing neuroinflammation to persist thereby contributing to neuropathic pain (observed in 50+% of people living with HIV). Expression of the HIV-1 proteins, trans-activator of transcription (Tat) and glycoprotein-120 (gp120) in mice is sufficient to activate microglia and induce neuropathic pain. Identification of therapeutics capable of attenuating Tat- and gp120-mediated inflammation is necessary. Clinical evidence suggests that minor- (CBD, THC and CBN), varinic- (CBDV), and/or acidic (CBGA and CBDA) phytocannabinoids in addition to terpenes (myrcene, β-caryophyllene, α-/β-terpinolene) may be beneficial for treating inflammatory pain and other adverse effects associated with HIV. We hypothesized that these compounds would ameliorate HIV-protein-mediated microglial

activation *in vitro* and neuropathic pain *in vivo.* **Methods:** Isolated minor cannabinoids and terpenes were prepared by the Univ. Mississippi Marijuana Research Laboratory. Primary human microglial activation state was observed and determined via microscopy 24 hours after exposure to Tat_{1-86} (50 ng/ml) and/or phytocannabinoids or terpenes (1 μM). Transgenic mice that expressed Tat or gp120 (or their non-transgene expressing controls) were treated with phytocannabinoids or terpenes diluted in an EtOH:cremaphor:saline (1:1:8) vehicle (0.5-59 mg/kg, i.p.) and assessed in an abdominal acetic-acid writhing assay. **Results:** CBD and myrcene significantly reduced activation of primary microglia in cell culture. Multiple cannabinoids, including CBGA, CBD and CBN, reduced visceral pain in Tat(+) mice. Intriguing sex differences were observed with several compounds, such as β-CP, significantly attenuating visceral pain in Tat(+) male but not female mice, and CBG attenuating visceral pain in female but not male mice. **Conclusion:** Together, this work demonstrates the anti-inflammatory capacity of phytocannabinoids and terpenes to attenuate neuroinflammation and to alleviate visceral pain associated with exposure to neurotoxic HIV proteins. Future experiments will assess the capacity for phytocannabinoids to alleviate additional modalities of pain including thermal algesia and *neuropathic nociception.*

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Program #/Poster #: 043.28

Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: R01 DA052851
University of Mississippi School of Pharmacy

Title: Cannabis constituents attenuate HIV replication and microglial activation in human cells

Authors: J. J. PARIS[1], F. MAHDI[1], M. M. RADWAN[2], A. S. WANAS[2], M. A. ELSOHLY[2], N. M. ASHPOLE[1];
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Abstract: People living with HIV report greater cannabis use than do seronegative individuals and some reports find cannabis-related benefits for anti-viremia. The mechanisms are not known, but some of the chemical constituents of cannabis may reduce HIV replication and improve immune function. In particular, Δ^9-tetrahydrocannabinol (Δ^9-THC) reduces HIV replication *in vitro* and demonstrates both potentially beneficial and harmful immune effects in macaques with simian immunodeficiency virus (SIV). Unlike Δ^9-THC, some cannabis constituents exert little-to-no affinity for the cannabinoid receptor type 1 (CB1) and instead act at the anti-inflammatory

CB2 receptor, or other non-cannabinoid receptors, and may exert greater anti-viremic efficacy. In support, CB2-selective agonists reduce HIV-1 viremia in cultured human monocyte-derived dendritic cells in a concentration-dependent manner. There are over 120 phytocannabinoid compounds in *Cannabis sativa L.* and many terpenes that may exert a more favorable anti-viremic/anti-inflammatory profile against HIV infection. Using HIV_{BaL}-infected human primary peripheral blood mononuclear cells, we screened the capacity for acidic, varinic, and minor cannabinoids as well as terpenes to attenuate HIV replication as assessed by p24 content at 3, 7, and 10 days post-infection. All compounds were screened at 1 μM concentration. Among acidic cannabinoids, cannabigerolic-acid (CBGA), cannabidiolic-acid (CBDA), cannabichromenic-acid (CBCA), and cannabicyclolic-acid (CBLA) significantly attenuated HIV replication. Among their metabolites, CBG, CBD, Δ^9- THC, cannabinol (CBN), and cannabidivarin (CBDV) also significantly attenuated HIV replication. Among the terpenes assessed, only the sesquiterpene, β-caryophyllene, and the monoterpene, myrcene, significantly attenuated HIV replication (with β-caryophyllene exerting much greater apparent efficacy). Cannabinoids and terpenes were then evaluated for effects against HIV-1 Tat-mediated microglial activation. CBDA, CBCA, THCA, CBNA, CBD, Δ^9- THC, α-pinene, β-pinene, terpinolene, and myrcene reduced the proportion of microglia that were activated by Tat, suggesting that the immunomodulatory benefits of these cannabis constituents may extend to the central nervous system. Together, these findings reveal several phytocannabinoids and terpenes, beyond Δ^9- THC, that may exert beneficial anti-viremic and anti-inflammatory profiles.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

Location: SDCC Halls B-H

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH R21 NS120806-01
Rutgers Busch Biomedical Collaborative grant
NIH NIGMS T32GM135141
NIH NCATS 1TL1TR003019

Title: Dysregulated neuro-immune interaction mediated by interferon activation after HIV-1 viral infection in human microglia-containing cerebral organoid

Authors: *A. STILLITANO[1], A. J. BORELAND[1,2], H.-C. LIN[1], Y. ABBO[1], R. XU[3], R. P. HART[3], P. JIANG[3], Z. PANG[1,2], A. RABSON[1];
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Abstract: Human Immunodeficiency Virus type-1 (HIV-1) Associated Neurocognitive Disorder (HAND) affects up to half of HIV-positive patients and causes long-term neurological consequences, including dementia. There are no effective therapeutics for HAND because of a lack of understanding of the pathophysiology of HIV-induced glial and neuronal functional deficits in humans. To address this unmet need, we have developed a model of HIV-1 central nervous system (CNS) infection using human-induced pluripotent stem cells (hiPSC)-derived mixtures of microglia, neurons, and astrocytes in both 2D cultures and 3D brain organoids to model HIV-1 infection of human microglial cells and identify neurological consequences. hiPSCs were differentiated into primitive microglial precursor cells (PMPs) and infected with HIV-1 virus in a 2D monoculture. We detected HIV p24 production in culture supernatant by ELISA in a time-dependent manner in two different CCR5-tropic virus strains (JRFL and YU2) as well as upregulation of key inflammatory cytokines such as CXCL10 and M-CSF. To further elucidate our findings, we used RNAseq to analyze whole-genome transcriptomics and discovered a robust interferon response in conjunction with upregulation of selected inflammatory cytokines. The induced expression of interferon-stimulated genes such as ISG15, RSAD2, and CXCL10 was confirmed by quantitative RT-PCR for both JRFL and YU2 infection. The infected PMPs were added to human microglia-containing cortical organoids and were shown to integrate into the organoid with persistent viral gene expression for at least two weeks. Ongoing efforts are focused on delineating underlying molecular and cellular mechanisms of HIV infection in the human CNS model The observation of robust interferon-stimulated responses in HIV-1 infected microglia may provide novel insights into the mechanisms of HAND and provide novel routes of therapy development.

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Poster

043. Neuroinflammation: HIV, SIV, and Zika

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Topic: C.07. Neurotoxicity, Inflammation, and Neuroprotection

Support: NIH grant R21HD096309

Title: In Utero Response to Prenatal Infection of a Novel Rat-Adapted Virus

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Abstract: Zika Virus (ZIKV) is a flavivirus that, in cases of in utero fetal infection, is known to cause microcephaly and other neurological and developmental disorders. Like humans, rats exhibit immunosuppression during pregnancy which allows the Zika Virus to infect fetuses in

utero. Building on our previously developed model of prenatal ZIKV infection, we are currently using a novel rat-adapted virus that has been passaged on rat cochlear microglial cells, which is expected to produce a stronger infection in rats than a human-derived virus. Pregnant rats were infected at gestational day 18 as previously established by our model. They were inoculated either with the virus, a diluent control, a UV-inactivated virus (iZIKV), or a non-neutralizing antibody combined with the virus. This antibody was included to increase infection in vivo through antibody-dependent enhancement (ADE). This process can drive entry of the virus into cells that express an Fc receptor, a receptor located on immune cells and involved in antibody recognition. We collected tissue from fetuses 24, 48, and 72 hours following maternal infection. Peripheral and brain tissue were collected to assess the response to viral infection in utero to determine timeline of fetal infection. Tissue from both males and females were collected although sex is not analyzed as a biological variable at this time due to incomplete power. Preliminary findings suggest that maternal immune response is not activated by ZIKV inoculation suggesting that any effects in the offspring are due to direct infection. Immune activation in the placenta is highest at 24 and 48 hours following infection. Together these results will help validate our model as etiologically relevant and will characterize the fetal immune response to ZIKV infection. Continuation of ZIKV research is imperative to understanding the full spectrum of neurological and immunological consequences of ZIKV.

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Poster

044. Brain Injury: Molecular Mechanisms II

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Program #/Poster #: 044.01

Topic: C.10. Brain Injury and Trauma

Support: 2022 Kangwon National University Hospital Grant

Title: Establish severe ischemia model with biomarker by unilateral common carotid artery occlusion in the gerbil

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Abstract: Compared to mice or rat ischemia model, CCA occlusion model(UOCCA) of gerbil have advantages of simplicity and reproducibility due to lack of the posterior communicating arteries. Previously we reported 30 min of UOCCA can be used to study mechanisms of infarction and/or regional selective neuronal death/loss. We established severe ischemia UOCCA of gerbil with biomarker.Male Mongolian gerbils were obtained at 6 months of age (body

weight, 70-75 g) from the Experimental Animal Center. the gerbils were carried out at left side for 1 hour/ 45minutes using aneurysm clips after a midline incision under general anesthesia. After surgery, neurological signs of the animals were observed as a previously reported method for quick evaluation of six neurological signs. Interleukin 6 and Tumor necrosis factor a were checked at 1hour, 24hour and 5 days after UOCCA for finding biomarker for severe ischemia. For histology in the cerebral cortex, striatum, thalamus and hippocampus were evaluated by H&E, CV and TTC at 5 days after UOCCA. This study reveals that 45 minutes of UOCCA induced infarcts in cerebral cortex, striatum, thalamus, and hippocampus depending in relation with ischemic symptoms in gerbils at 5 days after UOCCA. A substantial degree of reactive astrocytes was found although numbers of GFAP cells were different depending on the ischemic regions 5 days after UOCCA. IL-6 could be helpful for predicting severe ischemic injury. Compared to 45min UOCCA, 4 out of 7 gerbils died after 1hour UOCCA. As compared to high mortality, survived gerbils were recovered from transient neurological deficit and without severe ischemic lesion in H&E, TTC at 5 days after UOCCA.UOCCA of gerbil have been known for the advantages of simplicity and reproducibility. However, several reports were published about the variability of neurological outcome after UOCCA.
Comparted to high mortality from mild UOCCA model, it was reported that only 30-40% of animal had neurological deficits after UOCCA. It comes from combined agenesis of anterior communicating artery or not. Because of the importance of brain ischemia model with reproducibility, IL-6 could be helpful for predict severity of ischemic injury.

Disclosures: **C. Kim:** None. **M. Won:** None. **T. Lee:** None. **S. Lee:** None. **J. Kim:** None. **J. Bang:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.02

Topic: C.10. Brain Injury and Trauma

Support: R01-NS109585
R01-AG051902
F31-NS122471
T32-NS105864
P30-NS045758

Title: Traumatic brain injury alters sleep architecture and homeostatic responses to REM sleep disruption by sleep fragmentation

Authors: *Z. TAPP-POOLE[1], C. J. COTTER[1], S. HOULE[1], C. REN[1], Z. ZIMOMRA[1], J. F. SHERIDAN[2], J. P. GODBOUT[4], O. KOKIKO-COCHRAN[3];
[1]The Ohio State Univ., Columbus, OH; [2]Inst. for Behavioral Med. Res., [3]Neurosci., Ohio State Univ., Columbus, OH; [4]Neurosci., Ohio State Univ. Dept. of Neurosci., Columbus, OH

Abstract: Traumatic brain injury (TBI) impairs the ability to restore homeostasis in response to stress through hypothalamic-pituitary-adrenal (HPA)-axis dysfunction. Many stressors result in sleep disturbances, thus mechanical sleep fragmentation (SF) provides a physiologically relevant approach to study the effects of stress after injury. We have previously shown that 30 days of post-TBI SF exacerbates injury-induced inflammation and causes dorsal hippocampal-dependent behavioral dysfunction. We hypothesize that TBI alone would disturb sleep/wake-transitions and SF would exacerbate these responses, synergizing with TBI-induced sleep disturbances and acting as a stressor after injury to compromise recovery. To test this, equal numbers of male and female mice were given moderate lateral fluid percussion TBI or sham injury and implanted with a telemetry sensor. This sensor measured body temperature, home cage activity, rapid eye movement (REM) sleep, and non-REM sleep. Animals were then left undisturbed or exposed to daily, transient SF for 30-days post-injury (DPI). Acutely after injury, TBI increased non-REM sleep compared to shams during the light period 1-7 DPI, consistent with previous reports. This was associated with enhanced home cage activity of TBI mice during the dark period, indicating hyperactivity. SF selectively disrupted REM sleep during the SF period regardless of injury. Intriguingly, sham SF mice had decreased home cage activity during the SF period while TBI SF mice had the most. Chronically, TBI no longer influenced time spent in non-REM sleep from 23-30 DPI. TBI did, however, decrease home cage activity during the dark period. SF continued to selectively disrupt REM sleep during the SF period but interacted uniquely with sham and TBI groups. Sham SF mice had decreased body temperature and activity during the SF period, which could indicate enhanced sleep need and a compensatory homeostatic response to loss of REM sleep to decrease energy output. TBI SF mice had increased REM sleep during the dark period compared to TBI alone, which may reflect an alternative compensatory response. Altogether, these data demonstrate SF selectively disrupts REM sleep throughout chronic exposure and is associated with time- and injury-dependent changes in sleep architecture and homeostatic measures. Inability to appropriately compensate for REM sleep disruption after TBI could contribute to post-injury sequelae and worsen long-term outcome.

Disclosures: Z. Tapp-Poole: None. **C.J. Cotter:** None. **S. Houle:** None. **C. Ren:** None. **Z. Zimomra:** None. **J.F. Sheridan:** None. **J.P. Godbout:** None. **O. Kokiko-Cochran:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.03

Topic: C.10. Brain Injury and Trauma

Support: Abrexa Pharmaceuticals

Title: Zucker rats as clinically relevant model for POCD research

Authors: *K. OBERMAN[1], R. SCHOEMAKER[1], E. VILLAFRANCA[2];
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Abstract: A major complication in patients undergoing surgery is postoperative cognitive decline (POCD), an early form of dementia. Main risk factors are age and preoperative health. Previous research showed that although anti-inflammatory treatment was successful in young healthy rats with POCD, it appeared less effective in aged rats. Aged rats, bred in pathogen-free conditions with optimal food may not best represent the general aged patients that develops POCD. Therefore, we explored a potentially more clinically relevant model; Zucker rats, presenting several risk factors for POCD, including hypertension, insulin resistance, chronic low grade inflammation and mild cognitive impairment. Aim of this study was to assess POCD development in the Zucker rats. Male 25-30 weeks old Zucker rats were randomly allocated into two groups: Zucker no surgery (n=15) or Zucker surgery group (n=15). Male lean littermates served as control (n=14). Zucker surgery rats underwent major abdominal surgery. All animals where housed individually and locomotion sensors were placed above the cages to record activity. Post-operative day 7-11 behavioral testing was performed, including an Open Field test (OF), Novel Object and Novel Location Recognition test (NOR/NLR) and the Morris Water Maze (MWM) to assess exploratory behavior, object memory, spatial learning and memory, and cognitive flexibility, resp. Day 14, rats were sacrificed and blood and brain tissue were collected. Compared to lean rats, Zucker rats without surgery displayed an disturbed circadian rhythm, including less diurnality and more fragmentation. Moreover, Zucker rats had increased plasma triglycerides and showed impaired long term spatial memory in the MWM. After surgery Zucker rats showed a disturbed circadian rhythm, reflected in more day activity. In the OF, Zucker surgery rats payed less visits to the center at similar distance moved. Furthermore, short term spatial memory tended to be decreased, reflected in a worse performance in the NLR. No effects of surgery were observed in spatial learning capacity, cognitive flexibility and long-term spatial memory. In addition, neuroinflammation, obtained from microglia activity, and neurogenesis were not affected by surgery. Zucker rats displayed POCD risk factors, including disturbed circadian rhythm, increased triglyceride levels and impaired long term spatial memory. However, despite these risk factors, Zucker rats seemed to develop only mild POCD characteristics, compared to young healthy rats. Therefore, either the observed risk factors are not contributing to POCD, or the Zucker rat model may be less suitable for therapeutic studies.

Disclosures: K. Oberman: None. **R. Schoemaker:** None. **E. Villafranca:** A. Employment/Salary (full or part-time):; Abrexa Pharmaceuticals. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; Abrexa Pharmaceuticals. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abrexa Pharmaceuticals. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Abrexa Pharmaceuticals.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.04

Topic: C.10. Brain Injury and Trauma

Support: R21NS119991
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Brain & Behavior Research Foundation (NARSAD)
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NSF PRCEN undergraduate and graduate Fellowships
NEUROD-ID undergradute fellowship

Title: Closed-head injury increased avoidance in the absence of a fearful stimulus in rats.

Authors: *O. MARTÍNEZ GUZMAN, M. CACERES-CHACON, S. RODRIGUEZ-ROSADO, M. RIVERA-LÓPEZ, G. HERNANDEZ-BUSOT, D. SIERRA-MERCADO; Univ. of Puerto Rico Sch. of Med., San Juan, PR

Abstract: Concussion, the most common form of brain injury, may impair fear-related behaviors. One type of fear-related behavior, avoidance, occurs when there is a need to escape from difficult situations such as an aversive stimulus (i.e. foot shock) is presented in the presence of a reward (i.e. sucrose pellets). The effects of concussion on avoidance remain unknown. A concussion can be modeled in rodents with a closed head injury (CHI). In our model, a guide tube is placed above the head of anesthetized rats, and weight is dropped through the tube. In the current study, we hypothesize that CHI will impair avoidance behavior. One hour after CHI or Sham injury, locomotion behaviors were assessed in an open field to test for motion deficits, and CHI did not affect the distance traveled. In the platform-mediated avoidance, rats were conditioned in an operant chamber to auditory tones co-terminating with a mild foot shock. An acrylic platform in the opposite corner of the sucrose-delivering bar allowed rats to avoid the shocks. Animals that underwent a CHI spent more time on the platform throughout the test session during the absence of the tone, even though they learn previously that the absence of tone was a safe period. These results suggest that brain injury results in excess avoidance behavior ($p=0.0127$). On the other hand, previous studies showed that the infralimbic cortex (IL) is important for avoidance behaviors. One hour after the avoidance session rats were perfused transcardially and brains were removed for c-Fos immuno-labeling. Preliminary data of cFos cell counts show that CHI does not affect cellular activity in the IL of animals that suffered a CHI. The translational relevance of this work suggests that brain injury may contribute to mental health disorders since excess avoidance is characteristic of patients with fear and anxiety disorders.

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Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.05

Topic: C.10. Brain Injury and Trauma

Support: VA Polytrauma/TBI System of Care

Title: Functional recovery and neuronal plasticity are enhanced in rats receiving anti-Nogo-A antibody therapy following a traumatic brain injury

Authors: *B. POWERS[1], S.-Y. TSAI[1], R. FARRER[1], S. TON[1], S. CHAUDHARY[1], G. KARTJE[1,2];
[1]Edward Hines Jr. Veteran's Affairs Hosp., Hines, IL; [2]Mol. Pharmacol. and Neurosci., Loyola Univ. Chicago, Maywood, IL

Abstract: Traumatic brain injury (TBI) can cause sensory and motor functional deficits, and recovery is often slow and incomplete. Currently, there are no effective pharmacological treatments for recovery from TBI, but recent research has indicated great potential demonstrated by anti-Nogo-A antibody therapy. This antibody neutralizes Nogo-A, an endogenous protein that inhibits neuronal plasticity. We modeled TBI in adult male, Long Evans rats, using the controlled cortical impact (CCI) method, resulting in focal brain damage and motor deficits very similar to those observed in humans with a TBI. We hypothesized that treatment with anti-Nogo-A neutralizing antibodies following TBI would result in disinhibited axonal growth from the unlesioned cortex, the establishment of new compensatory synaptic connections, and improvement in functional outcome. First, rats were trained on the skilled forelimb reaching task, which requires the rats to reach through a small window to retrieve a food pellet. Rats were also assessed for baseline performance on the horizontal ladder rung walking task. Then, all rats were given a TBI and one week later were randomly divided into 3 groups: TBI only (n=7), TBI + anti-Nogo-A antibody (n=8), and TBI + control antibody (n=8). Testing on the skilled forelimb reaching task and horizontal ladder rung walking task resumed 3 days after TBI and continued until 8 weeks after TBI. Rats then received an injection of the anterograde neuronal tracer, biotinylated dextran amine (BDA), into the motor cortex contralateral to the TBI, to assess axonal plasticity. Our results indicate significant improvement on both skilled motor tasks in rats that received anti-Nogo-A antibody therapy. In addition, analysis of BDA-positive axons revealed cortico-rubral plasticity to the de-afferented red nucleus in rats in this treatment group. We conclude that anti-Nogo-A antibody treatment may improve functional recovery via neuronal plasticity to brain areas important for control of skilled movements, and this treatment shows promise to improve outcomes in humans who have suffered a TBI.

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Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 044.06

Topic: C.10. Brain Injury and Trauma

Support: An endowment for the Richard T. Anderson Chair in Neurosciences, University of Oklahoma College of Pharmacy (KMS)

Title: Upregulation of Nociceptin Orphanin FQ (N/OFQ) and its receptor correlates with sensory and vestibular dysfunction following mild and moderate traumatic brain injury (TBI) in rats

Authors: *O. N. AL YACOUB, H. O. AWWAD, Y. ZHANG, K. M. STANDIFER;
Dept. of Pharmaceut. Sci., Univ. of Oklahoma Hlth. Sci. Ctr., Oklahoma City, OK

Abstract: Vestibulomotor deficits and chronic pain are prevalent in patients with traumatic brain injury (TBI). We previously reported that the opioid receptor superfamily member, nociceptin/orphanin FQ (N/OFQ) peptide (NOP) receptor and its endogenous neuropeptide are involved in blast TBI-induced vestibulomotor dysfunction. The goal of this study was to investigate changes in N/OFQ and NOP receptor levels following mild (mTBI) or moderate TBI (modTBI) and its relationship to TBI-induced deficits in wildtype (WT) and NOP receptor knockout (KO) Wistar Han rats. Male rats (9-16 weeks) received a craniotomy with mTBI or modTBI controlled cortical impact to the left cerebral hemisphere or without (sham). Injury recovery was assessed using the modified neurological severity score (mNSS) on day 8 post-TBI; scores increase with severity. Vestibulomotor function was determined using the rotarod. Sensitivity to mechanical and thermal stimuli was assessed by measuring hind paw withdrawal threshold from pressure (PWT) and tail flick (TF) latency for removal from heat source, respectively. Brains were collected on day 8 following euthanasia. Tissue dissected from the ipsilateral (ipsi) hemisphere and the corresponding region from the contralateral (contra) hemisphere was homogenized for biochemical assays. N/OFQ and NOP receptor levels were measured using radioimmunoassay (RIA) and immunoblotting, respectively. Two-way ANOVA or Pearson's Correlation Analysis was performed (as appropriate) on data from each genotype. Results: TBI produced an injury severity-dependent increase in N/OFQ levels in the ipsi side but not the contra side of WT brain on day 8 (*$p<0.0005$ compared to sham rats; n=5-6). No differences in N/OFQ levels between sham and TBI or between ipsi and contra sides in any KO rat group were found (n=5-6). Levels of N/OFQ in either side of WT brain negatively correlated with rotarod performance (*$p=0.0003$, ipsi side; *$p=0.0032$, contra side; n=16), and positively correlated with mNSS scores (*$p=0.0141$, ipsi side; *$p=0.0033$, contra side; n=16) on day 8. Levels of N/OFQ in the ipsi side of WT brain negatively correlated with PWT and TF values (*$p<0.005$), consistent with higher sensitivity to sensory stimuli. There was no correlation between behavioral scores and N/OFQ levels in KO rats. NOP receptor expression was higher in tissue from the ipsi side of modTBI WT brain compared to the contra side (*$p=0.01$, n=5), with a significant effect of side. In conclusion, TBI increased N/OFQ and NOP receptor expression in brain tissue of WT rats 8 days post-TBI that correlated with sensory and motor dysfunction induced by TBI in WT but not NOP receptor KO rats.

Disclosures: O.N. Al Yacoub: None. **H.O. Awwad:** None. **Y. Zhang:** None. **K.M. Standifer:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

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Program #/Poster #: 044.07

Topic: C.10. Brain Injury and Trauma

Support: MOST Grant 111-2636-B-038-006-

Title: Zinc finger protein 179 promotes nerve regeneration and axon extension following traumatic axotomy

Authors: *J.-Y. CHUANG;
Taipei Med. Univ., Taipei, Taiwan

Abstract: Secondary axotomy/axon degeneration induced by traumatic brain injury (TBI) is considered as a key risk factor of the early onset of neurodegenerative diseases. Therefore, a detailed investigation of the mechanisms underlying TBI is thus important to identify effective treatments and improve the disease outcome. We recently discovered that Znf179, also known as RING finger protein 112 (Rnf112), acts as a novel neuroprotector and interacts with sigma-1 receptor (Sig-1R). After treatment with a Sig-1R agonist DHEAS, Znf179 increased its interaction with various cytoplasmic proteins, annotated to functions in “Protein Synthesis” and “Cellular Movement.” Moreover, we found an obvious localization of Znf179 on neurite growth cones (NGCs), and an increase in axonal outgrowth after Znf179 overexpression and DHEAS treatment. In addition, we have known histone deacetylase 6 (HDAC6) as a binding partner of Znf179. Treatment with MPT0B291, a potent HDAC6 inhibitor, induced the association of Znf179 and the translation factors polyadenylate-binding protein-1 (PABP), which may stimulate translation initiation and promote protein synthesis. Therefore, in the study, we found that the HDAC6/Sig-1R/Znf179 pathway plays a pivotal role in axonal regeneration and functional recovery after traumatic axotomy. Using a pharmacological strategy-induced Znf179 functions via inhibiting HDAC6 activation and altering Sig-1R signaling can facilitate intrinsic regenerative outgrowth after TBI.

Disclosures: J. Chuang: None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 044.08

Topic: C.10. Brain Injury and Trauma

Support: NIH 1R21NS111099
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Title: Characterization of a rat model of repetitive mild traumatic brain injury at 2 weeks post-injury

Authors: K. M. FRONCZAK, A. ROBERTS, M. PARRY, E. HOLETS, J. HENCHIR, C. DIXON, ***S. CARLSON**;
Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Repetitive mild traumatic brain injury (rmTBI) is a prominent public health concern, with a high prevalence in athlete and military populations, with linkage to debilitating chronic sequalae. It is well known that repeat concussive events are compounding in terms of susceptibility and resulting symptomology; however, the underlying pathophysiological mechanisms of rmTBI remain poorly understood. Developing reliable and well characterized preclinical models of rmTBI is important in the investigation of biomolecular injury mechanisms, as models can have varying parameters, effecting the pathology of the resulting injury. The fluid percussion injury (FPI) model is a reliable method of mTBI replication in rodent subjects, though it is currently underutilized in rmTBI research. In this study, we performed a novel characterization of a variation of the repetitive mild FPI (rmFPI) model, with a focus on understanding the influence of frequency of injury. We characterized the grated acute behavioral impairment and histopathology occurring in response to one, two or four mild FPI (1.25 atm) or sham control surgeries, implemented 24h apart (n=12 per injury status). Beam balance and Morris water maze performances revealed significant differences in outcomes between the four groups, with impairment severity increasing with additional injuries. Qualitive analysis of contusion formation, assessed by Cresyl violet staining (n=6 per injury status), revealed qualitative cell loss following four FPI only. These findings led us to further characterize the subacute pathophysiological outcomes of the dual FPI (dFPI) (n=6 per group), as this injury demonstrated compounding behavioral dysfunction compared to sham and single FPI, while showing limited overt lesion formation. dFPI led to no significant changes in synaptic density two weeks post-injury, as measured by synaptophysin, but did result in a striking increase in Iba-1-positive microglia in the thalamus, cortex and subcortical white matter, as quantified by Iba-1 immunoreactivity. With this examination, we provide an account of the subacute post-injury outcomes occurring in response to rmFPI, with these parameters, also demonstrating the reliability of the FPI model in rmTBI replication.

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Poster

044. Brain Injury: Molecular Mechanisms II

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Program #/Poster #: 044.09

Topic: C.10. Brain Injury and Trauma

Support: NHMRC Ideas Grant

Title: A new rat model of intimate partner violence (IPV)-related brain injury

Authors: M. SUN[1], ***S. SHULTZ**[2,3];
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Abstract: Intimate partner violence (IPV) is a serious societal and medical issue worldwide that has severe and long-term impacts on the lives of women. Of the many challenges faced by IPV survivors, brain injury as a result of the physical attacks is likely one of the most significant - with both short- and long-term impacts. Although initial studies suggest there is evidence of brain injury in over 90% of IPV survivors, the nature of this brain damage and how it contributes to their lived experience has been remarkably understudied. Traumatic brain injury (TBI) falls on a spectrum, ranging from mild to severe, with mild TBI (mTBI) being the most common in the general population as well as in IPV. However, it cannot be assumed that findings from other mTBI studies are generalisable to the brain injury that occurs in IPV. Rather, the pathophysiology of brain injury that occurs in IPV survivors is likely unique compared to other contexts because in IPV the mTBI typically occurs in combination with non-fatal strangulation (NFS), and this added hypoxic/ischemic insult may exacerbate mTBI pathophysiology. The aim of this study was to develop the first rodent model of combined mTBI and NFS. It was hypothesised that the combined injuries would result in worse behavioral outcomes and pathophysiology compared to the single injuries. Young-adult female rats were assigned to either a sham group, mTBI group, NFS group, or mTBI + NFS group. Rats were also assigned to either a 2h, 24h, 72h, or 1 week recovery time prior to being euthanised for post-mortem analysis. The mTBI was induced by the lateral impact device. The NFS was induced by placing the rat in a supine position and suspending a 500g weight across the trachea for 90s. Rats in all groups underwent neuromotor testing before and after the assigned injury, and the 1 week recovery group also underwent additional behavioral testing to assess cognitive and anxiety measures. The mTBI + NFS group had worse neuromotor outcomes acutely after the injury compared to the mTBI, NFS, and sham groups. The mTBI + NFS groups also had exacerbated and prolonged neuroinflammation when compared to all other groups. This new model of IPV-related brain injury could be a useful tool to provide translational insights into the understudied problem of brain injury in IPV survivors.

Disclosures: M. Sun: None. **S. Shultz:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.10

Topic: C.10. Brain Injury and Trauma

Support: I01BX004561-01A2

Title: Effects of mild traumatic brain injury on impulsivity and the serotonergic system in the brainstem

Authors: *V. STIRITZ[1,2], T. P. COMINSKI[1], C. W. YOE[1,2], E. R. BENNETT[1,2], M. GRINBERG[1,2], J. P. STAMOS[1], K. D. BECK[1,3];
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Abstract: Traumatic brain injury (TBI) is a growing public health concern, especially affecting those in the U.S. military. Most TBIs are mild in severity and can result in changes in impulsivity, consisting of impaired inhibition, emotional instability, and poor decision making. Impulsivity is considered a key risk factor for suicide. The acoustic startle response (ASR), a brainstem reflex, is suppressed following TBI in both humans and animals. Interestingly, the raphe nuclei in the brainstem are a major source of serotonin, an important modulator of impulsivity. Therefore, damage or dysfunction to the raphe nuclei may be driving the changes in impulsivity and suicidality exhibited following TBI. Rats sustained a single mTBI using the lateral fluid percussion model; changes in cognitive and motor impulsivity were assessed at 1 month and 3 months post-injury using the Go/No-Go and Delayed Discount paradigms. ASR was also assessed at these time points. A separate non-behavior cohort of rats sustained a mTBI and were sacrificed at 1 month and 3 months post-injury. Brains were extracted and tissue punches of brainstem nuclei were taken for qRT-PCR analysis to measure alterations in serotonergic and pro-inflammatory cytokine gene expression. In the Go/No-Go task, TBI animals demonstrated a slight increase in premature responses from 1 month to 3 months post-injury while sham controls showed a decrease in premature responses from 1 month to 3 month post-injury, suggesting an increase in motor impulsivity in TBI animals over time after injury. Changes in cognitive impulsivity following mTBI were not detected in the Delayed Discount task, however, behavioral testing is ongoing. As previously shown, ASR was suppressed in mTBI rats compared to sham controls post-injury. Lastly, our qRT-PCR results show a trend for decreased serotonin transporter gene expression in the medial raphe nucleus in TBI rats compared to sham controls 1 month post injury. Gene expression analysis of serotonin transporter, tryptophan hydroxylase, and the pro-inflammatory cytokines Il-1β, IL-1α; and TNF-α; in the brainstem 3 months post-injury are currently underway. This study serves to provide greater insight into the behavioral changes and molecular mechanisms caused by mTBI and their contribution to increased impulsivity and suicide.

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Poster

044. Brain Injury: Molecular Mechanisms II

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Program #/Poster #: 044.11

Topic: C.10. Brain Injury and Trauma

Support: NIH NS111378
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NIH NS116838

Title: Traumatic Brain Injury Alters the Gut-Derived Serotonergic System and Associated Peripheral Organs

Authors: *Z. YING[1], N. M. MERCADO[1], G. ZHANG[1], F. GOMEZ-PINILLA[1,2];
[1]Dept. Integrative Biol. and Physiol., [2]Dept. Neurosurgery, UCLA Brain Injury Res. Ctr., UCLA, Los Angeles, CA

Abstract: Most efforts to understand the pathology of traumatic brain injury (TBI) have been centered on the brain, ignoring the role played by systemic physiology. Gut-derived serotonin is emerging as a major regulator of systemic homeostasis involving various organs and tissues throughout the body. Here, we attempted to shed light on the roles occupied by gut-derived serotonin and its downstream metabolic targets in the systemic pathogenesis of TBI. Male C57BL/6J mice were subjected to a fluid percussion injury (FPI) and RT-qPCR was used to examine mRNA levels in intestine, liver, and adipose tissue. In the intestinal tract, TBI transiently downregulated enteric neuronal markers *Chat* and *Nos1* in the duodenum and colon, and altered colonic genes related to synthesis and degradation of serotonin, favoring an overall serotonin downregulation. There also was a decrease in serotonin fluorescence intensity in the colonic mucosa and reduced circulating blood serotonin levels, with concurrent alterations in serotonin-associated gene expression in downstream tissues after TBI (i.e., upregulation of serotonin receptor *Htr2a* and dysregulation of genes associated with lipid metabolism in liver and adipose). Levels of commensal bacterial species were also altered in the gut, and were associated with TBI-mediated changes in the colonic serotonin system. Our findings suggest that TBI alters peripheral serotonin homeostasis, which in turn may impact gastrointestinal function, gut microbiota, and systemic energy balance. These data highlight the importance of building an integrative view of the role of systemic physiology in TBI pathogenesis to assist in the development of effective TBI treatments.

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Poster

044. Brain Injury: Molecular Mechanisms II

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Topic: C.10. Brain Injury and Trauma

Support: ISCBIR Grant G00004807

Title: Regeneration of the Zebrafish Telencephalon Following Blunt Force Traumatic Brain Injury

Authors: *K. CLOGHESSY[1], D. R. HYDE[2];
[2]Biol. Sci., [1]Univ. of Notre Dame, Notre Dame, IN

Abstract: Blunt force traumatic brain injuries (bfTBIs) are a common injury that result in global and diffuse damage to the central nervous system (CNS). bfTBIs are associated with many different detrimental short- and long-term side effects, which can negatively impact quality of life. Current treatments for bfTBIs are lacking, and focus mainly on preventing chronic cell death, rather than regenerating lost neurons from an endogenous stem cell population. Zebrafish (*Danio rerio)* have a robust neurogenic capacity in the CNS, compared to mammals. Thus, it is an ideal to model elucidate mechanisms involved in endogenous neuronal regeneration, which could provide potential targets for treatment aimed at encouraging endogenous repair. Our lab modified the Marmarou weight drop model, allowing us to deliver reproducible and scalable blunt force injuries to the zebrafish telencephalon. The telencephalon contains the homologous region to the mammalian hippocampus, the center for learning and memory. The damaged zebrafish recapitulate several side effects commonly seen in the human population following bfTBI, including presence of subdural hematoma, increase in seizure rates, deficits in learning and memory, and increased apoptotic cell death. Data from several of these analyses will be presented. After a severe bfTBI, we observed a significant increase in the number of proliferating cells in the telencephalon from 48 to 96 hpi. The majority of these progenitor cells originate along the ventricle, differentiate into mature neurons and migrate into the pallium, where they are stable for at least 1 month post-injury. Most of these proliferating cells did not colocalize with known radial glial markers, which are the putative stem cell population in the zebrafish telencephalon. Additionally, little is known about the genetic mechanisms that control the regeneration response of the progenitor cells. To elucidate these mechanisms, as well as the identity of the progenitor cells, we performed a single-nuclear RNA-Seq (snRNA-Seq) of the zebrafish telencephalon throughout the regenerative time course following a severe bfTBI. The results of this snRNA-Seq analysis will be presented.

Disclosures: K. Cloghessy: None. **D.R. Hyde:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.13

Topic: C.10. Brain Injury and Trauma

Support: R01NS096143
F99NS125825

Title: Exploring immaturity in Neuronal Nuclei protein negative (NeuN-) membrane disrupted neurons after diffuse brain injury in rats

Authors: *M. L. HERNANDEZ[1], M. CHO[2], K. M. GORSE[2], A. D. LAFRENAYE[2];
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Abstract: Traumatic brain injury (TBI) has consequences that are associated with diffuse pathologies and can last for years following the initial insult. While TBI can precipitate a variety of diffuse pathologies, membrane disruption, primarily within neurons, has been shown to occur following injury. However, the underpinnings of TBI-induced neuronal membrane disruption remain unclear. Our previous findings indicated membrane disruption following central fluid percussion injury (CFPI) occurred at sub-acute (6 hours to 3 days post-injury) and late (2- and 4-weeks post-injury) timepoints in a dual-phased manner that s temporally divided by a reduction at 1w post-injury. Moreover, we found that a subset of these late membrane-disrupted neurons was negative for the marker of mature neurons, Neuronal Nuclear protein (NeuN-). Remarkably, the subset of NeuN- membrane disrupted neurons was significantly increased at 2w post-TBI. Notably, a consistent NeuN- neuronal population was observed within the lateral neocortex layers V and VI in all sham and injured animals. These data suggested a consistent subpopulation of immature neurons within the lateral neocortex regardless of injury. We hypothesize that the increase in membrane disrupted NeuN- neurons could indicate a reversion to an immature phenotype. Therefore, the current study investigated this NeuN- membrane disrupted subpopulation for immature markers, Doublecortin (DCX), and cFos within the temporal profile from 1d, 1w, and 2w using the CFPI model in adult male Sprague-Dawley rats (n=6/group). Overall protein levels of DCX and cFos were analyzed in the lateral neocortex using western blot analyses. To visualize membrane disruption, animals received an intracerebroventricular infusion of tagged cell-impermeable dextran 2h prior to experimental endpoints. Brain slices were co-labeled with fluorescent Nissl stain, NeuN, and immature markers (DCX and cFos) and were quantified for overlap with neurons demonstrating dextran uptake, indicating membrane disruption. Further evaluations of membrane disruption could provide insight into the mechanisms of diffuse pathology and therapeutic timeframes in TBI.

Disclosures: M.L. Hernandez: None. **M. Cho:** None. **K.M. Gorse:** None. **A.D. Lafrenaye:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.14

Topic: C.10. Brain Injury and Trauma

Support: National Football League NFL-LONG Study

Title: Dose-dependent differences in spatiotemporal profile of gene expression following single and repeated mild traumatic brain injury

Authors: *M. L. BOUCHER[1], K. WARREN[1], J. QIU[1,3], W. P. MEEHAN[2,3,4], R. MANNIX[1,3]; [1]Div. of Emergency Med., [2]Div. of Sports Med., Boston Children's Hosp., Boston, MA; [3]Dept. of Pediatrics, Harvard Med. Sch., Boston, MA; [4]The Micheli Ctr. for Sports Injury Prevention, Waltham, MA

Abstract: Traumatic brain injury (TBI) is a leading cause of disability and mortality in young people, yet lacks effective interventions. One of the most significant challenges in designing such interventions is the heterogeneity of injury types and injury response. There is evidence to suggest that injury patterns not only vary with injury dose (severity and frequency of injuries), but also vary by brain region and time point post-injury. In order to design effective treatments for TBI, thorough characterization of these differential responses is needed. Using the NanoString mouse neuroinflammation RNA-based gene panel, we quantified 770 genes to understand how expression patterns change in response to our mouse weight drop model of both single mild TBI and repetitive mild TBI. We also examined differences in brain region (cortex vs. hippocampus) and time point following injury (3 days post-injury vs. 10 days post-injury). Analyses were performed using R Studio and Ingenuity Pathway Analysis (IPA). Investigation of the effects of, and interactions between, the predictors (injury dose, brain region, time point) revealed four functional nodes – inflammation and immunity, metabolism and energy homeostasis, phagocytosis and apoptosis, and cell morphology, movement, and communication. While modification of these nodes was present in each injury condition, the specific genes and pathways, and their degree of activation or inhibition, changed as a result of the other predictors. Decision trees were also generated to identify genes and gene profiles that could discriminate between injury conditions. While this method is statistically different from IPA, it yielded accurate modeling and identified similar targets from the four functional nodes. Principal component analysis revealed clustering of samples based on brain region, while hierarchical clustering yielded clustering of samples based on time point post-injury, followed by brain region and then by injury dose. Genes utilized by the first three principal components mirrored those in the four nodes highlighted by IPA as well. Together, these results both highlight the relevance of the four functional nodes—inflammation and immunity, metabolism and energy homeostasis, phagocytosis and apoptosis, and cell morphology, movement, and communication—as well as indicate the way genes and pathways are differentially modified by injury dose, brain region, and time point. Further, these analyses provide both specific gene expression and pathway targets for further investigation, and underscore the importance of spatiotemporally targeted treatments for TBI.

Disclosures: M.L. Boucher: None. **K. Warren:** None. **J. Qiu:** None. **W.P. Meehan:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Hockey League Alumni Association, National Football League. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); ABC-Clio, Springer International, Wolters Kluwer. **R. Mannix:** B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current

grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; National Institutes of Health, Department of Defense, National Football League. C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Abbott Pharmaceuticals.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.15

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant P20GM109098
NIH T32 AG052375
West Virginia University Clinical and Translational Science Institute
West Virginia University Experimental Stroke Core
American Heart Association

Title: Mild traumatic brain injury leads to differing cerebrovascular impairments over time in male and female mice

Authors: *B. WHITEHEAD[1], R. VELAZQUEZ-CRUZ[2], A. ALBOWAIDEY[2], K. KARELINA[2], Z. WEIL[2];
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Abstract: Traumatic brain injuries (TBI) can produce lasting physical and cognitive impairment as well as cerebrovascular dysfunction. Clinically, TBI leads to increased incidence of ischemic stroke and experimentally produces worsened functional and pathophysiological stroke outcomes in mice. Due to the links among TBI, ischemic stroke, and cerebrovascular function, we hypothesized that TBI-induced derangements in vascular function mediated poor stroke outcomes. To test this hypothesis, we performed a mild closed-head TBI (mTBI) or control procedure on 6-8-week-old Swiss-Webster mice. We then performed a transient middle cerebral artery occlusion (MCAO) for one hour either 7- or 28- days post injury (DPI). Five days later we conducted behavioral testing to measure motor impairments and measured infarct volume via 2,3,5-Triphenyltetrazolium chloride (TTC) staining. We then performed immunohistochemistry (IHC) to measure levels of fibrin(ogen) deposits within the stroke hemisphere and contralateral hemisphere. In additional cohorts, we performed an mTBI, collected brains at 7- or 28- DPI, and conducted IHC to measure microglial responses on the cerebrovasculature via IBA1+ and CD31+ colocalization, as well as VEGF staining. Finally, we performed a blood-brain barrier permeability assay and IHC analysis to measure accumulation of extravasated tracer surrounding endothelial cells. Results from the TTC staining showed an increased infarct volume in both male and female mice at 7 DPI, with a persistent increase in female mice out to 28 DPI compared to sham injured animals (all $p<0.05$). IHC analyses revealed an increase in fibrin(ogen) deposits at both 7- and 28 DPI in the ipsilateral hemisphere of males and females

($p<0.05$), while contralateral hemisphere analyses found an increase at 7 DPI in female mice, and an increase at 28 DPI in male mice ($p<0.05$). IBA1 analysis revealed an overall increase in microglial cell area and vascular-associated microglia by 28 DPI in both male and female mice ($p<0.05$). VEGF analysis showed increases in total VEGF expression at 7 DPI in gray and white matter regions of the brain in both male and female mice. Intravascular VEGF increased in gray matter regions in male mice at 7 DPI, and extravascular VEGF increased in white matter regions in males at 7 DPI (all $p>0.05$). Finally, there was persistent accumulation of tracer surrounding blood vessels at both 7- and 28- DPI in male and female mice. Taken together, these data suggest sex differences in cerebrovascular responses that can result in different severities of stroke responses over time post-TBI.

Disclosures: B. Whitehead: None. **R. Velazquez-Cruz:** None. **A. Albowaidey:** None. **K. Karelina:** None. **Z. Weil:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

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Program #/Poster #: 044.16

Topic: C.10. Brain Injury and Trauma

Support: NIH R01NS107853
AHA 14SDG18730034

Title: Augmented TSPO expression and intracerebal hemorrhage: a role in brain damage?

Authors: *F. BONSACK, R. DASARI, A. THOMAS, S. SUKUMARI-RAMESH;
Pharmacol. and Toxicology, Med. Col. of Georgia, Augusta, GA

Abstract: Intracerebral Hemorrhage (ICH) is a subtype of stroke with devastatingly high rates of mortality and morbidity. Owing to the absence of effective treatment options, it is imperative to identify novel molecular targets for therapeutic intervention. To this end, based on our previous studies, we hypothesized that 18-kDa Translocator Protein (TSPO) could be a negative regulator of ICH-induced neuroinflammation, a critical regulator of secondary brain damage. To test this hypothesis, we employed TSPO knockout mice and ICH was induced using the collagenase injection method. ICH resulted in augmented expression of TSPO in microglia as well as macrophages in the ipsilateral brain striatum, as evidenced by immunohistochemistry as well as flow cytometry analysis. Functionally, male TSPO knockout mice exhibited significantly increased neurobehavioral deficits at day 3 post-ICH in comparison to experimental control. The induction of neurological deficits was associated with augmented neurodegeneration and brain cell death in male TSPO knockout mice, as evidenced by Fluorojade-B and TUNEL staining, respectively. Mechanistically, there was a significant increase in the striatal RNA expression of pro-inflammatory microglia/macrophage marker iNOS and a significant decrease in the expression of CD206, a marker for anti-inflammatory microglia/macrophages in TSPO knock

out mice in comparison to respective controls. Interestingly, there was no difference observed in neurological deficit scores in female TSPO knockout mice after ICH when compared to controls. Further studies are needed to elucidate whether there is a sex-based difference in the functional role of TSPO following ICH. Altogether, the data implicate a novel role of TSPO in ICH-mediated inflammatory brain damage in male subjects and TSPO induction after ICH could be an intrinsic regulatory mechanism to prevent exacerbated brain injury.

Disclosures: F. Bonsack: None. **R. Dasari:** None. **A. Thomas:** None. **S. Sukumari-Ramesh:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.17

Topic: C.10. Brain Injury and Trauma

Support: Department of Neurological Surgery, University of Wisconsin, Madison
NIH Grant RO1 NS102573

Title: Mmp-12 knockdown prevents secondary brain damage after stroke in mice

Authors: *V. ARRURI[1], A. CHOKKALLA[1], S. JEONG[2], B. CHELLUBOINA[1], S. L. MEHTA[1], K. VEERAVALLI[3], R. VEMUGANTI[1,4];
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Abstract: Post-stroke induction of matrix metalloproteinase-12 (MMP-12) promotes secondary brain damage. We previously reported that increased MMP-12 expression mediates blood-brain barrier disruption via tight junction protein degradation in rats after focal cerebral ischemia. Currently, we evaluated whether MMP-12 knockdown protects the post-stroke mouse brain and promotes better functional recovery. Adult male mice were injected with negative siRNA or MMP-12 siRNA (intravenous) at 5 min of reperfusion following 1h transient middle cerebral artery occlusion. MMP-12 knockdown significantly curtailed ischemic brain damage and improved the motor and cognitive functions. Mechanistically, MMP-12 knockdown ameliorated the post-stroke degradation of tight junction proteins zonula occludens-1, claudin-5, and occludin. Furthermore, MMP-12 knockdown significantly decreased the expression of inflammatory mediators including monocyte chemoattractant protein-1, tumor necrosis factor-α, and interleukin-6, and the expression of apoptotic marker cleaved caspase-3 after ischemia. Overall, the present study indicates that MMP-12 is a promising stroke therapeutic target.

Disclosures: V. Arruri: None. **A. Chokkalla:** None. **S. Jeong:** None. **B. Chelluboina:** None. **S.L. Mehta:** None. **K. Veeravalli:** None. **R. Vemuganti:** A. Employment/Salary (full or part-time):; William S. Middleton Veterans Administration Hospital, Madison, WI, USA.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.18

Topic: C.10. Brain Injury and Trauma

Support: NIH VA MERIT 1I01RX002705-01A1

Title: Contextual fear extinction is differentially altered by severity of traumatic brain injury

Authors: *E. R. HALTER[1,2], J. A. WOLF[1,2], C. ADAM[1], M. SERGISON[1];
[1]Univ. of Pennsylvania, Philadelphia, PA; [2]CMC VA Med. Ctr., Philadelphia, PA

Abstract: Traumatic Brain Injury (TBI) and Post-Traumatic Stress Disorder (PTSD) are two of the most common ailments afflicting Veterans. However, little is known about the comorbidity of these two disorders, or how they interact and progress over chronic time points. To study the effect of injury on fear memory acquisition and extinction, we subjected rats to a lateral fluid percussion injury (FPI) of 1.8 or 2.1 atm, creating a unilateral mild to moderate TBI. These animals were run through a contextual fear-conditioning and pattern separation paradigm at an acute timepoint two weeks post-injury (1.8atm n=5, 2.1atm n=5) or sham surgery (n=7). An additional chronic cohort was run at 6 months post-injury (1.8atm n=7, 2.1atm n=2) or sham surgery (n=5). All groups showed an equal affinity for the creation of contextual fear memories as evidenced by similar overall acquisition curves and corresponding high freezing levels in the acquisition context, context A, during the first extinction trial. Similar pattern separation abilities between groups were also displayed by significantly lower freezing levels in a similar, but novel context B, used only for extinction 2, and completed 5min after extinction 1. When a third extinction trial was completed one day later back in context A, differentiation was seen between cohorts. In both the acute and chronic timepoints, sham animals showed a significant reduction in freezing as compared to extinction 1. This suggests sufficient extinction memory creation and consolidation had occurred. At the same trial, injured animals at both timepoints did not show decreased freezing but instead displayed freezing at levels statistically similar to the first extinction trial in context A. In the 2.1atm injured groups, this high level of freezing in extinction 3 was much more pronounced than the 1.8atm injured animals, suggesting a corresponding larger injury effect. Both groups of injured animals did not show significant extinction in context A until extinction 4, one trial later than seen in the sham group. Our findings suggest that TBI slows the creation and consolidation of fear extinction memories but does not appear to affect the acquisition of contextual fear memories or pattern separation abilities on a short temporal scale. The mild to moderate injury may be disrupting the extinction process due to sleep deficits preventing the consolidation of the extinction memory. This effect appears to increase with injury size. Our data gives us a behavioral basis to further explore the mechanisms underlying this dysfunction using in vivo electrophysiological and continue expanding on the chronic injury time points using this behavioral paradigm.

Disclosures: **E.R. Halter:** None. **J.A. Wolf:** None. **C. Adam:** None. **M. Sergison:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.19

Topic: C.10. Brain Injury and Trauma

Support: NIGMS P20GM109089

Title: The Role of Spreading Depolarizations in the Acute Behavioral Deficits Associated with Single and Repeated Mild Traumatic Brain Injuries

Authors: *N. J. PINKOWSKI, B. FISH, C. J. MEHOS, R. A. MORTON;
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Abstract: An estimated 42 million individuals have a concussion or a mild traumatic brain injury (mTBI) every year. Individuals who suffer from an mTBI have acute symptoms that can include weakness, dizziness, and disorientation. It has been previously established in rodents that an mTBI can induce a spreading depolarization (SD) in the cortex. An SD is a massive loss of chemical and electrical potential that travels slowly through grey matter. Here we utilize a mild closed skull mTBI mouse model that can induce an SD, confirmed with intrinsic optical signal imaging through a transparent skull. To test for acute behavioral deficits animals are sequentially tested in an open field (10 minutes), escape and explore behavior, gross gait assessment, startle response, forceps grasp, balance beam (4-1cm square and 1 cm round), and inverted mesh (tested 13-23 minutes after injury). The number of tasks failed is reported as the neurological severity score (NSS). Our results show that an mTBI that induces a bilateral SD (n=15) is associated with behavioral deficits resulting in higher NSS than an mTBI that does not induce an SD (n=10, p=0.0002) and when compared to sham controls (n=27, p=0.0099). To determine if an SD is sufficient to produce similar behavioral deficits, we used an optogenetic approach to initiate SDs through the skull with light. Interestingly, optogenetically induced SDs produced similar behavioral deficits and NSSs (n=15, p= 0.9990 compared to impact). Previous work from our laboratory has shown that mTBI-induced SDs are associated with a prolonged period of reduced cerebral blood flow (CBF) with 30% below baseline at 30 minutes post-injury. We are targeting this 30-minute time point when the cerebral blood flow is lowest to indicate a period of vulnerability to worse outcomes from a second injury. The highest risk factor in having a concussion is history of a previous concussion. Repeated concussions are associated with worse symptoms and extended recovery. Here, mice receive 2 mTBI impacts with a 30-minute interval. Mice that had repeated mTBIs (n=21) had significantly higher neurological severity scores compared to mice with a single mTBI (n=18, p= 0.0453) or sham control mice (n=20, p= <0.0001). Three hours later the mice are re-tested on the NSS tasks to assess behavioral recovery. If mice have only a single mTBI, all behavioral deficits are recovered to sham level at 3 hours (p= >0.9999), but mice with repeated mTBIs at 30-minutes did not fully return to Sham

treated levels (p= 0.0410) indicating a prolonged period of behavioral deficits. Current work is investigating the role of SDs in the vulnerability to a secondary injury using optogenetics and fiber photometry.

Disclosures: **N.J. Pinkowski:** None. **B. Fish:** None. **C.J. Mehos:** None. **R.A. Morton:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

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Program #/Poster #: 044.20

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant 206180424

Title: Fear Extinction and Cortical Circuitry after Mild Traumatic Brain Injury

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Abstract: Traumatic brain injury (TBI) is a leading cause of death and disability in children and adults in the United States. 10-15% of mild TBI (mTBI) survivors develop neuropsychiatric disorders such as posttraumatic stress disorder, making them a significant public health concern. Notably, an inability to suppress fear and override fearful memories lies at the core of many neuropsychiatric disorders. This ability, known as fear extinction, is essential to mental health. Fear extinction requires learning and remembering that a fear-evoking object or situation is nonthreatening (i.e., safe) after it is repeatedly presented without an aversive consequence, thereby creating a retrievable extinction memory. The ability to retrieve fear extinction memories relies on the infralimbic cortex (IL) subregion of the medial prefrontal cortex (mPFC). Indeed, data suggests that the potentiation of IL neurons is necessary for fear suppression and the retention of fear extinction memories. While previous research shows fear extinction is impaired after mTBI in both humans and rodents, little is known of how the IL responds to mTBI. Importantly, our lab has demonstrated that mTBI decreased network activity in the prelimbic subregion of the mPFC, highlighting the region's vulnerability to mTBI. Using a well-established mouse model of mTBI, this work aims to determine whether mTBIs disrupt the IL neurocircuitry responsible for the capacity to extinguish fearful memories. We predict injured mice will show reduced IL network activity, a failure to generate long-term potentiation, and reduced excitability in IL neurons after injury. This work begins to outline the mechanism of injury-induced fear-based neuropsychiatric disorders, and lays the groundwork for the development of a treatment for mTBI survivors.

Disclosures: **C.E. Ubri:** None. **A.M. Farrugia:** None. **A.S. Cohen:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

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Topic: C.10. Brain Injury and Trauma

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SERB, GOI; EMR/2017/000621
SERB,GOI; CRG/2020/004971
SERB,GOI; CRG/2021/008295

Title: Repeated mild traumatic brain injury silences mitofusin-2 gene expression in the hippocampus via DNA methylation: Implications in mitochondrial dysfunction and cognition

Authors: *P. G. KULKARNI[1], N. BALASUBRAMANIAN[2], R. MANJREKAR[1], T. BANERJEE[3], A. J. SAKHARKAR[1];
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Abstract: Chromatin remodeling by DNA methylation is widely reported in the pathogenesis of neurodegenerative disorders. Mitochondrial dysfunction serves as a critical denominator of stress-induced neurodegeneration. However, the epigenetic mechanisms involved in the structural organization of mitochondria in the brain and their implications in trauma-induced secondary injuries are largely unknown. We have previously reported mitochondrial dysfunction due to oxidative stress induced by repeated mild traumatic brain injury (rMTBI) in the hippocampus (Balasubramanian et al., 2021, Molecular Neurobiology 58:1162-1184). Further, rMTBI-induced secondary injuries result from glutamate excitotoxicity. Herein, we probe the dysregulation of mitofusin-2 (*Mfn-2*) gene expression in the hippocampus due to DNA methylation induced by rMTBI and its manifestations in mitochondrial dysfunction using closed-head weight drop method (Adult male Wistar rats). Repeated MTBI was induced by dropping the weight on the closed head for 5 times (one hit on alternate days). The human neuroblastoma cell line, SH-SY5Y, was employed to study the effects of glutamate excitotoxicity (50 mM, 24 h) on DNA methylation and *Mfn-2* gene expression *in vitro*. While on one hand, rMTBI caused hypomethylation at the *Mfn-2* promoter and increased *Mfn-2* expression after 48 h of the last hit, on another hand, the DNA hypermethylation after 30 days lowered *Mfn-2* expression. Similarly, glutamate excitotoxicity induced DNA hypermethylation at the *Mfn-2* promoter and silenced the *Mfn-2* expression in SH-SY5Y cells. While rMTBI reduced the mitochondrial mass as tested by MitoTracker green assays, glutamate excitotoxicity heightened cellular and mitochondrial ROS levels as measured by DCFDA and MitoSOX assays, respectively. Similarly, mitochondrial membrane potential (MMP by JC1 dye) showed a concomitant reduction in glutamate-treated SH-SY5Y cells. To study the role of DNA methylation in *Mfn-2* gene expression, glutamate-treated SH-SY5Y cells and the rMTBI-exposed rats were administered with 5-Azacytidine (5-AzaC), a pan DNMT inhibitor. 5-AzaC treatment normalized DNA methylation and *Mfn-2* function in both, rMTBI-exposed rats as well as glutamate-treated cells. Moreover, 5-AzaC pre-

treatment protected the glutamate-exposed cells from deterioration in MMP and surge of ROS levels. Likewise, 5-AzaC treatment restored rMTBI-induced deficits in working and reference memory in the 8-Arm radial maze test. In conclusion, rMTBI-induced glutamate excitotoxicity may provoke neurodegeneration and cause memory deficits in the hippocampus by silencing the *Mfn-2* gene expression via DNA methylation.

Disclosures: P.G. Kulkarni: None. **N. Balasubramanian:** None. **R. Manjrekar:** None. **T. Banerjee:** None. **A.J. Sakharkar:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

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Topic: C.10. Brain Injury and Trauma

Support: NIH NINDS 1R01NS112693-01A1
HHS PHS Ruth L. Kirschstein NRSA T32NS077889
Lexington VA Merit BX003405

Title: Characterizing the Effect of 17β-Estradiol on Mitochondrial Dysfunction after Severe Controlled Cortical Impact in Mice.

Authors: *O. J. KALIMON[1,2,4], H. J. VEKARIA[2], V. G. VISWANATHAN[2], M. L. SPRY[2], E. P. BROWN[2], W. B. HUBBARD[2,3,4], P. G. SULLIVAN[1,2,4];
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[4]Lexington VA Healthcare Syst., Lexington, KY

Abstract: Traumatic brain injury (TBI) is caused by a blow, jolt, or penetrating injury to the head resulting in abnormal brain function. A large percentage of clinical and pre-clinical studies that included both males and females have found after severe TBI, females had better outcomes compared to male counterparts. Mitochondrial dysfunction is a hallmark of TBI and has been thoroughly studied in male rodent models of injury; however, little is known about these outcomes in females. No studies have examined the effect of 17β-estradiol (E2) on mitochondrial dysfunction after TBI, but data from outside the field identifies mitochondria as a target of E2 action. To understand the apparent sex-dependent outcomes after severe TBI, these studies explore the hypothesis that having E2 on board during injury will protect mitochondria from controlled cortical impact (CCI)-induced dysfunction.Total mitochondria from the uninjured cortex of male and female mice were treated with 0, 0.2, 20, or 2000nM E2 to determine whether mitochondria from females are more sensitive to E2 action than males. Mitochondria were treated with E2 immediately before measuring bioenergetics, Ca^{2+} buffering, or reactive oxygen species (ROS) production. Bioenergetics were measured after the addition of substrates and inhibitors of the mitochondrial electron transport chain. Ca^{2+} buffering was measured using CaG5N to measure free calcium and TMRE for membrane potential after adding boluses of

$CaCl_2$. ROS production was measured using the fluorogenic dye, DCF-DA. The results showed 2000nM E2 impaired bioenergetics and increased ROS production in mitochondria from both sexes; Ca^{2+} buffering was not impaired. There were no significant changes with the other doses.Ongoing studies are repeating these assays in mitochondria isolated from the injured cortex of male and female mice 24h post-severe CCI. This will determine whether direct administration of E2 differentially influences mitochondrial function in an injured state. We predict the addition of E2 to damaged mitochondria will exacerbate this dysfunction after CCI. Further, we have utilized ovariectomized mice implanted with a physiological dose of E2 (180µg/mL) to determine whether E2 administration prior to injury will protect mitochondria from CCI-related damage. The results showed nonsynaptic cortical mitochondria from E2-implanted mice had significant bioenergetic impairment after CCI compared to sham. There was no injury effect in nonsynaptic mitochondria from blank-implanted (sesame oil) groups, though the synaptic fraction showed this effect. These studies indicate E2 does not protect mitochondria from CCI-induced dysfunction.

Disclosures: O.J. Kalimon: None. **H.J. Vekaria:** None. **V.G. Viswanathan:** None. **M.L. Spry:** None. **E.P. Brown:** None. **W.B. Hubbard:** None. **P.G. Sullivan:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.23

Topic: C.10. Brain Injury and Trauma

Support: NIH/NINDS 1R01NSO96225-01A1
AHA19TPA34850047
Magale Foundation
Louisiana State University Research Council

Title: Mitochondrial dysfunction post-repetitive and mild traumatic brain injury

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Abstract: Traumatic brain injury (TBI) occurs due to an impact to the head that causes brain tissue alterations. Mild and repetitive TBI (rmTBI) accounts for the largest proportion of TBI-cases (estimated 82.3%), leading to long-term cognitive and behavioral impairment. The severity of TBI varies from mild to severe with repetitive and mild (rm) TBI accounting for the highest percentage of TBI-cases. There are no current treatment(s) for rmTBI, therefore, we sought to

identify novel signaling molecules/pathways that could be contributing to the progression of rmTBI. We employed a clinically relevant closed-head impact model (CHIMERA) that allows free rotation of the head upon impact. Our central hypothesis is that the loss of PRMT7 activity, mediates excitotoxicity, increased cellular death, disturbed mitochondrial dynamics and contributes to behavioral deficits. PRMTs are enzymes that catalyze the methylation of arginine residues (a constitutive post-translational modification) involved in transcription, translation, receptor trafficking, and protein stability. There are currently 11 known PRMT isoforms (PRMT1-11), with PRMT7 gene deletion in human patients causing neurological deficits such as intellectual disability and microcephaly, along with hyperexcitability and impaired social behaviors in murine *in vivo* models. We assessed diffuse axonal injury and demonstrated enhanced silver deposition (dark stained regions) throughout the brain. Using capillary electrophoresis, we observed protein arginine methyltransferase 7 protein expression was significantly decreased in the cortex at 3 (0.5815±0.04701) arbitrary units (AU) and 7 days (0.5696±0.03154) (AU) v. sham (0.7780±0.03064) (AU). Via LC/MS, we observed enhanced glutamate levels in the hippocampus 3 days (6714±107.8) (AU) after rmTBI. DRP1 protein expression was enhanced 3 (5.248±0.2749) (AU) and 7 days (5.720±0.2031) (AU) v. sham (2.571±0.1839) (AU) in the cortex; and 1 (4.120±0.1551) (AU), 3 (5.802±0.3690) (AU), and 7 days (4.861±0.4092) (AU) after rmTBI in the hippocampus v. sham (2.790±0.1325) (AU). Utilizing Seahorse XF24 analyzer to measure oxygen consumption rates, we observed a significant decrease 1 day (70.28±3.476) (pmol/min) in ATP-linked respiration followed by an increase 3 days (102.1±4.991) (pmol/min) post-rmTBI. Furthermore, maximal respiration was enhanced 3 days (176.7±8.430) (pmol/min) after post-rmTBI mice (via Seahorse XF24). Learning, working memory, and locomotor skills were significantly impaired post-rmTBI. Overall, our data suggest PRMT7 may influence mitochondrial health and contributes to the pathological progression of rmTBI.

Disclosures: **C.H. Acosta:** None. **C.T. Citadin:** None. **G. Clemons:** None. **W. Carr:** None. **M. Udo:** None. **C.Y. Wu:** None. **R.H. Lee:** None. **H. Lin:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.24

Topic: C.10. Brain Injury and Trauma

Support: R01 NS115876

Title: Lysosomal accumulation of lipids leads to autophagy inhibition in microglia and macrophages and contributes to inflammation after traumatic brain injury

Authors: *A. MEHRABANI-TABARI[1], N. HEGDEKAR[1], Y. MOREL[1], L. MULLER[2], C. SARKAR[1], M. KANE[2], J. JONES[2], M. LIPINSKI[1];
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Abstract: Protracted inflammation observed in both resident microglia and infiltrating macrophages after traumatic brain injury (TBI) correlates with poor prognosis. Our data suggest that autophagy is inhibited in those activated mononuclear phagocytes and that this inhibition contributes to the prolonged inflammatory phenotype after TBI. However, the mechanism of autophagy inhibition in microglia and macrophages after TBI has yet to be determined. Data from our laboratory including immunofluorescent staining, DESI-MSI lipid imaging, and LC-MS/MS lipidomic analyses of samples from controlled cortical impact (CCI) mouse model of TBI show that inhibition of autophagy correlates with accumulation of lipids and formation of lipid droplets in both infiltrating macrophages and resident microglia. Specifically, our lipidomic data demonstrate accumulation of neutral lipids typically found in lipid droplets in FACS-purified microglia and macrophages from the TBI brain. MSI data show accumulation of cholesteryl esters in perilesional tissue of the TBI brain. Finally, immunofluorescence data demonstrate lipid droplet accumulation in microglia and macrophages and correlation with inhibition of autophagy after TBI. Accumulation of lipid droplets in microglia and macrophages after TBI is reminiscent of myelin-laden foam macrophages reported in multiple sclerosis and lipid droplet associated microglia (LDAM) observed in the aged brain. We hypothesized that excessive phagocytosis of complex cholesterol species including myelin debris and oxidized LDL (oxLDL) that was also detected in the TBI brain, causes observed lysosomal dysfunction and autophagy impairment. We tested this in vitro in microglia and macrophage cell lines and bone marrow derived macrophages (BMDM). Treatment with either myelin or various cholesterol species including oxLDL, caused lipid accumulation, autophagy inhibition, and exacerbated proinflammatory responses induced by LPS. Our data suggest that myelin and cholesterol phagocytosis by myeloid cells inhibits autophagy and exacerbates inflammation after TBI.

Disclosures: A. Mehrabani-Tabari: None. **N. Hegdekar:** None. **Y. Morel:** None. **L. Muller:** None. **C. Sarkar:** None. **M. Kane:** None. **J. Jones:** None. **M. Lipinski:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.25

Topic: C.10. Brain Injury and Trauma

Support: R01 NS115876

Title: Dysregulation of lipid metabolism and lysosomal function link TBI to brain aging and neurodegeneration

Authors: C. SARKAR[1], N. HEGDEKAR[1], S. BUSTOS[1], A. MEHRABANI[1], Y. MOREL[2], J. JONES[2], M. KANE[2], **M. M. LIPINSKI**[1];
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Abstract: Abstract: Epidemiological studies link history of traumatic brain injury (TBI) to development of neurodegenerative diseases later in life. While the contributing mechanisms remain unclear, it is hypothesized that TBI may accelerate detrimental cellular changes occurring during normal brain aging. A potential candidate is the autophagy-lysosomal pathway essential for maintenance of proteostasis in neurons and regulation of inflammatory responses in immune cells including microglia. Autophagy function declines during brain aging and in neurodegenerative diseases. Our recent data demonstrate that autophagy is also inhibited after TBI and contributes to unfavorable outcomes. To investigate the mechanisms contributing to neurodegeneration after TBI, we compared brain aging trajectory for up to 18 months in mice with and without exposure to TBI at 2 months of age. Our RNA-seq analyses identified several sets of genes with differential expression patterns in the aging brain with and without prior TBI exposure. Pathway analysis of a group of genes which expression was elevated following TBI and remained increased during aging as compared to age-matched sham mice, revealed enrichment for genes involved in inflammation and lipid metabolism. The upstream regulators included LPS and progranulin (Grn), both previously shown to be involved is formation of lipid droplet associate microglia (LDAM) in the aged brain. Consistently, we observed presence of lipid droplet accumulating microglia in the injured brain, which persisted for at least 4 months after TBI. Further supporting perturbation of lipid metabolism, our LC-MS/MS lipidomic analyses of lysosomes purified form the aged brain demonstrated persistent changes in lipid composition between age-matched mice with and without prior TBI. Lipid classes increased in lysosomes of aged mice exposed to TBI included sphingomyelin (SM), which accumulation is known to cause lysosomal dysfunction in the lipid storage disease, Nieman Picks disease. Consistent with TBI and aging induced lysosomal dysfunction, brain aging in mice was associated with decrease in autophagy, which was further exacerbated in mice exposed to TBI. Together, our data indicate that TBI leads to persistent perturbation of lysosomal lipid homeostasis and autophagy-lysosomal dysfunction, which may contribute to accelerated brain aging and development of neurodegeneration in the injured brain.

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Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.26

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant RF1NS110637
NIH Grant R01NS110635

Title: Age related changes in plasma extracellular vesicles contribute to neuroinflammation and neurodegeneration in the brain after spinal cord injury

Authors: *J. WU, N. KHAN, Z. LEI, Y. LI, R. RITZEL, A. I. FADEN;
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Abstract: Spinal cord injury (SCI) leads to secondary brain neuroinflammation and neurodegeneration that increases dementia risk. Preclinical studies support that cognitive behavioral deficits are exacerbated with age and worse in males. However, the mechanisms contributing to SCI-induced brain dysfunction are not well understood. Cell-to-cell signaling through extracellular vesicles (EVs) has emerged as a critical mediator of neuroinflammation, including at a distance through circulation. We have previously shown that SCI in young adult (YA) male mice leads to robust changes in plasma EV count and microRNA (miR) content. Here, our goal was to investigate the impact of age, biological sex, and aging on EVs and brain after SCI. YA or aged mice received T10 moderate contusion injury. Total plasma EVs were isolated by ultracentrifugation. Particle count and size distribution were assessed by nanoparticle tracking analysis, while individual EV protein expression was quantified by Western blot. EV miR cargo was examined using the Fireplex® assay. The transcriptional changes in the brain were assessed by a NanoString nCounter Neuropathology panel. In shams, plasma EVs from aged mice had decreased count and a smaller peak size relative to YA. At 2 m post-injury, there was no difference in particle count or size distribution between YA and aged mice. However, aged animals showed increased expression of EV markers CD63 and CD81 with SCI. Acute alterations in miR content of plasma and spinal cord EVs were largely similar between young and aged mice. Next, YA mice were subjected to SCI followed by 19 m post-injury. In plasma, males had increased EV and lipoprotein markers after injury. In the brain, we observed strong sex-dependent differences in the transcriptome of the cortex and hippocampus (Hi) after SCI. Males had a pronounced reduction in genes related to axonal structure and myelination in the cortex. In contrast, females showed an improved oxidative stress and unfolded protein response profile. Decreased expression of certain cytokines in the Hi may contribute to a reduced anti-inflammatory environment that is detrimental to neuronal survival and adult neurogenesis. Across both regions and sexes, gene expression related to homeostatic microglia were reduced. Chronic SCI also led to increased EV particle counts in the brain and modified their miR content. Alterations in EV miRs paralleled those reported with neurodegenerative disease, depression, and inflammatory processes. Collectively, these studies are the first to describe changes in circulating EVs after chronic SCI and in aged animals and support a potential EV-mediated mechanism for SCI-induced brain changes.

Disclosures: J. Wu: None. **N. Khan:** None. **Z. Lei:** None. **Y. Li:** None. **R. Ritzel:** None. **A.I. Faden:** None.

Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.27

Topic: C.10. Brain Injury and Trauma

Support: NIH RF1NS110637 (JW)
NIH R01NS094527 (JW)

Title: Spinal cord injury in aged mice exacerbates neuropathological changes in both spinal cord and brain leading to worsened neurological dysfunction

Authors: *Z. LEI, B. KRISHNAMACHARY, N. KHAN, R. M. RITZEL, Y. LI, H. LI, A. I. FADEN, J. WU;
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Abstract: Approximately 20% of all spinal cord injuries (SCI) occur in persons aged 65 years or older. Older patients with SCI have different features with regard to neurological characteristics after injury. Recent large-scale longitudinal population-based studies showed that patients with SCI are at a higher risk of developing dementia than non-SCI patients, indicating that SCI is a potential risk factor for dementia. Age is known to potentiate inflammation and neurodegeneration at the injured site leading to impaired recovery from SCI. However, no research has been aimed at studying the mechanisms of SCI-mediated cognitive impairment in the elderly. In the present study, young adult (3-month-old) and aged (18-month-old) male mice were subjected to moderate contusion SCI at T10, and their functional outcomes were evaluated using a battery of neurobehavioral tests including motor function [Basso Mouse Scale (BMS)], cognition [Y-maze, novel object recognition (NOR)], and depression [novelty-suppressed feeding (NSF) and social recognition (SR)]. BMS assessment of locomotor function showed marked impairment in aged mice compared to young animals, which were correlated with reduced spared white matter and increased lesion volume. At 2 months post-injury, aged mice displayed worse performance in neurobehavioral tests, as evidenced by a lower % of spontaneous alteration in the Y-Maze task, reduced novelty preference in the NOR test, increased sociability deficits in SR, and elevated latency to detect food in NSF, indicating exacerbated impairment of cognition and depressive-like behavior in aged mice compared to young animals. NanoString analysis with the neuropathology panel identified several age- and injury-specific genes that were differentially expressed in the brain after SCI, including those involved with the inflammation, neurodegeneration, and autophagy pathways. These findings were further validated by qPCR and microscopy. Flow cytometry demonstrated increased microglia and myeloid and lymphocytes infiltration at the injured site from young mice which was exacerbated with age. Moreover, SCI in aged mice altered microglial function and dysregulated autophagy function in both microglia and neurons of the brain, resulting in neurodegeneration compared to young animals. Taken together, our data indicate that aging exacerbates neuropathological changes in both the injured spinal cord and remote brain region that is associated with poorer functional outcomes. Thus, our studies provide innovative cellular and molecular perspectives on the pathophysiology of age-related deficits after SCI.

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Poster

044. Brain Injury: Molecular Mechanisms II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 044.28

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R21NS117867
NIH Grant 5R01NS115876

Title: Peroxisomal ether phospholipid synthesis is disrupted in the brain after TBI

Authors: *C. SARKAR[1], N. U. HEGDEKAR[1], S. BUSTOS[1], Y. MOREL[2], A. MEHRABANI[1], N. LINGENFELTER[1], J. JONES[2], M. M. LIPINSKI[1];
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Abstract: The brain is highly enriched in lipids, which play an important role in maintaining structural integrity and regulating cellular and subcellular functions. Changes in lipid homeostasis and metabolism are detrimental for neuronal survival and functions and have been implicated in different neurodegenerative diseases including traumatic brain injury. Previously we demonstrated alteration in lysosomal lipid composition in the mouse cortices following controlled cortical impact (CCI)-induced TBI. In this study, we investigated how ether phospholipids that contain an ether bond at the sn-1 position of their glycerol backbone, are regulated in the mouse brain following brain injury. Ether phospholipids constitute almost 20% of total brain lipids. They play crucial roles in membrane raft formation, membrane trafficking and cell signaling, and provide protection to the membrane structure against oxidative stress. We determined the levels of ether phospholipids in the brains of sham and TBI mice using liquid chromatography tandem mass spectrometry (LC-MS/MS) based lipidomic analysis. Our data showed a significant decrease in ether phospholipids abundance in the injured mouse cortices as compared to the sham mice. This is most likely due to the deregulation in ether phospholipid synthesis in the brain following TBI. Peroxisome, a single membrane bound organelle serves as the site for the initiation of ether phospholipids synthesis. Its resident enzymes glyceronephosphate O-acyltransferase (GNPAT) and alkylglycerone phosphate synthase (AGPS) are involved in generating ether phospholipid precursor, which is transported to endoplasmic reticulum for the completion of ether phospholipid synthesis. We detected a significant decrease in the GNPAT level and marked cytosolic accumulation of AGPS in the injured cortices of mice suggesting impairment in peroxisomal ether phospholipid synthesis following TBI. We fed TBI mice with 1-O-octadecylglycerol (OAG), an ether phospholipid precursor to restore ether phospholipid level in the injured brain. We detected a decrease in NLRP3 expression and observed functional improvement in these mice as compared to the untreated injured mice. Taken together, our data demonstrate that peroxisomal ether phospholipid synthesis is disrupted in the injured brain which might be a contributing factor for the functional declines after TBI.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.01

Topic: C.10. Brain Injury and Trauma

Title: Exosomes encapsulated in micron cellulose sheet for the improvement of sensory and motor functions and neuroregeneration after sciatic nerve injury in rats

Authors: *K. D. SHARMA[1], R. D. HILL[2], J. WESTMORELAND[3], J. P. MORE, II[4], J. A. HESTEKIN[5], J. Y. XIE[6];
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Abstract: Exosomes secreted by stem cells possess great therapeutic potential in neuroregeneration with a variety of signaling molecules such as growth factors to change the extracellular milieu and impact the functions of adjacent cells. In this study, we tested the effect of exosomes secreted by human bone marrow-derived mesenchymal stem cells in the functional recovery and neuroregeneration of sciatic nerve after crush injury. Exosomes can be quickly metabolized in the plasma when administered systemically that may produce undesirable side effects. Thus, we adopted a new micron cellulose film to confine exosomes at the injury site for more concentrated and sustained treatment with minimal systemic distribution. Micron cellulose was partially oxidized into TEMPO (2,2,6,6-tetramethylpiperidinyl-1-oxyl) cellulose with enhanced stability compared to unmodified cellulose. The TEMPO cellulose raw materials were then cast into 200 μm thin films with porous structures to hold the exosomes. Adult male and female rats received left sciatic nerve crush for 30 s. Exosomes (20 μl, 3 μg protein-equivalent) were infused directly onto the injured nerve or encapsulated inside the TEMPO cellulose films and then wrapped around the injured nerve segment. The sensory and motor functions of the hind limbs were assessed at various days post-injury. Animals in the film+exosome and film groups showed faster recovery of the ipsilateral hindlimb locomotion compared to the injury control group. The treatment of film+exosome also prevented the development of tactile allodynia induced by nerve crush. Immunohistochemical studies showed better regeneration of axons in the film+exosome-treated group as well. We have also examined the conduction of the injured nerve and the regeneration of the myelin sheath after crush injury via electromyography (EMG) recording and Western Blot, respectively. EMG was recorded from the gastrocnemius muscle while the uninjured sciatic nerve trunk above the crushed site was stimulated at 0.1 Hz 0.7 mV. There was a mild improvement of muscle contraction at 28 days after the injury in rats with Exosome + Film treatment. Western blot results did not show an apparent increase in myelin basic protein in the nerve samples taken at the injury site or distal to it 28 days post-injury. No observable side effect was noticed. While the combination of TEMPO cellulose and exosomes accelerated the functional recovery after nerve injury, further work is warranted to determine whether it could be useful to promote neuroregeneration.

Disclosures: K.D. Sharma: None. **R.D. Hill:** None. **J. Westmoreland:** None. **J.P. More:** None. **J.A. Hestekin:** None. **J.Y. Xie:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.02

Topic: C.10. Brain Injury and Trauma

Title: Novel method to monitor the progress of peripheral nerve regeneration using lower leg circumference

Authors: D. ALEXANDER[1], A. SALCIDO[1], K. LIMON[1], L. NGO[1], N. KYRITSIS[2], R. A. ABRAMS[3], *J. KOFFLER[1];
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Abstract: Three percent of all trauma cases involve peripheral nerve injury. When there is segmental nerve loss, and primary repair is not feasible, nerve autografting is necessary, often harvested from the leg. Current methods of assessing repair success are limited, require lengthy examinations, and subjective to examiner biases. Here we report a novel quantitative method to detect functional regeneration using measurements of the lower leg circumference in a 3cm-long injury model in the rabbit sciatic nerve. Denervated muscles are atrophied over time and as regeneration progresses, they regain muscle mass, which allowed us to monitor the progress of regeneration over time.24 female New Zealand White rabbits underwent a sciatic nerve transection followed by a sciatic nerve autograft (n=12) or a lesion only control (n=12). We measured leg circumference of both legs every two weeks up to 16 weeks post-surgery. Surgical leg measurements were normalized to non-surgical leg per timepoint. During the first 12 weeks after injury the circumference of injured leg decreased due to muscle atrophy. Lesion only treated animals injured leg circumference was 77.76%±1.23% (mean±SEM) of the healthy leg, while autograft treated animals leg circumference was 77.16% ±1.23% (mean±SEM). In the following weeks, bulk muscle volume of lesion only animals continued to decline and reached 76.05% ±1.58% (mean±SEM) at the end of the study at week 16, while the autograft treated animals showed a significant increase in muscle circumference to 83.77% ±2.18% (mean±SEM) at 16 weeks ($p<0.005$, Mixed model analysis followed by Tukey's post hoc analysis).Differences in the circumference of legs are commonly documented to evaluate muscle weakness during physical therapy to treat leg injuries, e.g. after knee surgery. In this work we used this quantitative test and applied it to monitor nerve regeneration after peripheral nerve injury repair procedure in experimental animals. We demonstrate that this inexpensive and noninvasive method can be readily performed in the clinic, providing physicians with a tool to monitor over time the success of nerve repair surgery.

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Poster

045. Peripheral Nerve Trauma

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.03

Topic: C.10. Brain Injury and Trauma

Support: CAPES Doctoral Scholarship 88882.453978/2019-01

Title: Ayahuasca promotes anti-allodynic effects in the partial sciatic nerve ligation model of neuropathic pain in mice by GABAergic and serotonergic mechanisms

Authors: *P. S. S. LAURIA[1], E. L. WÂNDEGA[1], L. S. ABREU[2], J. M. GOMES[3], M. S. SILVA[3], R. C. SANTANA[4], V. L. C. NUNES[5], R. D. COUTO[1], M. B. P. SOARES[6], C. F. VILLARREAL[1];
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Abstract: Neuropathic pain is a challenging condition since it is often unresponsive to available analgesics. Ayahuasca (AYA) is a ritualistic brew mostly known for its psychoactive properties. Recent studies have shown that AYA promotes antidepressant effects by serotonergic mechanisms. Considering the role of serotonergic neuromodulation in pain transmission, this study investigated the antinociceptive effects of AYA in the partial sciatic nerve ligation model of neuropathic pain (PSNL) (Animal Ethics Committee CEUA/EMEVZ 26/2020). The antinociceptive potential of oral administrations of AYA (0.024 - 3 mL/kg) was initially screened in the formalin test using male Swiss mice (n = 6 per group). AYA dose-dependently reduced the pain-like behaviors of mice in the late phase of the test. Male C57BL/6 mice (n = 6 per group) submitted to PSNL were orally treated with AYA (0.024 - 3 mL/kg) once or twice a day for 14 days. The PSNL model was marked by tactile allodynia as a result of nerve injury, shown by a drop in nociceptive thresholds assessed with von Frey filaments. Single exposures to AYA reduced allodynia dose-dependently from 5 to 10 h after treatments. Daily treatments once a day for 14 days at the maximum effective dose (0.6 mL/kg) promoted consistent anti-allodynic effect. When given to mice twice a day, AYA (0.6 mL/kg) completely reversed allodynia, maintaining a plateau of antinociception from days 5 to 14. Antagonism assays were performed to investigate possible anti-allodynic mechanisms of AYA, whose effect was partially reversed by methysergide (5 mg/kg) and bicuculline (1 mg/kg), but not by naloxone (5 mg/kg), phaclofen (2 mg/kg), or rimonabant (10 mg/kg). Daily treatments with AYA (0.6 mL/kg every 12 h) did not affect motor function or exploratory activity of mice, evaluated by rota-rod and open field tests, respectively. Toxicity of multiple exposures to AYA was investigated by determining hematological and serum biochemistry (AST, ALT, urea, creatinine) profiles, water and food intake, variation in body mass, and histology of organs. Daily treatments with AYA (0.6 mL/kg every 12 h) did not alter any of the evaluated parameters for toxicity. The chemical profile of AYA obtained by liquid chromatography-mass spectrometry revealed the presence of harmine,

harmaline, harmol, harmalol, tetrahydroharmine, 7-hydroxy-tetrahydroharmine, dimethyltryptamine, and 5-hydroxy-dimethyltryptamine. This is the first demonstration of the antinociceptive properties of AYA and its therapeutic potential in the treatment of neuropathic pain. Its mechanisms involve activation of GABAergic and serotonergic systems.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.04

Topic: C.10. Brain Injury and Trauma

Support: UCI Orthopaedic Department Seed Grant

Title: Understanding Human Motor Endplate Degeneration after Peripheral Nerve Injury

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Abstract: In humans, there is limited functional recovery after peripheral nerve injury (PNI) even with nerve repair surgery. One reason is time-dependent degeneration of denervated motor endplates (MEPs) at neuromuscular junctions (NMJs). In animal models, MEP degeneration begins with morphological changes from normal pretzel shapes to intermediate and plaque shapes followed by degeneration. Prior studies have demonstrated that human MEPs have different morphology, size, and density than rodent MEPs. Moreover, our recent studies indicate that unlike rodents, some MEPs in humans persist even >6 months post-PNI. These results suggest that degeneration of denervated human MEP may not be analogous to murine MEP degradation. Here, we use a new methodology for NMJ visualization to assess human NMJ degeneration in PNI patients. Denervated and innervated muscle samples were obtained in patients undergoing standard of care surgery 3-6 months post-PNI. Samples were prepared either (1) by immunostaining sections for α-BTX, neurofilament (NF) and synaptophysin (SYN) or (2) clearing the whole muscle sample with CUBIC R1 solution and immunostaining with NF, SYN and acetylcholine receptor (AChR)-α. Muscle samples were imaged with a Keyence BZ-X810 microscope; image stacks of MEPs were collected, and rendered in 3D in Imaris for morphometric analyses with ImageJ . MEPs in denervated tissue exhibited morphological degenerative changes when compared to innervated muscle including a mix of abnormal morphologies ranging from pretzel to fragmented. At similar time points post-injury, some patients had greater NMJ degeneration with a higher proportion of plaque MEPs compared to others. MEP morphology was better defined in samples immunostained for AChR-a than with a-

BTX. CUBIC-clearing prior to immunostaining allowed for excellent antibody penetration, enabling 3D reconstruction and morphometric quantification of MEP integrity in biopsied muscle. Our results indicate that human NMJ degeneration does not follow the same timeline as in rodents. Of note, our studies indicate that preserved MEPs in humans predict greater functional recovery with nerve repair surgery. Because human NMJ degeneration shows tremendous variation, some patients may have potential for recovery even if surgery is performed >6 months post-injury. These data suggest a role for pre-operative muscle biopsy prior to reconstructive surgery after a significant nerve injury.

Disclosures: R. Gupta: None. **V. Chen:** None. **L. Gonzales:** None. **T.R. Johnston:** None. **O. Steward:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.05

Topic: C.10. Brain Injury and Trauma

Support: CIHR

Title: Endocannabinoid CB1 receptor regulates neuromuscular junction degeneration and reinnervation following nerve injury

Authors: *R. PIOVESANA[1], L. BELLOCCHIO[2,3], G. MARSICANO[2,3], R. ROBITAILLE[1,4]; [1]Univ. of Montreal, Montreal, QC, Canada; [2]INSERM, U1215 NeuroCentre Magendie, Bordeaux, France; [3]Univ. of Bordeaux, Bordeaux, France; [4]Ctr. Interdisciplinaire de Recherche sur le Cerveau et l'Apprentissage (CIRCA), Montreal, QC, Canada

Abstract: After complete axonal transection, a complex and finely regulated process starts the removal of damaged tissue and the reparative process begins; the axonal compartment of neurons undergoes a number of degenerative processes, followed by a reinnervation process. In addition to the complex events occurring in the damaged axon, there is also a well-orchestrated sequence of reparative events occurring at the neuromuscular junction (NMJ). Perisynaptic Schwann cells, glial cells at the NMJ, are important for NMJ repair.
Cannabinoids are often used in the treatment of neuropathic pain related to nerve injury and despite evidence for their roles in axonal guidance and synapse formation during development, their involvement in nerve injury response remains poorly defined and mostly related to neuropathic pain control. Here we report novel roles of CB1 receptor at NMJs during degeneration/innervation processes following nerve injury. CB1 receptors are expressed in different muscles (e.g. EDL and Soleus) and are upregulated immediately after nerve crush ($p<0.05$). During the degeneration shortly after injury, CB1 antagonist treatment (AM251, IP injections, 3 mg/kg) caused an acceleration in the process,
with an increased percentage of degenerated NMJs 18h post-injury.

This occurred without alteration in the c-jun expression in EDL.
In the reinnervation process following complete nerve injury, AM251 treatment greatly reduced reinnervation as indicated with a significant percentage of partial innervated and denervated NMJs ($p<0.05$). The increased percentage of denervated NMJs was accompanied by a decrease of mono and poly-innervation, likely due to a delay in the reinnervation. Moreover, this CB1 regulation could be mediated via c-Jun pathway as suggested by a significant decrease of c-Jun expression ($p<0.05$).
These data highlight a novel role of CB1 receptor at NMJ and in the control of NMJ degeneration and reinnervation after nerve injury. A better understanding of these mechanisms could help address the inadequate NMJ maintenance observed in motor neuron-related neurodegenerative diseases.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

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Program #/Poster #: 045.06

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R21NS111334-01

Title: The influence of Schwann cells and exosomes on locomotion after repair of long-segmental peripheral nerve defect in the rat

Authors: *E. L. ERRANTE[1], T. SMARTZ[1], E. SCHAEFFER[4], J. OSTERLUND OLTMANNS[4], D. G. WALLACE[4], A. KHAN[2], S. S. BURKS[3], A. D. LEVI[3];
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Abstract: Peripheral nerve injury (PNI) occurs in approximately 3% of trauma patients. It is characterized by a loss of cellular and axonal integrity, producing deficits in motor function. Many PNIs are not amenable to repair with traditional techniques; however, cell therapies, particularly those that utilize Schwann cells (SCs), offer the promise of neural tissue and functional replacement. One component that is secreted by SCs includes exosomes, which carry cellular signaling molecules that can facilitate intercellular communication. They have shown promise in PNI, with studies showing SC-derived exosomes to contain the appropriate exosomal protein markers, associate to axons in high concentrations, and are able to improve the nerve regeneration process. Although the laboratory has had success using SCs in both preclinical and clinical treatment settings, SCs present their own set of issues; thus, it is imperative to find a better treatment strategy. The present project compared SCs to SC-derived exosome treatments

in second-generation collagen conduits, which have an internal lattice that improves presentation of cells to the axonal stumps. The aim of our study was to investigate if implanted SCs and SC-derived exosomes in conduits improve axonal regeneration and locomotor recovery after repair of a severe PNI. Adult male and female Fischer rats were divided into groups that included reversed autograft, SCs, and SC-derived exosomes, with all rats undergoing surgery that produced a significant gap in the sciatic nerve. Animals underwent locomotor assessment once every two weeks for the entirety of the 16-week experiment. At the end of the experiment, biochemical, immunohistochemical, electrophysiological recordings, and electron microscopy will be performed on all rats and their nerve grafts. Preliminary data indicates that exosomes can be inserted and visualized in the conduits, a novel observation to the author's knowledge. Further, preliminary data indicates that SCs are able to improve locomotor performance on a Catwalk compared to controls ($p<0.05$) and that nerve regeneration is improved with this treatment; however, comparing SCs to exosomes is still ongoing. Overall, preliminary data indicates that exosomes are visible and can be inserted into the complex collagen conduits for the first time. Additionally, SCs are able to produce functional improvement, as measured by a Catwalk. While data collection is still ongoing through the use of more behavioral testing and histological analyses, we are hopeful that we will be able to identify the best treatment method for a faster and improved degree of functional recovery after severe PNI.

Disclosures: E.L. Errante: None. **T. Smartz:** None. **E. Schaeffer:** None. **J. Osterlund Oltmanns:** None. **D.G. Wallace:** None. **A. Khan:** None. **S.S. Burks:** None. **A.D. Levi:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.07

Topic: C.10. Brain Injury and Trauma

Support: NIH Grant R21NS111334-01

Title: The influence of Schwann Cells and exosomes on pain outcomes after repair of a long-segmental peripheral nerve defect in the rat

Authors: *T. M. SMARTZ, E. L. ERRANTE, S. JERGOVA, S. S. BURKS, A. KHAN, A. D. LEVI;
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Abstract: Traumatic peripheral nerve injuries (PNI) are a very common and sometimes devastating group of disorders. PNI are challenging to satisfactorily treat with current surgical techniques and can lead to many debilitating symptoms. Neuropathic pain is common after PNI, which is usually accompanied by hypersensitivity to mechanical stimuli. To address the therapeutic shortcomings and neuropathic pain after PNI, we were interested in an approach using second-generation collagen conduits, which have a longitudinally oriented internal lattice.

This structure improves presentation of cells to the regenerating axonal stumps. While the laboratory has had immense success using these novel conduits in combination with Schwann cells (SCs), we were interested in comparing this condition to use of conduits with SC-derived exosomes. Exosomes are vesicles that can transfer macromolecules. They are secreted from many different cells, including SCs, and carry cellular signaling molecules to facilitate intercellular communication. SC-derived exosomes have been shown to increase peripheral nerve regeneration. Thus, the aim of the present study was to assess SCs and SC-derived exosomes in order to assess their abilities as a treatment method and their impact on neuropathic pain after a rodent model of a severe PNI. Adult male and female Fischer rats underwent surgery where the sciatic nerve was severed to create a large segmental gap. Conduits were then loaded with either exosomes or SCs and implanted to assist in nerve regeneration. After, every 2 weeks they were assessed for pain/sensation markers for the duration of the 16 week study. Specifically, a subset of rats underwent conditioned place preference training while all rats underwent von Frey filament testing and tests of temperature sensitivity. While data collection is still ongoing, preliminary data show that exosomes can be inserted and visualized in the second-generation collagen conduits, which is the first time this has been noted to our knowledge with the use of these tubes. Further, preliminary behavioral tests using pain/sensitivity measures show a difference in performance between controls and rats that have received an injury ($p<0.05$); however, the long-term direction of this difference has yet to be established. While data collection is still ongoing with additional behavioral testing and histological analysis, we are confident that we will be able to determine a better treatment method for both severe PNI and the neuropathic pain that is often produced from such injuries.

Disclosures: **T.M. Smartz:** None. **E.L. Errante:** None. **S. Jergova:** None. **S.S. Burks:** None. **A. Khan:** None. **A.D. Levi:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.08

Topic: C.10. Brain Injury and Trauma

Title: Transcription factors EB and E3 contribute to the differentiation of Schwann cells after peripheral nerve injury

Authors: *A. PATEL, C. HEFFERNAN, A. TORRES, A. MARQUEZ, P. MAUREL, R. DOBROWOLSKI, H. A. KIM;
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Abstract: After peripheral nerve system (PNS) injury, distal myelinating and Remak Schwann cells (SCs) temporarily convert to repair SCs to aid axon regrowth and remyelination. The molecular mechanisms underlying repair SC formation and function are incompletely understood. Transcription factors EB and E3 (TFEB and TFE3) are members of the MiT-TFE

family that play overlapping roles in regulating cellular clearance, integrated stress response, and stem cell pluripotency (among others). Recently, TFEB has been shown to be a negative regulator of oligodendrocyte myelination. SC TFEB function has also been implicated in myelin degradation following PNS injury. To define the role of TFEB and TFE3 in repair SC function, we generated desert hedgehog (DHH) Cre driven SC specific TFEB knock-out on a global TFE3 deficient background (dKO). Sciatic nerves were transected and collected at 3, 5, or 7 days post injury (DPI), with n=3 for all experiments. Morphological analysis of semi-thin cross sections of the distal nerves indicates a delay in myelin degradation, by an increased percentage of D-type fibers, at 7DPI compared to age-matched controls. Interestingly, injury-induced expression of Runx2 and p75, repair Schwann cell markers, was impaired early on (3DPI) in the mutant mice. Immunohistochemical analysis revealed a significant decrease in Runx2+ cells and a loss of p75+ repair-Schwann cell specific morphology in the mutant mice. Furthermore, Western blotting shows that dKOs are unable to maintain expression of c-Jun, a transcription factor known to control many aspects of repair SCs. RNA sequencing data from 5DPI nerves indicate an overall decrease in repair Schwann cell-specific gene expression profile (Sox2, Runx2, p75, etc.) and a sustained expression of myelinating Schwann cell genes (Krox20, MBP, Cdh1, etc.) in the mutant mice. Altogether, our data indicate that members of MiT-TFE family proteins TFEB/TFE3 play a role in repair Schwann cell formation following PNS injury.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.09

Topic: C.10. Brain Injury and Trauma

Support: EPSRC, grant number EP/R004463/1

Title: Modelling Regenerative Angiogenesis to Inform Peripheral Nerve Injury Treatment Strategies

Authors: *M. BERG[1], O. GUILLEMOT LEGRIS[2], D. ELEFTHERIADOU[2], J. B. PHILLIPS[2], R. J. SHIPLEY[3];
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Abstract: Peripheral Nerve Injuries (PNIs), affect more than 1M people p.a. in Europe and the USA. Paralysis and loss of sensation are hallmarks of severe PNIs, involving pain and loss of autonomy for patients. Large-gap PNIs cannot repair unaided and generally require a surgical intervention. Autografts are used to bridge between severed nerve stumps, inducing donor site morbidity and often yielding underwhelming functional recovery. Engineered Neural Tissue

(EngNT) constructs are being developed to address these challenges. They are cylindrical anisotropic cellular hydrogels that mimic nerve tissue and provide a supportive microenvironment to accelerate regeneration, whilst enabling spatial seeding of therapeutic cells and other regenerative factors. Therapeutic cells, under low-oxygen conditions, release pro-angiogenic factors such as vascular growth factor, spatial gradients of which act as chemical cues for the formation of new micro-vessels. A key question is then to find which therapeutic cell type, density and spatial distribution will lead to the best revascularisation and be able to sustain long term regeneration. This puzzle is challenging to solve with experiments alone, so we propose to integrate the approach with mathematical modelling. We develop a system of diffusion-reaction equations that we parameterise against in-vitro data, using Bayesian inferences. Doing so allows us to 1) describe the interplay between the therapeutic cells and their microenvironment, including cell proliferation, nutrient consumption, and growth factor secretion and 2) to consider uncertainties stemming from both the experimental setup and the formulation of the model. We overlay this description with a model for vessel dynamics that includes vessel growth, blood flow and molecular exchanges with the surrounding microenvironment. Coupling these two models allows us to reproduce the rich spatio-temporal dynamics between blood flow, angiogenic processes, oxygen delivery, growth factors and cell population during nerve repair. Simulations are performed for different therapeutic cell types and for a range of cell-seeding densities and spatial distributions to explore the impact on angiogenesis and cell survival. Results predict the spatio-temporal distribution of oxygen and growth factor throughout the EngNT, and the complex interplay between these distributions, the therapeutic cell population, and the vasculature. This computational-experimental approach allows us to explore a range of scenarios and identify new cell seeding strategies that may accelerate vascularisation of a repair construct to be taken forward to in vivo experimental testing.

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Poster

045. Peripheral Nerve Trauma

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Program #/Poster #: 045.10

Topic: C.10. Brain Injury and Trauma

Support: US Department of Defense grant W81XWH-17-0402
University of Wyoming Sensory Biology COBRE NIH Grant 5P20GM121310
National Institute of General Medical Sciences NIH Grant P20GM103432

Title: Graft orientation affects outcomes in complex peripheral nerve injuries

Authors: *J. ALLGOOD, J. BUSHMAN;
Univ. of Wyoming, Univ. of Wyoming, Laramie, WY

Abstract: Potential title: Graft orientation affects outcomes in complex peripheral nerve injuries Nearly 80 km of peripheral nerves extend and branch throughout the human body. Injuries to peripheral nerves are the most common cause of persistent neuronal dysfunction in the human population since regeneration is rarely restorative. Despite the highly branched structure and organization of the peripheral nervous system, almost all studies address only peripheral nerve injuries that are a single sharp bisection or a linear segmental defect. How regeneration may occur when the injury encompasses a branch point is unknown. This is presumably because there have not been any effective treatments to address branched injuries owing to a number of complications, the least of which being preferential motor axon reinnervation. This is a phenomenon where motor axons are recruited to preferentially reinnervate branches already containing motor axons, causing the remaining branch to suffer. Nerves with mixed axon morphology are particularly impacted. Our group has begun to study the regeneration of injuries through branched segmental defects using a combination of autografts and allografts, with dedicated or mixed axon morphology, in inbred and outbred rat strains and in combination with targeted localized immunosuppressive strategies. The injury model is a 2 cm complex segmental defect of the sciatic nerve that includes the peroneal and tibial branch point. After removal of this section, experimental groups included (1) autograft in the pre-injury orientation, (2) autografts in a switched orientation (auto-switch) where the peroneal branch within the autograft was sutured to the tibial branch and vice versa, (3-4) allografts of the sciatic branch in both the original and switched orientations and (5-6) allografts of the femoral branch, which contain dedicated motor or sensory branches, in both orientations. Outcome measures included compound muscle action potentials (CMAPs), gait analysis using the Noldus CatWalk system, end point nerve morphometry, muscle mass and retrograde labeling from each branch. Data indicates that outcomes are significantly influenced by graft source and orientation which provides the first framework for how graft material should be selected to repair complex branched segmental peripheral nerve defects.

Disclosures: **J. Allgood:** None. **J. Bushman:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

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Program #/Poster #: 045.11

Topic: C.10. Brain Injury and Trauma

Support: The General Insurance Association of Japan
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The Japan Agency for Medical Research and Development JP21gm6210004

Title: Pdgfr alpha expressing fibloblasts in epineurium promote the repair of the lesion site after peripheral nerve injury by enchancing endothelial cell migration via chrdl1 secretion

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Abstract: Because clinical outcomes after peripheral nerve transection injury (PNI) are still unsatisfactory, an effective therapy needs to be developed. Despite the recent advancement in understanding the repair mechanisms after PNI, the repair process of the lesion site, especially at the immediate post-injury period, remains to be elucidated. The purpose of the present study is to unveil the molecular and cellular mechanisms underlying the repair process of the lesion site after PNI. Time course study revealed that, from 1 to 4 days after transection injury in rat sciatic nerve, macrophages, epineurium-derived PDGFRα expressing fibroblasts (Epi-PDGFRα-Fibs), and Epi-derived endothelial cells (Epi-ECs) were migrating into the lesion site sequentially and that the transected Epi bridged the lesion site prior to the bridging of the transected parenchyma. When a transection injury was created with a lack of an Epi, the parenchyma failed to bridge the lesion site. The single cell RNA sequence on cells accumulating at the lesion site 2 days after injury revealed that the lesion site consisted of 12 cell populations including three types of Fibs and one type of ECs and that only Fibs highly expressed PDGFRα. When their PDGFRα function was inhibited with the functional blocking antibodies, the migration of Epi-ECs into the lesion site was impaired, resulting in the delay of the bridging of the Epi and parenchyma. Further, the transplantation of Epi-PDGFRα-Fibs into the lesion site promoted the migration of Epi-ECs into the lesion site, resulting in the acceleration of the repair of the Epi and parenchyma. The additional RNA sequence analysis demonstrated that Epi-PDGFRα-Fibs express CHRDL1, which is a soluble factor to antagonize BMP4, significantly greater than skin derived PDGFRα-Fib. Immunolabeling study confirmed that macrophages and Epi-PDGFRα-Fibs in the lesion site expressed BMP4 and CHRDL1 respectively. Lastly, an administration of CHRDL1 to the lesion site promoted the migration of Epi-ECs into the lesion site and enhanced the bridging of the epineurium as well as the parenchyma. These findings indicate that the repair of the lesion site after PNI requires the bridging of the Epi prior to that of the parenchyma and that Epi-PDGFRα-Fibs contribute to the repair of the Epi by promoting the migration of Epi-ECs via secreting CHRDL1 to inhibit macrophage derived BMP4. This unveils a novel repair mechanism of the lesion site at the immediate PNI period and sheds light on an indispensable role of specific Fibs in the Epi after PNI.

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Poster

045. Peripheral Nerve Trauma

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Program #/Poster #: 045.12

Topic: C.10. Brain Injury and Trauma

Support: NRF-2016R1D1A1B01014190 from National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology

Title: Epigenetic regulation of GAP-43 gene expression in Schwann cells facilitates axonal regeneration after Sciatic nerve injury

Authors: *K.-J. KIM[1], E. KIM[2], U. NAMGUNG[2];
[1]Daejeon UNiversity, Daejeon, Korea, Republic of; [2]Daejeon Univ., Daejeon, Korea, Republic of

Abstract: Previous studies have well documented that the production of axonal growth-associated protein GAP-43 is increased in axons after peripheral nerve injury. GAP-43 expression is also induced from a certain type of Schwann cells, but its function in Schwann cells has not been clearly defined. Given that Schwann cells undergo phenotypic changes accompanying multiple gene expressions after peripheral nerve injury, here we investigated the involvement of the epigenetic mechanism of GAP-43 expression in Schwann cells. The expression of GAP-43 mRNA and protein, which was induced from Schwann cells 3 - 7 days after nerve injury, was upregulated by the treatment of trichostatin A (TSA), a histone deacetylase (HDAC) inhibitor. Interestingly, TSA also induced GAP-43 expression and Schwann cell proliferation in the intact sciatic nerve whereas its effects on Schwann cell proliferation were less remarkable after nerve injury. To understand the molecular basis on TSA-induced proliferation of GAP-43-expressing Schwann cells, we analyzed the expression of cell division cycle 2 (Cdc2) in Schwann cells. Cdc2 expression was induced from Schwann cells after sciatic nerve injury and also increased by TSA treatment in the intact nerve. It was noted that Cdc2 was produced from the type of Schwann cells expressing both GAP-43 and myelin basic protein (MBP) in the sciatic nerve given injury and/or TSA treatment. Transfection of Schwann cells with a plasmid construct overexpressing GAP-43 shRNA resulted in decreased neurite outgrowth in co-cultured DRG neurons. In contrast, Schwann cells treated with TSA showed increased GAP-43 signals and enhanced neurite outgrowth of cocultured DRG neurons. Finally, immunofluorescence staining of NF-200 protein in the longitudinal nerve sections showed that TSA administration facilitated distal elongation of regenerating axons. Our data suggest that epigenetic regulation of GAP-43 gene expression in proliferating Schwann cells may play a role in promoting axonal regeneration after peripheral nerve injury.

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Poster

045. Peripheral Nerve Trauma

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Program #/Poster #: 045.13

Topic: C.10. Brain Injury and Trauma

Support: Postdoctoral Fondecyt grant to Génesis Vega H 3210751.
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Title: Targeting necroptosis to halt mitochondrial dysfunction and axon degeneration in chemotherapy-induced peripheral neuropathies

Authors: *G. VEGA[1], P. BRICEÑO[2], E. VERDIN[1], F. COURT[2];
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Abstract: Axonal degeneration program (AxDP) occurs in many diseases of the nervous system and is an important target for neuroprotection. Necroptosis, a programmed form of necrosis, has been linked to AxDP in the aged brain and neurodegenerative conditions such as Parkinson's and Alzheimer's disease. However, the exact role of necroptosis during the AxDP in peripheral neuropathies is unclear. Peripheral neuropathies (PNs) are currently the most common neurodegenerative disorders associated with cancer survivors treated with neurotoxic chemotherapeutic agents. Most of the available treatments for neuropathic pain in PNs have moderate efficacy and present side effects that limit their use; therefore, other therapeutic approaches are needed. We asked whether necroptosis activation is related to mitochondrial dysfunction during chemotherapy-induced axonal degeneration in peripheral neurons. To test this, we used an *in vitro* model of dorsal root ganglion neurons (DRGs) from E14 mice embryos and an *in vivo* model of OIPN (oxaliplatin-induced peripheral neuropathy) in adult and old mice. We measured necrosome activation, mitochondrial activation and its role in key features of AxDP. Our data showed an early activation of the necrosome in oxaliplatin-treated DRGs explants, leading to mitochondrial dysfunction and axonal degeneration. Pharmacological and genetic inhibition of the necrosome prevents early hallmarks of AxDP, mitochondrial dysfunction and late axonal fragmentation *in vitro*. In addition, *in vivo* experiments showed that pharmacological and genetic inhibition of necroptosis prevented axonal loss in skin and cornea and prevented thermal and mechanical hypersensitivity in mouse treated with oxaliplatin. In summary, our data suggest that in chemotherapy-induced PNs necroptosis activation triggers mitochondrial dysfunction, which ultimately leads to axonal degeneration. The study provides novel therapeutic targets and potential interventions against axonal degeneration in OIPN.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.14

Topic: C.10. Brain Injury and Trauma

Support: Queen's University, Department of Surgery

Title: A Detailed Characterization of the Mouse Median Nerve Injury Model for the Study of Peripheral Nerve Injury and Recovery

Authors: M. TOPLEY[1], A. BOYLE[1], A.-M. CROTTY[1], M. D. KAWAJA[2], *J. HENDRY[1];
[2]Biomed. and Mol. Sci., [1]Queen's Univ., Kingston, ON, Canada

Abstract: **Introduction** Hindlimb nerve injury models are some of the most common methods to study nerve regeneration. However, hindlimb nerve injuries do not reflect the deficits of upper extremity injuries, they also are prone to autophagy and involve elaborate functional analysis. Mouse models offer flexibility in studying molecular mechanisms through a wide array of transgenic strains. Furthermore, forelimb nerve injury models allow the study of more clinically representative recovery. This study presents a mouse median nerve injury model that details motor and sensory neuron populations, distal histomorphometry and electrically stimulated grip strength characteristics. **Methods** Two cohorts of 6 C57Bl/6 mice underwent median nerve transection in the axilla. Animals then underwent retrograde labeling of the regenerating median nerve 5mm distal to the transection site at either 5- or 8-days post injury. Retrograde labeling involved exposing the transected tip to a retrograde neurotracer for 1 hour to delineate the sensory neurons in the dorsal root ganglia and motoneurons in the ventral spinal cord. In the same procedure as retrograde labeling the 1mm segment of median nerve distal to the cut site was harvested, fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and processed for histomorphometric analysis using a custom macro with Clemex Vision Pro software. A third cohort of uninjured C57Bl/6 mice (n=4) underwent stimulated grip strength for baseline estimates of mouse grip strength normalized to body mass. This was performed under anesthesia and percutaneous delivery of a low voltage current to stimulate tetanic contraction of digit flexors and measuring the force required to break the grip using a Bioseb grip strength meter. **Results** The retrograde staining revealed that the sensory and motor neuron pools for the uninjured mouse median nerve were 2093 +/- 156 and 245 +/- 27, respectively. The greatest proportion of sensory arose from the C7 and C8 root levels. Lastly, histomorphometry revealed, the average number of myelinated axons was 813, myelin thickness was 1.8 μm, total fiber diameter was 5.6μm and g-ratio was 0.56. Functionally, we observed that the mean normalized values of mouse stimulated grip strength was a mean of 0.422 gram of grip per gram of body mass. **Conclusion** Taken together, the motor and sensory neuron pools, histomorphometry parameters, and grip strength, serve as a baseline reference to assess the functional and histological recovery of nerve regeneration. Furthermore, the use of a mouse median nerve injury allows for the use of transgenic manipulations, enhancing the ability to explore molecular pathways that regulate nerve injury.

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Poster

045. Peripheral Nerve Trauma

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Topic: C.10. Brain Injury and Trauma

Support: DoD Grant OR180077 W81XWH-19-2-0054
Lone Star Paralysis Foundation

Title: Polyethylene glycol fusion repair of severed sciatic nerves both restores sensory axon connectivity and reorganizes afferent terminal fields in the spinal cord

Authors: *E. HIBBARD[1], L. ZHOU[2], C. YANG[2], K. VENKUDUSAMY[2], G. D. BITTNER[2], D. R. SENGELAUB[1];
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Abstract: Peripheral nerve injuries are the most common type of nerve trauma, and result in the rapid degeneration of the distal nerve segments and immediate loss of motor and sensory functions. Behavioral recovery is typically poor. We use a plasmalemmal fusogen, polyethylene glycol (PEG), to immediately fuse closely apposed axolemmas of severed proximal and distal axons. Axons repaired by PEG fusion do not undergo Wallerian Degeneration, and PEG-fused animals exhibit rapid and extensive behavioral recovery. We previously reported that sciatic motor axons proximal to the injury and repair were successfully fused to distal motor axon segments. PEG-fusion of severed axons is non-specific, i.e., spinal motoneurons in PEG-fused animals were found to project to appropriate as well as novel target muscles. In the current study, we examined the consequences of PEG-fusion for sensory axons of the sciatic nerve. We hypothesized that PEG-fusion of sensory axons would also be nonspecific, resulting in a similar restoration of appropriate connectivity as well as reorganizations in sensory terminal fields in the spinal cord. Sensory labeling was examined in young adult male and female rats (Sprague-Dawley). At 2-21 days after either a unilateral single-cut or allograft repair of the sciatic nerve with PEG, we anterogradely labeled sensory afferents from the dorsal aspect of the hindpaw using a bilateral intradermal injection of WGA-HRP (0.3 µl, 2%). This hindpaw area is innervated by the peroneal branch of the sciatic nerve, and sensory axons terminate in a punctate area in the middle of the L3-L4 dorsal horn. Ipsilateral to the injury and PEG-fusion repair, sensory afferents were found in the appropriate area of the dorsal horn as well as in atypical medial areas. Contralateral to the injury and PEG-fusion repair, the sensory afferents from the unoperated nerve were found to expand both mediolaterally and rostrocaudally into areas within the dorsal horn beyond those labeled in unoperated control animals. We have previously demonstrated that the restoration of appropriate connectivity for motor axons after PEG-fusion repair is likely a consequence of their topographic organization within the sciatic nerve. Thus, the appropriate sensory labeling seen after PEG-fusion is likely due to a similar topographic organization of sensory axons within the nerve. Furthermore, PEG-fusion produces a reorganization of terminal fields both ipsilateral and contralateral to the repair. This central reorganization may contribute to the improved behavioral recovery seen after PEG-repair, supporting the use of this novel repair methodology over currently available treatments.

Disclosures: E. Hibbard: None. **L. Zhou:** None. **C. Yang:** None. **K. Venkudusamy:** None. **G.D. Bittner:** None. **D.R. Sengelaub:** None.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.16

Topic: C.10. Brain Injury and Trauma

Support: NIBIB/NIH, DP2EB028110
NINDS F99NS124174

Title: Nanotransfection-driven neurogenic reprogramming in skeletal muscle provides protection following denervation

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Abstract: Peripheral nerve injuries (PNI) can cause motor and sensory deficits that are short-lived or long-lasting and permanent. These injuries can be addressed at the soma in the dorsal or ventral roots, the focal injury, or downstream at the axon terminals. A limiting step of functional recovery can be seen in the rate of regeneration that is ~1 mm/day (~1 inch/month). This rate is crucial role in regaining function of denervated skeletal muscle that has a 12–18-month window for complete reinnervation to occur before fibrosis and scar formation becomes too severe. We propose the local induction of neurons in skeletal muscle can temporarily innervate the motor endplates to extending the window for functional recovery. Local gene delivery to peripheral nerves and tissues has the potential to enable novel therapies for PNI and overcome translational hurdles (e.g., reliance on viral vectors and cytotoxicity). We developed a novel and facile approach for non-viral cargo delivery into nerves and skeletal muscle in a highly deterministic and benign manner.
C57BL/6J mice underwent sciatic nerve transection at the mid-point of the bicep femoris. Cleanroom fabricated, nanochannel-platforms controllably nanoporated cell membranes on the triceps surae (TS) surface to electrophoretically deliver a neuron-inducing plasmid DNA cocktail. For five weeks post injury, the mice were assessed for electromyographic evidence of fibrillation, a phenomenon that occurs in muscle fibers when neuromuscular junctions (NMJ) are unoccupied. Histology of the TS and cell experiments were used to analyze NMJ morphology and cell reprogramming efficiency, respectively. Fibrillations were analyzed with Kaplan-Meier and show earlier resolution in the treated group compared with those receiving sham (90% vs 50%, respectively). The TS was collected from both limbs, weighed, and preserved for histological purposes. Muscle weights reveal no change with both groups showing ~30% reduction of muscle mass in the denervated limb. Histology of NMJ revealed a significant shift toward immature morphologies in the sham group. Experiments with the C2C12s have revealed their potential direct neuronal reprogramming.These results suggest myoblasts have direct neuron conversion potential that can prove protective after transection injuries. Ongoing studies are focusing on evaluating the timeline of neuronal induction, evaluating other electrophysiological outcomes, and related cellular/molecular pathways. Moreover, this study supports the viability of targeted gene delivery and cell-based therapies to enhance muscle preservation following a severe/chronic nerve injury.

Disclosures: **J. Moore:** None. **J. Albert:** None. **J.M. Winograd:** None. **I. Valerio:** None. **W.D. Arnold:** None. **D. Gallego-Perez:** None.

Poster

045. Peripheral Nerve Trauma

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.17

Topic: C.10. Brain Injury and Trauma

Support: NIH SBIR 7R44NS097113-05

Title: In vivo efficacy of a novel nerve coaptation device as a suture-less alternative for repairing peripheral nerve defects

Authors: *G. BENDALE[1], L. DANIEL[1], M. SMITH[1], I. DEBRULER[1], M. FERNANDES-GRAGNANI[1], R. CLEMENT[2], J. MCNEICE[2], F. GRIFFITTS[2], M. SONNTAG[2], J. GRIFFIS[2], I. P. CLEMENTS[2], J. ISAACS[1];
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Abstract: Though microsuture neurorrhaphy is the accepted clinical standard treatment for severed peripheral nerves, this technique requires high microsurgical proficiency and still often fails to provide adequate nerve approximation. Entubulation utilizing commercially available conduits may technically enhance nerve alignment and potentially provide a pro-regenerative microenvironment, but still requires precise suture placement. In response, we have developed Nerve Tape®, a suture-less coaptation device that incorporates tissue grabbing Nitinol microhooks within a flexible porcine small intestine sub-mucosa (SIS) backing. These tiny microhooks engage the outer epineurium of the nerve while the SIS backing wraps the coaptation to provides a stable, entubulated repair. We hypothesized that the Nerve Tape would provide enhanced axon regeneration compared to commercially available conduits or microsuture only assisted repairs. Eighteen male New Zealand White rabbits underwent a tibial nerve transection, immediately repaired with either 1) Nerve Tape, 2) conduit (AxoGuard Nerve Connector) plus anchoring sutures or 3) four 9-0 nylon microsutures spaced 90° apart within the epineurium. At sixteen weeks post-injury, the nerves were re-exposed to test sensory and motor nerve conduction, measure target muscle weight and girth, and perform nerve tissue histology. There were no statistically significant differences in nerve conduction velocity, gross morphology, or muscle characteristics between the three repair groups. Average total axon counts distal to the repair were 13223 ± 2390 for Nerve Tape, 13279 ± 1306 for conduit repair, and 12673 ± 1394 for microsutures (not statistically different; p=0.88). Average g-ratio for nerve tape, conduit repairs, and microsuture repairs were 0.5, 0.47, and 0.55 respectively (not statistically different; p=0.49). Axon counts proximal to the repair were 10819 ±1449 for Nerve Tape, 11945±2690 for conduit (AxoGuard), and 12053±1629 for microsutures and average g-ratios were 0.62, 0.6 and 0.64 respectively (not statistically different). Our data demonstrates that Nerve Tape offers similar efficacy compared to conduit assisted and microsuture only repairs, and is a suture-less alternative that will potentially increase the consistency and technical quality of peripheral nerve repairs.

Disclosures: **G. Bendale:** None. **L. Daniel:** None. **M. Smith:** None. **I. deBruler:** None. **M. Fernandes-Gragnani:** None. **R. Clement:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **J. McNeice:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **F. Griffitts:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **M. Sonntag:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **J. Griffis:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. **I.P. Clements:** A. Employment/Salary (full or part-time):; BioCircuit Technologies. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual Property. **J. Isaacs:** 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 SBIR Grant for the reported study. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Intellectual Property.

Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.18

Topic: C.10. Brain Injury and Trauma

Support: CDMRP: W81XWH2010277
PRMRP: W81XWH2110197

Title: Targeting macrophages using nanoparticles to study inflammation driven pain sensitivity in neuromuscular-polytrauma model

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Abstract: Individuals that suffer polytrauma injuries to skeletal muscle and nerves tissues develop chronic pain and aberrant muscle formation that can lead long recovery periods without adequate treatment. Chronic inflammation is often targeted as a key mediator with NSAIDs to improve muscle repair, though there is little evidence that muscle damage contributes to chronic pain. To study inflammation and pain-associated with muscle damage, we developed two novel rodent models using a closed-air piston system and nerve injury model. Firstly, to demonstrate that muscle injury contributes to pain, we crushed the left-gastrocnemius muscle with varying degrees of magnitude (1mm, 3mm, 5mm). Based on Von Frey performance and future therapeutic studies, the polytrauma model was developed using 1mm and 3mm muscle crush models combined with the well validated nerve injury model, Chronic Constriction Injury (CCI). Inflammation was tracked *in vivo* and *ex vivo* using dual-labeled nanoparticles in both models.

Varying the magnitude of muscle crush, we observed immediate and dose-dependent allodynia in muscle crush groups. Despite a significant change in grip strength and print area from baseline performance at 3 days post-injury (dpi), there were no differences among muscle crush groups for these behaviors. Polytrauma injuries showed muscle crush dependent increase in allodynia with mechanical sensitivity diminishing at 21 and 49dpi, respectively. Similar to single injury models, we observed acute grip strength changes, yet no disruptions in gait or any differences between polytrauma groups. Tracking inflammation *in vivo*, we observed accumulation of nanoparticles at site of injury at 3dpi which diminished at 14 and 28dpi for muscle crush and polytrauma injury models. Macrophage and T-cell analyses in targeted tissues showed preferential uptake of nanoparticles in CD68 expressing macrophages. Since NSAIDs are widely used to reduce inflammation associated with soft tissue injuries, we loaded nanoparticles with the selective COX-2 inhibitor, Celecoxib, and performed a treatment time course with 1mm-CCI polytrauma subjects. Reductions in pain sensitivity, grip strength and macrophage accumulation at the site of injury were dependent upon the treatment timepoint, suggesting the effect of COX-2 inhibition in macrophages varies over the course of the response to polytrauma injury. Here we established reproducible inflammation and mechanical sensitivity in two novel models of muscle crush and polytrauma. Our research demonstrates that targeting macrophage-driven mechanical sensitivity may reduce recovery time after neuromuscular-polytrauma injury.

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Poster

045. Peripheral Nerve Trauma

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Topic: C.10. Brain Injury and Trauma

Support: United States Department of Defense Award No. W81XWH-18-1-0321
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United States Department of Veterans Affairs, Rehabilitation Research and Development Service Merit Review Award # 5 I01 RX003566-02

Title: Targeted peripheral nerve stimulation to improve residuum tissue health in diabetic amputees

Authors: *H. S. MORGAN[1,2], R. J. TRIOLO[1,2], H. CHARKHKAR[1,2];
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Abstract: Diabetes is the leading cause of lower-limb amputations in the United States. Diabetic peripheral neuropathy (DPN) damages peripheral nerves in the extremities, causing loss of sensory, motor, and autonomic function. Because of the continued proximal progression of the

disease, diabetic amputees tend to have poorer prognoses with a significant risk of re-amputation. The goal of this pilot study was to evaluate the feasibility of using peripheral nerve stimulation paradigms established for restoring sensation in traumatic amputees to improve perfusion of the residual limb. A methodology for using thermographic imaging to quantify blood flow changes based on skin surface temperature was also developed. Electrical stimulation was delivered to the peripheral nerves in the residual limb of three individuals with traumatic transtibial amputation via chronically implanted 16-contact nerve cuff electrodes. Stimulation parameters and cuff contacts were selected based on reported location, intensity, and quality of elicited sensations; contacts for which elicited sensations corresponded to the lateral side of the residuum were used in this experiment. Sensations reported included pressure, tingling, and pulsing. Thermographic images of the residual limb were captured at regular intervals while the participants were seated with their prosthesis removed. The trial consisted of a baseline period, followed by a period of stimulation during which current was delivered in short intervals via relevant contacts, and a post-stimulation recovery period. Visual markers were placed at locations of interest on the lower limb and an image processing script using feature detection and tracking was developed in MATLAB (MathWorks, Inc.) to extract the temperature values at these selected locations over the course of the experiment. This methodology compensated for natural limb movement of the participant and allowed the temperatures of large areas to be monitored simultaneously. Based on preliminary data, an increase in temperature during the stimulation period was observed only in the lateral region of the residuum targeted by the electrical stimulation and not in the medial region. These observations indicate that peripheral nerve stimulation can transiently affect blood flow in the extremities and further that this effect can be directed toward target areas. Further data collection is underway to establish statistical significance. Future work includes measuring microvasculature perfusion and oxygenation under the same stimulation conditions to validate any changes in the blood flow due to peripheral nerve stimulation.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.20

Topic: C.10. Brain Injury and Trauma

Title: Trigeminal and Peripheral Electrical Stimulation to Augment Peripheral Nerve Regeneration in a Murine Forelimb Gap Repair Model

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Abstract: Peripheral nerve injuries are a common problem that incur significant morbidity and loss of function to patients. One method to improve outcomes in nerve regeneration is electrical stimulation (ES), which has been shown to speed axonal growth when administered peripherally and evoke targeted neuroplasticity in the cortex when administered to cranial nerves. The aim of this study is to demonstrate that the trigeminal nerve can be used as a novel target for ES to improve functional outcomes of peripheral nerve injury in a rat forelimb injury model. After being trained to proficiency in a reach and grasp task, 48 male Lewis rats were randomized into 4 groups: 1) sham injury, 2) nerve injury with sham ES, 3) nerve injury with intraoperative peripheral ES, and 4) nerve injury with trigeminal ES during post injury rehabilitation. Nerve injuries consisted of complete transection of the median and ulnar nerve 1cm proximal to the medial epicondyle of the humerus with a 2mm gap repair. Rats in group 3 were fitted with a bipolar cuff electrode proximal to the repair and stimulated (biphasic, cathode leading 100us pulses, current amplitude 80% muscle activation threshold, 20Hz) for 1 hour. Following stimulation, the cuff electrode was removed and the site closed. All groups were allowed 6 weeks of recovery prior to the start of a 6-week rehabilitation phase which consisted of the rats being retrained on the same reach and grasp task performed pre-injury. At week 5 of recovery, rats in group 4 were implanted with a bipolar cuff electrode on the supraorbital branch of the trigeminal nerve. During rehabilitation these rats received 500ms of stimulation (biphasic, cathode leading, 200us pulses, current amplitude 80% of side effect threshold, 30 Hz) immediately following each successful reach and grasp. Median peak pull force and success rates were collected at pre-injury baseline during the 6-week rehabilitation period for all groups. Mechanosensory thresholds were assessed prior to injury and every two weeks during the recovery and rehabilitation phases. Pull force, success rate and mechanosensory threshold changed significantly due to injury compared to baseline measures. Preliminary results show that rats who received either trigeminal or peripheral stimulation outperformed sham stimulated animals after 6 weeks of rehabilitation. These data suggest that trigeminal stimulation may have similar mechanisms to vagus nerve stimulation, which has been demonstrated to evoke targeted neuroplasticity and improve functional outcomes in animal models. The trigeminal nerve offers a promising target for ES as it can be stimulated without the need for surgical implant of an electrode.

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Poster

045. Peripheral Nerve Trauma

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 045.21

Topic: C.10. Brain Injury and Trauma

Support: NIH

Title: Varying the dosage and timing of vagus nerve stimulation can differentially impact recovery from peripheral nerve injury.

Authors: *A. D. RUIZ[1], K. MALLEY[3], T. DANAPHONGSE[5], C. MOTA BELTRAN[4], M. WHITE[4], S. HUSAIN[4], A. NABEEHA[4], A. YOUSAF[4], I. WAMBUGU[4], N. VALMIKI[4], S. A. HAYS[2];
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Abstract: An estimated 30 million people in the United States experience some form of peripheral neuropathy. Treatment of peripheral nerve injury is focused on management of the symptoms, which fail to provide sufficient benefits in many patients. Vagus nerve stimulation (VNS) paired with rehabilitative training has been shown to enhance recovery after various neurological injuries, including traumatic peripheral nerve damage. Improved recovery is mediated by VNS-dependent facilitation of synaptic plasticity. Although VNS reliably results in increased recovery of motor and sensory function, optimization of the therapy is necessary for effective clinical translation. This study comprehensively characterizes the effect of varying the timing and dosing of VNS therapy in a model of nerve injury. Animals with a median and ulnar nerve injury received tactile therapy paired with VNS in various paradigms. In the first experiment, the number of daily and weekly VNS pairings was varied across total therapy length. Results show that enhanced recovery of somatosensation requires that VNS must be delivered many times a day, with daily repetition, across many weeks. In the second experiment, VNS stimulations were delivered either, as a block before, concurrent with, or as a block after tactile therapy. Results shows that VNS must be delivered concurrently during rehabilitation to most effectively promote recovery of somatosensation after peripheral nerve injury. These findings directly inform the clinical translation of this promising approach.

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Poster

045. Peripheral Nerve Trauma

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Program #/Poster #: 045.22

Topic: C.10. Brain Injury and Trauma

Support: NIH R01 NS094384
R01 NS103803

Title: Moderate intensity vagus nerve stimulation promotes somatosensory recovery.

Authors: *K. MALLEY, A. RUIZ, T. DANAPHONGSE, F. AHMAD, S. BAGHDADI, M. MIAN, J. MONTEFALCON, R. RAPHI, B. STANISLAV, A. SULE, S. HAYS, M. KILGARD; Univ. of Texas at Dallas, Univ. of Texas at Dallas, Richardson, TX

Abstract: Vagus nerve stimulation (VNS) represents a novel neuromodulation approach to facilitate rehabilitative interventions for neurological injuries. When short bursts of stimulation are paired with particular motor exercises, VNS enhances synaptic plasticity in the trained networks and promotes significantly greater motor recovery than equivalent rehabilitative training without stimulation. VNS has received FDA approval to improve recovery of motor function after stroke, and recent preclinical studies and a pilot clinical study indicate a similar intervention enhances recovery of somatosensory function. Optimization of VNS therapy is a key step in translation of this approach. In this study, we characterized the relationship between VNS stimulation intensity and somatosensory recovery. Animals with a median and ulnar nerve injury received tactile therapy paired with VNS at either 0 mA, 0.4 mA, 0.8 mA, or 1.2 mA. Right forepaw somatosensation was assayed with an automated esthesiometer. We found a significant effect of both stimulation intensity and time on withdrawal thresholds. Only 0.8 mA VNS paired with tactile rehabilitation significantly improved withdrawal thresholds compared to equivalent rehabilitation without stimulation. Groups that received 0.4 mA and 1.2 mA VNS showed a modest trend toward improvements compared to Rehab alone. 0.4 mA VNS resulted in significantly less recovery compared to 0.8 mA VNS, and, similarly, 1.2 mA VNS displayed a trend towards less recovery than 0.8 mA VNS. Together, these findings demonstrate that 0.8 mA VNS significantly improves recovery of forelimb withdrawal thresholds after median and ulnar nerve injury. Additionally, consistent with previous studies, lower and higher stimulation intensities fail to produce the degree of recovery observed with moderate intensity stimulation. These results support previous studies and inform the clinic to most effectively deliver VNS therapy.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.01

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CAPES (process 88887.369624/2019-00)
CNPq (process 303050/2021-7)
FAPESP (process 2018/05006-0)

Title: Neuroprotection and glial modulation by transgenic human embryonic stem cells (hESCs) overexpressing FGF2 after avulsion and reimplantation of spinal motor roots

Authors: B. H. M. LIMA[1], S. KYRYLENKO[2], R. S. FERREIRA JR.[3], B. BARRAVIERA[3], ***A. L. OLIVEIRA**[1];
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Abstract: Traumatic peripheral nerve injuries are the most frequent in individuals of working age, generating disabilities and high costs to the health and social security system. Spinal cord root avulsion, in particular, modifies cellular homeostasis generating retrograde changes in the axotomized motoneuron microenvironment, leading to degenerative outcomes, including neuronal loss and glial reactivity. Such events lead to permanent functional loss, although may be prevented to a certain extent if root reimplantation and immunomodulation are performed timely. Given this, cell therapy constitutes an alternative to enhance pharmacological treatments, which have not yet proven to be sufficient, to minimize the deleterious outcome following CNS/PNS interface nerve root damage. Thus, in the present work, human embryonic stem cells (hESCs), bioengineered to overexpress FGF2 (18KDa, 23KDa, 31KDa), were used in combination to reimplantation of ventral roots after avulsion, by using a heterologous biopolymer of fibrin. For that, female Lewis/HsdUnib rats (n=70) were subjected to L4-L6 (sciatic nerve) motor root avulsion and reimplantation, associated with hESCs therapy. Acute effects of trauma were studied two weeks post-injury by Nissl staining (neuronal survival) and immunohistochemistry to detect synapse preservation, astroglial and microglial attenuation. Long-term recovery was monitored for twelve weeks via the walking track test (CatWalk system), combined with *ex-vivo* morphological analysis. When compared to the group without cell therapy, a significant degree of neuroprotection was observed following hESCs engraftment with the heavy molecular weight isoform (73%±4%, mean±SEM, ****$p<0.0001$), together with the preservation of the synaptic coverage (synaptophysin immunolabeling, 79%±4%, ****$p<0.0001$). Nevertheless, loss of glutamatergic inputs was depicted by the downregulation of VGLUT-1 staining (48%±5%, *$p<0.05$). Furthermore, even with the increase in microglial expression (869%±62%, **$p<0.01$), astroglial reactivity was attenuated (179%±7%, ***$p<0.001$), putatively contributing to the reduction of glial scar formation, thus facilitating the regrowth of motor axons towards the reimplanted roots. Concurrently, hESCs therapy enhanced functional recovery, evaluated by the peroneal functional index. Therefore, transgenic hESCs can be considered an effective delivery system of neurotrophic factors, rescuing axotomized motoneurons, and modulating the glial response after spinal cord root injury.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.02

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH grant 1R15NS125565-01
State of New Jersey Commission on Spinal Cord Research grant CSCR14ERG002
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Title: Elegant signaling: two plexins and three semaphorins guide neuronal regeneration

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Abstract: Extracellular signaling proteins that mediate neuronal growth cone guidance during development are well positioned to be involved in neuronal regeneration and recovery from injury. Semaphorins and their receptors, the plexins, are a family of highly conserved proteins involved in axon pathfinding and synapse formation during development. The *Caenorhabditis elegans (C. elegans)* genome encodes for only 3 semaphorins and 2 plexin receptors, compared to 20 semaphorins and 9 plexins in the mammalian nervous system. The transmembrane semaphorins SMP-1 and SMP-2, signal through their receptor PLX-1, while the secreted semaphorin MAB-20, signals through PLX-2.
We took advantage of the small number of signaling components in *C. elegans*, its natural ability to regenerate or regrow neuronal processes after injury and the capability to precisely disconnect single neurites using femtosecond laser microsurgery, to investigate the role of semaphorin signaling in neuroregeneration *in vivo*. We described the transcriptional neuronal expression patterns of all semaphorins and plexins by co-expressing fluorescent protein driven by their promoters in the NeuroPAL strain, which allows for unambiguous identification of individual neurons. We identified expression patterns for both receptor promoters in subgroups of motoneurons in the ventral nerve cord that extend dorsoventral commissures, which we targeted for microsurgery. We investigated the regrowth and reconnection of motoneuron neurites, and the recovery of locomotion behavior following laser microsurgery in plexin-knockout strains. Regrowth and reconnection were more prevalent in the absence of each plexin, while recovery of locomotion surpassed regeneration in all genotypes. These results suggest that the secreted and membrane-bound semaphorin signaling pathways both restrict regeneration but in distinct processes that likely include spatial specificity and recurrent signals.
To identify specific locations of semaphorin activity and determine its spatiotemporal response to injury, we are generating transgenic animals with fluorescently tagged secreted semaphorin and animals in which a split fluorescent protein indicates the apposition of the plexin (PLX-1) with membrane bound semaphorins (SMP-1 and 2).

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 046.03

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H. Neilsen Foundation

Title: Single nucleus RNA sequencing studies demonstrate that different subtypes of V2a propriospinal neurons respond differently to spinal cord injury

Authors: *C. THAPA[1], P. CHATURVEDI[2], I. WALLING[1], S. M. BAUMGARTNER[3], S. A. CRONE[3];
[1]Univ. of Cincinnati, Cincinnati, OH; [2]Developmental Biol., [3]Neurosurg., Cincinnati Children's Hosp. Med. Ctr., Cincinnati, OH

Abstract: Propriospinal neurons are a heterogenous population of neurons that are critical for recovery after spinal cord injury. These neurons can be categorized based on differences in gene expression, neurotransmitter type, developmental origin, spatial location and functional properties. We hypothesize that the different propriospinal neurons respond differently to injury and undergo different changes during recovery. Understanding these differences can lead to identification of therapeutic targets to help restore function after injury. Our lab has previously shown that the V2a class of propriospinal neurons is important for recovery of breathing following spinal cord injury. Using single nucleus RNA sequencing, we show that V2a neurons are a heterogenous population of neurons that can be resolved into 12 clusters based on gene expression patterns. We further demonstrate that different subtypes of V2a have spatially distinct locations in the spinal cord (e.g. medial versus lateral) and thus likely receive different inputs and perform distinct functions. Five days following a C2-hemisection spinal cord injury, we observed there are more downregulated genes than upregulated genes in V2a neurons. We found pathways that were consistently up or downregulated after injury across all V2a clusters using gene enrichment analysis. In addition, we identified changes in pathways/genes after injury that were cluster specific. These results support our hypothesis that different types of neurons respond differently to spinal cord injury. The identified pathways/genes may help in identifying novel therapeutic targets to promote recovery of function after spinal cord injury.

Disclosures: C. Thapa: None. **P. Chaturvedi:** None. **I. Walling:** None. **S.M. Baumgartner:** None. **S.A. Crone:** None.

Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 046.04

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Characterizing the context-dependent roles of DLK and LZK in injured corticospinal motor neurons

Authors: *C. CHAVEZ-MARTINEZ[1], N.-S. SAADIQ[1], A. MARTINEZ[1], H. J. KIM[1], Y. JIN[2], B. ZHENG[1];
[1]Neurosciences, Univ. of California San Diego, La Jolla, CA; [2]Div. Biolog Sci, Neurobio. Section, Univ. Of California San Diego Neurosciences Grad. Program, LA Jolla, CA

Abstract: The corticospinal tract (CST) is one of the principal pathways for voluntary movement, and is made up of corticospinal motor neuron (CSMN) axons that descend from the cortex into the spinal cord. Previously, we have shown that two mitogen-activated protein kinase kinase kinases (MAP3Ks), dual leucine zipper kinase (DLK) and leucine zipper bearing kinase (LZK), promote regeneration in CSMNs following spinal cord injury. Here, we investigate the pro-apoptotic roles of DLK and LZK in injured CSMNs, using multiple injury models in combination with genetic loss-of-function strategies.

Disclosures: C. Chavez-Martinez: None. **N. Saadiq:** None. **A. Martinez:** None. **H.J. Kim:** None. **Y. Jin:** None. **B. Zheng:** None.

Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.05

Topic: C.11. Spinal Cord Injury and Plasticity

Title: Leucine Zipper-bearing Kinase (LZK) is a regulator of cytoskeleton organization and astrocyte cell migration

Authors: *M. HEMATI GOURABI;
Neurosci., Spinal Cord and Brain injury Res. Ctr., Lexington, KY

Abstract: After Spinal cord injury, reactive astrocytes migrate toward the injury site to form the astrocytic scar. Our lab discovered leucine zipper-bearing kinase (LZK) as a major positive regulator of astrocyte reactivity to injury. This study examines the role of LZK in the regulation f astrocyte cell migration. Astrocytes were isolated from tamoxifen-inducible, astrocyte-specific LZK knockout (KO) mice and *4-Hydroxytamoxifen was applied to induce gene deletion in vitro*. We assessed cell migration by scratch assay, lamellipodia characterization, microtubule acetylation, and filamentous to globular actin ration. Astrocytes lacking LZK showed decreased cell migration, reduced length of lamellipodia, and lower levels of polymerized actin and acetylated tubulin. These results suggest that LZK promotes astrocyte migration by regulating

tubulin and actin dynamics in cytoskeleton rearrangement. Pathways through which LZK causes these cytoskeletal changes are under investigation.

Disclosures: M. Hemati Gourabi: None.

Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.06

Topic: C.11. Spinal Cord Injury and Plasticity

Support: U54AG062334
R01NS121533

Title: Dynamic regulation of RNA-binding proteins after axonal injury

Authors: B. J. HARRISON[1], J. MATTA[2], ***P. WARD**[2];
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Abstract: Individuals with spinal cord and peripheral nerve injuries are faced with many life-long physiological challenges. Functional deficits and challenges persist in part because the peripheral nervous system (PNS) possesses only a limited ability to regenerate, and axon regeneration in the central nervous system (CNS) is greatly limited. Researchers are finding new ways to support axon regeneration in the PNS and CNS, and possible treatment options include targeting RNA-protein interactions. RNA binding proteins are involved in many facets of nervous system function. The morphology of neurons that project to spinal cord from peripheral nerves is unique in that the combined mass of the axon and terminal processes massively exceeds the neuronal soma. mRNAs, mitochondria, and proteins are continuously transported along the axon to support and maintain these distal parts of the neuron. In addition, remote nerves and termini require the ability to translate mRNAs on demand, to respond rapidly to changing circumstances, such as injury. RNA binding proteins regulate the transport, storage, and local translation of specific mRNAs. Herein, we examine the dynamic regulation of RNA-binding proteins after injury, identify novel target RNA-binding proteins that may be involved in axon regeneration, and discuss the significance of RNA binding protein involvement in axon regeneration, with a specific emphasis on those that result in functional recovery.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.07

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS083983

Title: Single cell sequencing reveals transcriptional responses of corticospinal tract neurons to axotomy and to forced expression of candidate pro-growth factors

Authors: *E. BATSEL[1], M. G. BLACKMORE[2], S. TESTOR[3], Z. BEINE[3], I. VENKATESH[4]; [1]Marquette Univ. Dept. of Biol. Sci., Milwaukee, WI; [2]Biomed. Sci., Marquette Univ., Milwaukee, WI; [3]Marquette Univ., MILWAUKEE, WI; [4]Ctr. for Cell and Mol. Biol., Hyderabad, India

Abstract: Axonal connectivity by long-distance projection neurons such as corticospinal (CST) is achieved during development in two phases. The first is characterized by the growth of lead axons that is rapid (>1mm/day), fasciculated, linear, and travels distances of centimeters. In the second, numerous collateral sprouts emerge from points along the initial axon shaft and grow individually through adjacent grey matter. These are highly synaptogenic, often slow-growing (10s of microns per day), and extend only millimeters from their point of exit. After spinal cord injury in the adult, mature CST neurons display a spontaneous sprouting response that resembles this second phase of developmental growth but do not re-initiate the initial mode of long-distance axon elongation, constraining recovery. To better understand the adult injury response, we examined transcriptional changes in mature CST neurons as they responded to spinal axotomy and compared it to the pattern of gene expression in embryonic neurons during rapid axon elongation. Cell nuclei of CST neurons were retrogradely labeled by spinal injection of AAV2-retro-H2B-mScarlet, animals were left uninjured or subjected to spinal injury, and one week later the cell nuclei of CST neurons were purified by fluorescence-activated nuclei sorting and analyzed by single nuclei sequencing (10X Chromium). Corresponding single-nuclei data were derived from publicly available datasets from the cortex at embryonic day 18, an age in which projection neurons are engaged in rapid axon elongation. As expected, adult CST neurons showed large differences in gene expression compared to E18 neurons, with many hundreds of genes detected as at least two-fold up-or downregulated. More than 100 strongly downregulated transcripts were linked to ontological terms such as axonogenesis, cytoskeleton, or translation, indicating a potential contribution to axon growth. Spinal injury, however, triggered very muted changes in transcript abundance and almost no re-expression of embryonic, growth-relevant transcripts. These data support the hypothesis that the absence of rapid axon elongation in adult CST neurons after injury may reflect in part the low abundance of transcripts that support initial axon elongation during embryogenesis. Accordingly, using the same single-cell sequencing platform, ongoing work is systematically testing whether forced re-expression of candidate pro-regenerative transcription factors in adult CST neurons leads to re-expression of a targeted set of embryonic, growth-relevant transcripts.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Program #/Poster #: 046.08

Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant NS108189
W.F Keck Foundation grant

Title: Luminescent reporters to track AAV cargo expression in living mice

Authors: *O. STEWARD[1], M. METCALFE[5], K. H. COLE[2], K. K. NG[3], L. P. HALBERS[3], C. C. CHEN[3], A. LUPTAK[6], J. A. PRESCHER[4];
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Abstract: Viral vector-based delivery of gene modifying cargos is being used increasingly for tests of therapeutic candidates for a variety of neurological disorders. A current bottleneck for development of approaches using viral vectors is that initial timing and dosing studies currently require time-consuming and costly post-mortem assays involving multiple animals at multiple time points. Such parametric studies could be carried out more quickly and efficiently, with major cost savings and huge reduction of animal numbers, if viral vector cargo expression could be quantitatively assessed over time in living animals. Repeated assessment in living animals would be especially relevant for tracking regulated (on-off) expression of viral vector cargos. Here we report initial studies using bioluminescent reporters (molecular lanterns) to track expression of AAV cargos over time in living rodents using non-invasive imaging techniques. Bioluminescence is the biology of fireflies where an enzyme (luciferase) acts on a substrate (luciferin) to generate photons. We engineered two AAV constructs that express firefly luciferase (Fluc): 1) AAV2/Fluc; 2) AAV2-*retro*/Fluc-GFP, which enables remote transfection of cells of origin of the corticospinal tract via retrograde transport following injections of the vector into the spinal cord. Vectors were injected into the cortex or spinal cord of mice. To assess luminescence, mice received IP injections of luciferin and photon emission was assessed while mice were lightly anesthetized in an In Vivo Imaging System (IVIS). Light produced by AAV-encoded Fluc in response to IP injections of the substrate luciferin was readily detected through the skull and scalp in living mice allowing quantification of photon flux. Luminescence ramped up over the first 5 days post-injection and then remained relatively stable with repeated imaging. IVIS imaging of acute brain slices from mice with AAV2/Fluc-injections into the cortex revealed robust focal luminescence at the injection site. With AAV2-*retro*/Fluc-GFP injections into the spinal cord, luminescence in brain was generated from retrogradely-transduced spinally-projecting neurons identified by immunostaining for GFP. Parametric studies are ongoing to define relationships between AAV genome copy number and photon generation.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

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Program #/Poster #: 046.09

Title: WITHDRAWN

Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 046.10

Topic: C.11. Spinal Cord Injury and Plasticity

Support: Kentucky Spinal Cord and Head Injury Research Trust (KSCHIRT)
National Institute of Neurological Disorders and Stroke (NINDS) NS121193

Title: Astrocytes promote acute survival of CNS macrophages and motor recovery after SCI

Authors: *T. CAO[1], M. HEMATI GOURABI[1], W. FENSKE[1], X. XU[1], A. MILLS[1,2], L. BAUR[1,2], M. CHEN[1,3];
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Abstract: Spinal cord injury (SCI) results in permanent disability and affects more than 250,000 individuals in the US. Astrocytes, together with microglia and monocyte-derived macrophages (referred to as CNS macrophages), coordinate wound healing after SCI. Much of how these cells orchestrate wound compaction remains to be understood. We found that astrocytes produce colony stimulating factor 1 (CSF1), which is vital for microglia and macrophages' proliferation, survival, and phagocytic activity. In this work, we aim to examine the effects of astrocyte-derived CSF1 on the cellular dynamics of astrocytes, microglia, and macrophages at the lesion site in acute and chronic stages of SCI, modeled by complete crush at thoracic level T8 in age-matched mice. After 7- and 60-days post-injury, the cell composition of the injury site is examined by flow cytometry. SCI reduced astrocyte and microglia cell numbers at 7 days post-injury. At 7 days after SCI, mice with CSF1 deletion in adult astrocytes showed a trend toward reducing microglia, macrophage, astrocyte and lymphocyte cell numbers at the lesion site. At the chronic time point of 60 days after SCI, however, astrocytic CSF1 knockout mice trended towards higher cell numbers of all three cell types, suggesting a mechanism compensating for their early loss. Strikingly, astrocyte-specific CSF1 gene deletion efficiency negatively correlates with astrocytes and microglia cell numbers at the lesion site on 7dpi. Correlated to the number of astrocytes and Basso Mouse Scale, regular horizontal ladder testing over 8 weeks showed

worsened hindlimb motor recovery in astrocytic CSF1-knockout mice. These results indicate that astrocyte-derived CSF1 supports the acute survival of CNS macrophages and functional recovery following SCI.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Craig H Neilsen Foundation. postdoctoral fellowship grant 2019
C33266GG SCIRB IDEA,

Title: Targeting epithelial-to-mesenchymal transition-like signaling in astrocytes to improve outcomes following SCI

Authors: *A. VIVINETTO[1], A. BERNSTEIN[1], J. W. CAVE[2], E. HOLLIS[1,3];
[1]Burke Neurolog. Inst., White Plains, NY; [2]InVitro Cell Res., Fort Lee, NJ; [3]Feil Family Brain and Mind Institute, Weill Cornell Med., New York, NY

Abstract: Following spinal cord injury (SCI), astrocytes create a boundary that surrounds the lesion and reduces secondary degeneration during the sub-acute period. Strategies aimed at accelerating boundary formation offer the opportunity to enhance these beneficial actions of astrocytes and improve neurological outcomes after SCI. Developing such strategies, however, requires a detailed understanding of the molecular mechanisms that regulate astrogliosis and boundary formation. The cellular changes that occur during astrogliosis share features with cells undergoing wound healing processes in non-neural epithelium. Epithelial wound healing is regulated by a molecular signature known as the epithelial-to-mesenchymal Transition (EMT). We have found that the Zeb family (Zeb1 and Zeb2) of canonical EMT transcription factors are selectively overexpressed in astrocytes after SCI. Furthermore, conditional deletion of Zeb2 in astrocytes resulted in decreased astrogliosis, increased lesion volumes, and decreased recovery of motor function after moderate contusive SCI. Based on these findings, we decided to evaluate the impact of pharmacological EMT modifiers on astrocyte reactivity and functional recovery after SCI. We modulated EMT-like signaling in vivo by delivering recombinant RANKL. Binding of receptor activator of nuclear factor-kappaB ligand (RANKL) and its ligand RANK facilitates EMT and both RANK and RANKL are upregulated in astrocytes after SCI. Our preliminary results indicate that RANKL infusion affects functional recovery after SCI. Together, these results show that EMT factors are up-regulated in astrocytes after SCI and suggest that modulation of the astrocytic EMT-like response could be used to optimize boundary formation, preserve function, and improve outcomes following SCI.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: Grant-in-Aids for JSPS Research Fellow JP20J12345
Grants-in-Aids for AMED-CREST 18gm1210005h0001

Title: Single-nucleus RNA sequencing revealed ependymal-like cells induced by spinal cord injury in neonatal mice

Authors: *I. IKEDA_YORIFUJI, H. TSUJIOKA, T. YAMASHITA;
Osaka Univ., Grad. Sch. of Medicine, Osaka Univ., Suita, Japan

Abstract: [Background] The central nervous system of adult mammals has limited regenerative potential, and spinal cord injury (SCI) often causes permanent motor disfunction. In contrast, injured spinal cord tissue can be restored and neural function can be significantly recovered in neonates. A scar is formed at the SCI lesion in adult rodents; however, in neonates, scar structure and severe inflammatory response are not observed, and axon regrowth is achieved in the lesion. Bulk RNA-seq of the adult and neonatal injured spinal cord has shown their different pathological inflammatory reactions after SCI. However, different cellular composition in the lesion between adults and neonates has not been well characterized. [Methods/Results] We performed dorsal spinal cord hemisection at T10 using needles for 7-week-old adult and postnatal-day-1 neonatal mice. To compare the tissue repair processes in adults and neonates after SCI, we performed immunohistochemistry at the lesion using antibodies against the fibrotic scar marker and astrocyte marker. The percentage of these marker positive area significantly increased in the adult SCI at 7 days post injury (d.p.i.) compared to adult sham group, but did not increase in the neonate SCI group. To characterize the cellular transcriptional profiles of adults and neonates at the lesion, we performed single nucleus RNA-seq (snRNA-seq) using isolated nuclei from 500-µm-wide piece of sham and injured spinal cord at 7 d.p.i.. We analyzed data for 4,076 nuclei in total of 4 groups and identified 18 clusters. Based on expression of maker genes of ependymal cells (*Enkur*) and a subset of radial glial cells, we defined the cells which are enriched in neonatal SCI as ependymal-like cells. To reveal unique character of injury-induced neonatal ependymal-like cells, we evaluated the differentially expressed genes in ependymal-like cells of adults and neonates. Gene ontology terms upregulated in neonatal SCI were not shared with the previously reported physiological ependymal cells of adults and neonates. Histological analysis revealed that *Enkur*+ cells line the central canal in sham animals of adults and neonates, and these cells were enriched in the lesion only in neonates. We further confirmed that *Enkur*+ cells were positive for the cellular markers of ependymal cells, astrocytes and radial glia. [Conclusion] We revealed the age-dependent cellular transcriptomes after SCI using snRNA-seq.

In neonates, the number of ependymal-like cells was significantly increased after SCI. Injury-induced neonatal ependymal-like cells might possess the unique character as neural stem cells, compared with healthy ependymal cells of adults and neonates.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Program #/Poster #: 046.13

Topic: C.11. Spinal Cord Injury and Plasticity

Support: CIHR RN416617
CIHR RN388149

Title: Spinal Lumbar V3 Interneurons Are Crucial In The Recovery Of Locomotion After SCI

Authors: *H. ZHANG[1], A. KHATMI[1], D. PATEL[1], Y. ZHANG[2], D. J. BENNETT[1], K. K. FENRICH[1];
[1]Neurosci. and Mental Hlth. Inst. and Fac. of Rehabil. Med., Univ. of Alberta, edmonton, AB, Canada; [2]Med. Neurosci., Dalhousie Univ., Halifax, NS, Canada

Abstract: Many rehabilitation practices, such as locomotor training to activate spinal motor circuits, have been combined with electrical spinal cord stimulation (epiSES) to activate sensory afferents, with the aim to reorganize spinal motor circuitry and improve recovery after spinal cord injury (SCI). Currently, however, the major limitation to advancing these potential rehabilitation strategies is that the underlying mechanisms and neuroplastic changes caused by locomotor training and epiSES remain poorly understood. Genetically identified spinal interneuron (IN) populations have been shown their distinctive roles in sensory-motor functions, yet very few of them have been investigated in SCI models. Among them, V3 INs, one of the major groups of excitatory spinal neurons that play crucial roles in generating robust and stable locomotor activities, have shown great potential as a therapeutic target to treat SCI. Firstly, we found that after spinal cord staggered hemisection at T10 and then T8, V3OFF mice (in which the VGlut2 expression is deleted thus silencing the V3 INs) still had severely impaired hindlimb locomotion six weeks after the 2nd surgery, while wild-type mice already started spontaneous walking. Furthermore, to check the V3 INs contribution to the sensory integration that improves the recovery of locomotion after SCI, the V3OFF and wild-type mice receive 6 weeks of treadmill training, 20 minutes a day and 5 days a week. We find that V3OFF mice still cannot recover proper locomotion that is identified with good left-right hindlimb alternation and vigour angular movement in each joint. Surprisingly, with epiSES training every other day for six weeks after SCI, V3OFF mice still could not recover the locomotion. EpiSES mainly evokes bilateral flexion twitch in V3OFF mice instead of the good locomotion that we have seen in wild-type mice under epiSES. In the end, optogenetic stimulation applied to lumbar V3 INs that express

ChR2 leads to substantial recovery of locomotion after SCI. Therefore, we hypothesize that V3 INs are crucial for locomotor recovery after SCI by mediating cutaneous or/and proprioceptive sensory feedback that assists walking.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NINDS 1R21NS111192-01
NINDS F30 NS122478-01
NIH T32 GM140935

Title: An intrinsic clock drives axon growth decline during human development

Authors: *B. PENG[1], E. A. N. THOMPSON[1], K. RODRIGUEZ[1], P. C. KLOSA[1], Z. P. ARNDT[1], S. KHULLAR[2], R. LU[3], C. S. MORROW[1], B. TEEFY[3], D. WANG[2], A. M. M. SOUSA[1], B. BENAYOUN[3], D. L. MOORE[1];
[1]Neurosci., [2]Biostatics and Med. Informatics, Computer science, Univ. of Wisconsin- Madison, Madison, WI; [3]Gerontology, USC, Los Angeles, CA

Abstract: Spinal cord injury (SCI) leads to life-long disability, with limited treatment options. After injury, central nervous system (CNS) axons fail to regenerate due both to extrinsic and intrinsic factors. Rodent studies have revealed a developmental regulation of axon growth ability, such that embryonic CNS neurons extend long axons, whereas postnatal CNS neurons cannot. Yet, whether this is similar in human CNS neurons is unknown. Recently, our lab generated an *in vitro*, age-relevant, human model to identify novel intrinsic factors which regulate axon growth. Our direct reprogramming protocol transdifferentiates human fibroblasts directly into induced neurons (Fib-iNs), skipping pluripotency which restores cells to an embryonic state. Using human fibroblast samples from 8 gestational weeks to 72 years-old, we confirmed that Fib-iNs maintained the original cell's age. Further, we found that early fetal Fib-iNs grew longer neurites relative to late fetal and postnatal ages, mirroring the age-dependent decrease in regenerative ability during development in rodents. Interestingly, these neurons are environmentally naïve, suggesting an intrinsic aging clock may drive changes in neurite growth ability. Using RNA-seq on Fib-iNs, we identified dramatic transcriptional shifts between ages with high versus low intrinsic growth ability. We are currently screening genes to identify novel therapeutic candidates for SCI.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: NIH Grant R01NS083983

Title: Brain-wide anatomical and transcriptional analyses of the supraspinal connectome in naïve and spinally injured mice

Authors: Z. WANG[1], Z. BEINE[2], P. TSOULFAS[3], ***M. BLACKMORE**[2];
[1]Biomed. Sci., [2]Marquette Univ., Milwaukee, WI; [3]Univ. of Miami, Miami, FL

Abstract: The supraspinal connectome is essential for normal behavior and homeostasis and consists of numerous sensory, motor, and autonomic projections from brain to spinal cord. Study of supraspinal control and its restoration after damage has focused mostly on a handful of major populations that carry motor commands, with more limited consideration of dozens more that provide autonomic or crucial motor modulation. To facilitate a broader anatomical understanding, we have combined optimized viral labeling, 3D imaging, and registration to a mouse digital neuroanatomical atlas to rapidly visualize and quantify supraspinal neurons in dozens of defined locations throughout the brain. This approach offers a unified, three-dimensional view of the supraspinal connectome and a practical means to quantify population-by-population changes in connectivity after injury or disease. In parallel we combined retrograde labeling of supraspinal cell nuclei with fluorescence activated nuclei sorting and single cell sequencing to transcriptionally profile diverse supraspinal populations. We identified numerous transcriptionally distinct cell types and used a combination of established and newly identified marker genes to assign an anatomical location to each. To validate the putative marker genes, we visualized selected transcripts and confirmed selective expression within lumbar-projecting neurons in discrete supraspinal regions. Combined, these twin resources aim to spur progress by broadening understanding of essential but understudied supraspinal populations.

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Poster

046. Spinal Cord Injury: Mechanisms of Regeneration and Repair

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Topic: C.11. Spinal Cord Injury and Plasticity

Support: R37 NS047484
RO1 NS105961

Title: Ryksignaling in astrocytes modulates glial border formation and coordinates cellular interactions after spinal cord injury

Authors: *Z. SHEN, B. FENG, V. SILVIA, J. WANG, T. WOO, Y. ZOU;
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Abstract: Astrocyte morphology dramatically changed after spinal cord injury (SCI). However, the local signaling mechanisms which regulate the astrocyte morphology and lesion border formation are still not fully understood. Here we found that Ryk, a Wnt receptor, is induced in the reactive astrocytes in acutely injured spinal cords. Conditional knockout Ryk in astrocyte dramatically changed astrocyte morphology, accelerated astrocyte border formation and reduced lesion volume. Using single-cell RNA sequencing, we found that Ryk cKO lead to a wide-range of changes of cell-cell signaling in a series of cell types. Also Ryk cKO accelerated differentiation of astrocytes and microglia to resolve inflammation. Furthermore, we showed Ryk cKO partly reduced fibrosis, increased neuronal protection and increased preservation of motor function in long term. Taken together, our results suggest that Ryk is a signaling hub which may coordinate the injury responses of multiple cell types and Ryk inhibition represents a promising therapeutic strategy to accelerate lesion border formation, attenuate secondary damage and improve functional recovery after SCI.

Disclosures: Z. Shen: None. **B. Feng:** None. **V. Silvia:** None. **J. Wang:** None. **T. Woo:** None. **Y. Zou:** None.

Poster

047. Amygdala and Pain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 047.01

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261

Title: Role of exogenously induced neuroimmune activation in the rodent right and left central amygdala

Authors: *G. S. WELCH[1], P. PRESTO[1], B. MENDOZA[1], V. NEUGEBAUER[1,2,3];
[1]Dept. of Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. & Therapeut., [3]Garrison Inst. on Aging, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain is one of the costliest healthcare problems within the U.S., and despite therapeutic intervention, continues to affect tens of millions of people every year. To develop novel therapeutic strategies, knowledge gaps involving pain mechanisms within the brain must be further explored. Increasing evidence has demonstrated a role for neuroimmune signaling factors in the pathogenesis of chronic pain. Within the brain, the amygdala, a bilateral limbic structure, is of particular interest due to its involvement in the emotional-affective dimensions of pain and pain modulation. Preliminary evidence has suggested pain-related functional lateralization in the amygdala, particularly within its central nucleus (CeA). Whereas a pro-nociceptive phenotype has been characterized in the right CeA across various pain models, the left CeA may have an anti-nociceptive influence on pain modulation. However, little has been explored with regard to pain-related lateralization of neuroimmune signaling mechanisms in the CeA, particularly following exogenous stimulation. This study explored the effects of exogenously induced neuroinflammation, localized either to the right or left CeA, on pain-related behaviors. Micro-injected lipopolysaccharide (LPS), a component of gram-negative bacteria and a strong immunostimulant, was used to probe this neuroinflammatory state. We hypothesized that LPS-induced neuroimmune activation within the right but not left CeA would produce pain-like behaviors, mimicking the right-hemispheric lateralization of pronociceptive pain modulation to the right CeA in pain conditions. LPS was stereotaxically delivered directly into either the left or right CeA of male rats. One week post drug delivery, sensory thresholds (von Frey test) and anxiety-like behaviors [elevated plus maze (EPM) and light/dark box (LDB) test] were measured. Intra-CeA LPS injections produced anxiogenic responses in the EPM and LDB but an increase in sensory withdrawal thresholds, regardless of hemisphere. The results suggest that while exogenous activation of neuroimmune signaling mechanisms in either the right or the left CeA can generate affective behaviors, it can also create an antinociceptive phenotype that is dissimilar to endogenous activation in pain models. Our findings support the use of LPS as a tool to investigate neuroimmune signaling mechanisms within the amygdala. We hope that further mechanistic insight into the functional lateralization of pain processing in the amygdala will aid in developing therapeutic strategies to alleviate the burden of chronic neuropathic pain.

Disclosures: G.S. Welch: None. **P. Presto:** None. **B. Mendoza:** None. **V. Neugebauer:** None.

Poster

047. Amygdala and Pain

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Support: NIH Grant NS038261
NIH Grant NS118731

Title: Differential pain-related synaptic and excitability changes in amygdala CRF and non-CRF neurons in acute and chronic phases of a neuropathic pain model

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Abstract: The central nucleus of the amygdala (CeA) plays a key role in pain modulation and pain-related emotional-affective aspect of pain. The CeA contains a high level of corticotropin releasing factor (CRF)-expressing neurons, suggesting important roles of these neurons in pain. Our recent study revealed that manipulations of CeA-CRF neurons can modulate neuronal activity in the spinal cord and pain-related behaviors. However, pain-related cellular and synaptic changes in CeA-CRF neurons at different phases of neuropathic pain conditions remain to be determined. Here we sought to determine any differences in pain-related changes in CeA-CRF and CeA-non-CRF neurons between acute and chronic phases of a neuropathic pain model using brain slice patch-clamp recording. A Cre-dependent adeno-associated viral vector AAV5-EF1a-DIO-mCherry was stereotaxically injected into the CeA of transgenic Crh-Cre rats (original breeding pairs kindly provided by Dr. Robert Messing, UT Austin) to identify CeA-CRF neurons in brain slices. Whole-cell patch-clamp recordings were obtained from mCherry-positive CRF neurons or mCherry-negative non-CRF neurons in the capsular division of the CeA (CeC) in brain slices from sham control and neuropathic rats one (acute phase) or four (chronic phase) weeks after L5 spinal nerve ligation (SNL model). In current-clamp mode, neuronal excitability was measured by injecting depolarizing currents. In voltage-clamp mode, excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) were evoked by electrical stimulation of the basolateral amygdala (BLA) or presumed parabrachial (PB) input. We found increased EPSCs of PB inputs and excitability of CeA-CRF neurons at the acute, but not chronic, stage of SNL. In contrast, the excitability of non-CRF neurons in the CeC was increased at the chronic, but not acute, stage SNL. These results suggest that CeA-CRF neurons undergo pain-related changes at the acute, but not chronic, phase of SNL. In addition, our data suggest that excitability changes in non-CRF neurons in the CeC could play a key role in chronic phase of SNL.

Disclosures: T. Kiritoshi: None. **V. Yakhnitsa:** None. **V. Neugebauer:** None.

Poster

047. Amygdala and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grants NS038261
NIH Grants NS106902
NIH Grants NS109255

Title: Chemogenetic manipulation of amygdala kappa opioid receptor neurons modulates amygdala neuronal activity

Authors: *G. JI[1,2], T. KIRITOSHI[1], V. YAKHNITSA[1], E. NAVRATILOVA[4], F. PORRECA[4], V. NEUGEBAUER[1,2,3];
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Abstract: The amygdala has emerged as a key brain region that modulates aversive responses to stress, fear, and pain. The amygdala encompasses several functionally and structurally distinct nuclei. The lateral subdivision of the central nucleus (CeA) receives direct nociceptive input from parabrachial area (PB) as well as contextual pain information from thalamo-cortical systems via lateral-basolateral amygdala (BLA). We and others have demonstrated in rodent models that pain conditions promote neuroplasticity in the amygdala, including enhanced excitatory neurotransmission at the PB-CeA and BLA-CeA synapses and increased excitability of the CeA neurons. These neuroplastic changes ultimately increase CeA output and amplify pain responses in chronic pain states. In this study, we used chemogenetic manipulation of amygdala kappa opioid receptor (KOR) neurons to analyze the downstream neuronal effects of amygdala KOR neurons in chronic neuropathic pain (SNL) and sham control mice.For chemogenetic inhibition or activation of KOR+ neurons in the CeA, a Cre-inducible viral vector encoding Gi-DREADD (hM4D) or Gq-DREADD (hM3D) was injected stereotaxically into the right CeA of transgenic KOR-Cre mice. Brain slices containing the right amygdala were obtained from sham and neuropathic KOR-Cre mice at 3-4 weeks after vector injection, and whole-cell patch-clamp recordings were performed to test the effect of deschloroclozapine (DCZ, a selective DREADD actuator) on CRF and non-CRF CeA neurons. Chemogenetic inhibition of KOR neurons in sham KOR-Cre mice expressing Gi-DREADD with DCZ significantly decreased inhibitory transmission at the PB-CRF synapse, resulting in a shift of inhibition/excitation balance towards increased excitatory drive in sham mice. Chemogenetic activation of KOR neurons in SNL KOR-Cre mice expressing Gq-DREADD with DCZ significantly increased inhibitory transmission at the PB-CRF synapse and decreased excitability (depolarization-evoked action potentials).The data suggest that KOR neurons in the CeA modulate amygdala activity by exerting an inhibitory tone on downstream CeA neurons, including presumed amygdala output neurons. Blockade of KOR signaling in the CeA could have potential therapeutic implications.

Disclosures: G. Ji: None. **T. Kiritoshi:** None. **V. Yakhnitsa:** None. **E. Navratilova:** None. **F. Porreca:** None. **V. Neugebauer:** None.

Poster

047. Amygdala and Pain

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Program #/Poster #: 047.04

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS118731

Title: mGlur2 and mGluR3 activation mediate different neuronal and synaptic functions of CeA neurons in an arthritis pain model

Authors: *M. MAZZITELLI[1], V. NEUGEBAUER[1,2,3];
[1]Dept. of Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. & Therapeut., [3]Garrison Inst. on Aging, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: The amygdala is critically involved in the emotional-affective components of pain and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions and receives purely nociceptive information via the external lateral parabrachial nucleus (PB) and polymodal information from thalamo-cortical networks via the basolateral nuclei of amygdala (BLA). Gi/o-coupled group II metabotropic glutamate receptors (mGluR2 and 3) can decrease neurotransmitter release and regulate synaptic plasticity in different brain regions. Subtype-specific modulation of amygdala (CeA) neurons is an important knowledge gap that is addressed here in an arthritic pain model. Brain slice physiology was performed to determine the effects of a group II mGluR agonist (LY379268) alone or in combination with a mGluR2 selective NAM (VU6001966) to activate mGluR3, and of an mGluR2 selective PAM (LY487379) on CeA neurons. A viral vector encoding muscarinic-based Gi-DREADDs fused with mCherry under the control of GFAP promoter was injected into the CeA to chemogenetically inhibit astrocytes. A viral vector coding channelrhodopsin 2 (ChR2) fused to YFP under the control of CaMKII promoter was injected into the PB for optical activation of glutamatergic inputs to CeA. BLA-CeA inputs were activated by focal electrical stimulation. Whole-cell patch-clamp recordings in brain slices from arthritic rats (5-6 h postinduction of a kaolin/carrageenan-monoarthritis) were made in the lateral division of the CeA (CeL) to investigate neuronal excitability, monosynaptic excitatory postsynaptic currents (EPSCs), and glutamate-driven inhibitory postsynaptic currents (IPSCs) evoked from PB and BLA inputs. LY379268 decreased neuronal excitability, EPSCs and IPSCs. LY487379 resulted in decreased EPSCs, whereas selective activation of mGluR3 (combination of LY379268 with VU6001966) mimicked the effects of the group II mGluR agonist. Chemogenetic astrocyte inhibition decreased neuronal excitability, but had no effects on EPSCs or IPSCs, providing evidence for a critical contribution of astrocytes to the inhibitory effects of mGluR3 on CeL neuronal excitability in the pain condition. The results suggest that mGluR2 and mGluR3 modulate CeA functions in pain differently and that glial-neuronal interaction mediated by astrocytic mGluR3 plays a critical role in amygdala pain mechanisms. These new insights into neuronal and non-neuronal mGluR function in the amygdala may help identify appropriate therapeutic strategies for pain management.

Disclosures: M. Mazzitelli: None. **V. Neugebauer:** None.

Poster

047. Amygdala and Pain

Location: SDCC Halls B-H

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Program #/Poster #: 047.05

Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261

Title: Hmgb1 silencing in the amygdala inhibits pain-related behaviors in a sex-specific manner in a rat neuropathic pain model

Authors: *B. MENDOZA[1], P. PRESTO[1], I. PONOMAREV[1,2], V. NEUGEBAUER[1,2,3]; [1]Dept. of Pharmacol. and Neurosci., [2]Ctr. of Excellence for Translational Neurosci. and Therapeut., [3]Garrison Inst. on Aging, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Chronic pain is a complex illness that can lead to disability and functional impairments. Pain-related neuroplasticity including in the brain alters information processing and makes it difficult to develop effective treatment options that modify specific signaling pathways in the progression from acute to chronic pain. Improving the understanding of this transition from acute to chronic pain is required for the development of effective therapeutics that modify and alleviate the underlying cause of chronic pain instead of simply masking the symptoms. To investigate the intricacies between sensory, cognitive, and emotional-affective dimensions that form the experience of pain we focus on neuroplasticity in the amygdala, a limbic brain region that has proven to play a key role in the modulation and amplification of emotional-affective elements of pain. Mechanisms of amygdala plasticity in the transition from acute to chronic pain represent a current knowledge gap that we aim to address by studying neuroimmune signaling mechanisms that are largely unknown in brain in the context of pain. High motility group box 1 (Hmgb1) is a “proinflammatory” protein that is involved in the crosstalk between neuronal and glial cells in the spinal cord, but its function in the amygdala remains to be determined. We tested the hypothesis that increased Hmgb1 is involved in the initiation of pain-related amygdala plasticity and that this signaling molecule can be inhibited to control amygdala function and alleviate neuropathic pain. Transcriptomic analysis revealed an upregulation of Hmgb1 in the central nucleus of the amygdala (CeA) of adult chronic neuropathic rats after spinal nerve ligation (SNL). Stereotaxic injection of Hmgb1 siRNA in the right CeA of adult male and female rats as a 1-week posttreatment to SNL surgery decreased mechanical hypersensitivity (von Frey test and paw compression tests) in both sexes but produced a male-predominant reduction in anxiety-like behaviors (open field and elevated plus maze) and a female-predominant decrease in emotional-affective responses (audible and ultrasonic vocalizations). Subsequent mRNA expression analysis revealed a decrease in Hmgb1 expression in the right but not left CeA following siRNA posttreatment in both male and female rats. These results indicate that while Hmgb1 may play a sex-specific role in pain-related neuroimmune signaling within the CeA, the inhibition of Hmgb1 in the amygdala may serve as a potential target to alleviate chronic neuropathic pain in both sexes.

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Poster

047. Amygdala and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS118731

Title: Reduced SK2-channel expression in central nucleus of amygdala in chronic neuropathic pain. Evidence for epigenetic mechanism

Authors: *V. A. YAKHNITSA, O. PONOMAREVA, J. THOMPSON, K. PRUITT, V. NEUGEBAUER;
Texas Tech. Univ. Hlth. Sci. Ctr., Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX

Abstract: Pain and pain-induced emotional affective disorders contribute to neuroplasticity in the central nucleus of amygdala (CeA). Under normal conditions, small conductance calcium activated potassium (SK2) channels are abundantly expressed in the amygdala and inhibit neuronal excitability by mediating the medium afterhyperpolarization (mAHP), shunting excitatory synaptic transmission, and enhancing inhibitory synaptic transmission. In brain slices obtained from neuropathic rats (left L5 spinal nerve ligation model, SNL, 4 weeks post-surgery) excitability in capsular CeA neurons was increased while amplitude of mAHP was decreased compared to sham controls. Here, using immunohistochemistry, western blotting, and real time polymerase chain reaction (q-PCR) we demonstrate that amygdalar SK2-channel dysfunction in SNL state is due to reduced expression of SK2 channels. We further test the hypothesis that SK2 gene promoter methylation alters expression of SK2 channels in CeA neurons in chronic neuropathic pain. Number of immunohistochemically labeled SK2-positive particles was reduced in SNL rats compared to sham controls. Western blotting revealed decreased levels of both SK2-S and SK2-L isoforms of SK2 protein by ~50% in the CeA of SNL rats. q-PCR demonstrated reduced relative levels of SK2 subunit mRNA isolated from the CeA of SNL rats compared to samples from sham rats, suggesting pretranscriptional SK2 channel downregulation. The methylation profile of SK2 gene promoter region was assessed by bisulfite sequencing methods. Overall, low methylation levels were observed in both SNL and sham rats, however increased methylation of CpG island of SK2 promoter region in the CeA was detected in SNL group. Interestingly, methylated CpG sites identified in the SNL rat CeA samples were different from those seen in the sham rats. A total of 11 CpG sites ranging from position -832 to -383 relative to the transcription start site of SK2 gene were methylated in SNL rats. Only 3 sites at these positions were found to be methylated in the sham rat CeA (-759, -676, and -383). We found that sites -834, -514, -505, -467, and -389 CpG sites were specifically methylated in the sham rat CeA but were not methylated in the SNL rats. The data demonstrate reduced expression of SK2 channel function in CeA in neuropathic pain. Observed changes in methylation in pain model suggest reduced gene expression by epigenetic mechanism.

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Poster

047. Amygdala and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261

Title: Transcriptomic profiling of the central and basolateral amygdala in a rat model of chronic neuropathic pain

Authors: *P. PRESTO[1], G. JI[1,2], I. PONOMAREV[1,2], V. NEUGEBAUER[1,2,3];
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Abstract: Chronic pain is a pervasive healthcare issue comprised of complex interactions between sensory, cognitive, and emotional-affective dimensions. Together, this intricate interplay presents a challenge to the identification of effective therapeutic strategies. One obstacle to the discovery of successful treatment options arises from a lack of full understanding of the mechanisms and targets involved in the transition to a chronic pain state. Therefore, mechanistic insights into pain-related signaling processes are critical to identify new molecular targets for translational research and evidence-based medicine. Gene expression analysis provides a sensitive measure of cellular function, and abnormal changes in gene expression may ultimately impact behavior and disease states. Transcriptomic analysis provides crucial insight into actively expressed genes and transcripts from cells in various conditions, rendering this method an appealing approach to the discovery of novel molecular targets. Transcriptomic profiling in the periphery and spinal cord has revealed an upregulation of many transcription factors and cytokines in neuropathic pain, though pain-related gene expression profiles within the brain are overwhelmingly understudied. A limbic brain region, the amygdala has emerged as a key player in the emotional-affective aspects of pain and pain modulation. Changes in amygdala activity have been observed in pain models and neuroplasticity within the amygdala has been linked to pain-related behaviors. However, the molecular signatures of pain-related amygdala plasticity that may drive these behaviors remain to be determined. Here we characterize the amygdala transcriptional profile of adult male rats at the chronic stage of neuropathic pain. Tissues containing either the basolateral (BLA) or central (CeA) nucleus of the amygdala were collected for RNA sequencing 4 weeks after spinal nerve ligation (SNL) or sham surgery. Within the BLA, pathway and biological function enrichment analysis revealed differential expression in genes coding for GABAergic receptor signaling, calcium regulation, and long-term potentiation. In the CeA, differentially expressed genes included those related to opioid prodynorphin and proopiomelanocortin pathways, corticotropin releasing factor receptor signaling, and vasopressin synthesis. SNL surgery also promoted an upregulation in the CeA of genes related to neuroimmune signaling, such as *Hmgb1*, *Gfap*, and *Tnfr1*. Together these

findings provide mechanistic insight into pain-related amygdala function that may guide the development of novel therapeutic strategies for neuropathic pain relief.

Disclosures: **P. Presto:** None. **G. Ji:** None. **I. Ponomarev:** None. **V. Neugebauer:** None.

Poster

047. Amygdala and Pain

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant NS038261
NIH Grant NS106902
NIH Grant NS109255

Title: Chemogenetic manipulation of amygdala kappa opioid receptor neurons modulates neuropathic pain behaviors

Authors: *N. ANTENUCCI[1], G. JI[1,2], T. KIRITOSHI[1], E. NAVRATILOVA[4], F. PORRECA[4], V. NEUGEBAUER[1,2,3];
[1]Dept. of Pharmacol. and Neurosci., Lubbock, TX; [2]Ctr. of Excellence for Translational Neurosci. and Therapeut., [3]Garrison Inst. on Aging, Texas Tech. Univ. Hlth. Sci. Ctr., Lubbock, TX; [4]Dept. of Pharmacol., Arizona Hlth. Sci. Center, Univ. of Arizona, Tucson, AZ

Abstract: The amygdala is an important neural substrate for the emotional-affective dimension of pain. The central nucleus (CeA) serves major amygdala output functions and receives highly processed nociceptive and affected-related information from the lateral-basolateral network (LA-BLA). The CeA contains mostly gamma-aminobutyric acid neurons, some of which also co-express neuropeptides such as corticotropin-releasing factor (CRF) and dynorphin, an endogenous agonist of kappa opioid receptors (KORs). The dynorphin/KOR system in the amygdala is critical for the averse-affective behaviors in stress- or injury-induced pain conditions. In this study, we used chemogenetic manipulation of amygdala KOR neurons to determine the contribution of KOR+ neurons in the CeA to pain behaviors in chronic neuropathic pain (SNL) and sham control mice. For chemogenetic inhibition or activation of KOR+ neurons in the CeA, a Cre-inducible viral vector encoding Gi-DREADD (hM4D) or Gq-DREADD (hM3D) was injected stereotaxically into the right CeA of transgenic KOR-Cre mice. Emotional-affective behaviors (vocalizations), mechanosensitivity (electronic von Frey anesthesiometer), and anxiety-like behaviors (light-dark box test) were assessed in SNL and sham control mice. Chemogenetic inhibition of KOR+ neurons (to mimic the effect of a KOR agonist) in the CeA in sham mice induced pain behaviors measured as decreased withdrawal threshold in the electronic von Frey test, and decreased the frequency of entries into the light box of light-dark test. In contrast, chemogenetic activation of KOR+ neurons (to mimic the effect of a KOR antagonist) in the CeA in SNL mice reduced pain behaviors measured as increased withdrawal threshold in the

electronic von Frey test, decreased the ultrasonic vocalizations, and increased the frequency and duration in the light box.The data suggest that KOR neurons in the amygdala regulate amygdala output and pain behaviors by exerting an inhibitory tone on amygdala output neurons. Their inhibition would cause disinhibition and facilitate averse-affective pain-like behaviors. Blockade of KOR signaling in the CeA could have potential therapeutic implications.

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Poster

047. Amygdala and Pain

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Program #/Poster #: 047.09

Topic: D.02. Somatosensation – Pain

Support: NS038261

Title: Amygdala mGluR2 and mGluR3 modulate different aspects of arthritis pain-related behaviors

Authors: *V. NEUGEBAUER, M. MAZZITELLI;
Texas Tech. Univ. Hlth. Sci. Ctr., Texas Tech. Univ. HSC, Lubbock, TX

Abstract: Pain is a multidimensional experience with an important averse-affective dimension. The amygdala, a limbic brain area, is critically involved in the emotional-affective aspects of behaviors and in pain modulation. The central nucleus of amygdala (CeA) serves major output functions, and neuroplasticity in the CeA is mechanistically linked to pain-related behaviors in different pain conditions. The activation of Gi/o-coupled group II metabotropic glutamate receptors (mGluRs), which include mGluR2 and mGluR3, can decrease neurotransmitter release and regulate synaptic plasticity. mGluR2/3 have emerged as potential targets for neuropsychiatric disorders and they can inhibit pain-related processing and behaviors, but the contribution of mGluR2 and 3 in the amygdala to pain-related behaviors remains to be determined. This knowledge gap was addressed here in a rodent model of arthritis pain. Audible (nocifensive response) and ultrasonic (averse-affective response), mechanical withdrawal thresholds and anxiety-like behaviors were measured in adult rats 5-6 h after the induction of a kaolin/carrageenan-mono-arthritis in the left knee joint. The following drugs were stereotaxically administered by microdialysis into the CeA of arthritic rats: a group II agonist (LY379268), a PAM selective for mGluR2 (LY487379) or a combination of a group II mGluR agonist (LY379268) with a NAM selective for mGluR2 (VU6001966). Selective activation of mGluR2 decreased vocalizations and increased mechanical withdrawal thresholds, while selective activation of mGluR3 had similar inhibitory effects on emotional-affective responses but not mechanosensitivity in the arthritis pain condition. Group II mGluR activation improved the open-arm choice in the elevated plus maze in arthritic rats, suggesting anxiolytic effects, which

were mimicked by selective activation of mGluR3, but not mGluR2. Our data suggest that mGluR3 mediates the anxiolytic effects of group mGluR function whereas mGluR2 plays a critical role in the modulation of mechanosensory aspects (antinociceptive properties) of arthritic pain. Both subtypes are involved in the beneficial effects of group II mGluRs on emotional-affective responses (vocalizations) in arthritis pain. The data identify distinct roles of mGluR2 and mGluR3 in amygdala (CeA) in different aspects of pain modulation, which may guide subtype-selective therapeutic strategies.

Disclosures: V. Neugebauer: None. **M. Mazzitelli:** None.

Poster

047. Amygdala and Pain

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Program #/Poster #: 047.10

Topic: D.02. Somatosensation – Pain

Support: MOST 110-2314-B-002-165-MY3

Title: Deciphering emotional versus non-emotional components between positive and negative expectancy modulation of pain

Authors: *H.-Y. TSAI[1], M.-T. TSENG[2];
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Abstract: Positive expectations (i.e., expecting decreased pain) and negative expectations (i.e., expecting increased pain) toward noxious stimulations respectively alleviating and exacerbating human pain perceptions; however, psychological factors involved in pain expectations remain unclear. By applying the functional magnetic resonance imaging technology, we aim to investigate emotional versus non-emotional neural mechanisms underlying pain expectations. Thirty-one participants were instructed to use emotion regulation strategies to down-regulate expectation-related emotions in a cue-based expectancy paradigm. As indicated by subjective emotional ratings and skin conductance responses, participants successfully reduced their anxiety toward negative expectations and pleasantness toward positive expectations when applying emotion regulation strategies. We observed that expectancy effects on pain were diminished when emotions reduced. Additionally, the reduction in emotional ratings significantly predicted the expectancy effect, supporting an emotional component in the pain expectation. The non-emotional neural mechanism, which was unbiased by emotional states, for positive and negative expectations respectively engaged the anterior insular cortex and the rostral anterior cingulate cortex, with these two regions exhibiting interrelated activation. The emotional mechanism was dissociable between positive and negative expectations: positive expectations involved the ventral medial prefrontal cortex (vmPFC) tracking the pleasantness and co-activating with the

periaqueductal gray (PAG); by contrast, negative expectations recruited the amygdala and thalamus subserving the modulation of anxiety on the nociceptive processing. Furthermore, emotions also modified the encoding of aversive prediction error in the vmPFC for positive expectations and PAG for negative expectations. Taken together, the current study identifies that emotional modulations on the nociceptive processing and prediction error signalling subserve the emotional mechanism underlying pain expectations.

Disclosures: H. Tsai: None. **M. Tseng:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

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Topic: D.02. Somatosensation – Pain

Support: Irish Research Council Postgraduate Scholarship

Title: Sex differences in somatosensory sensitivity in healthy human participants following exposure to acute psychological stress

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Abstract: The endocannabinoid (eCB) system is involved in the modulation of pain and the stress response. Stress has a complex, modulatory influence on pain and can result in stress-induced analgesia or stress-induced hyperalgesia. The aim of this study was to investigate the effect of acute psychological stress on somatosensory sensitivity (using quantitative sensory testing [QST]), and eCB /*N*-acylethanolamine (NAE) levels, in healthy human volunteers. A further aim was to investigate the relationship between eCB/NAE levels in plasma, serum and saliva. Twenty-six healthy volunteers consented to procedures approved by the NUI Galway Research Ethics Committee (14 female, 12 male; mean age ± SEM: 26 ± 0.8 years). Using a within-subjects design, participants were exposed to acute psychological stress using the Montreal Imaging Stress Task (MIST) or a non-stressful control version of the MIST on two separate days, followed by QST comprising a standardized battery of tests to determine thermal (cool, warm) detection and pain (cold, heat) thresholds and heat pain tolerance. Blood and saliva samples, heart rate (HR), and state-anxiety scores were obtained at 3 timepoints throughout the study: baseline, post-MIST and post-QST. Quantification of 2-arachidonoylglycerol (2-AG), anandamide (AEA), *N*-oleoylethanolamide (OEA) and *N*-palmitoylethanolamide (PEA), was carried out by LC-MS/MS. State-anxiety scores were significantly higher following the MIST,

compared to its control. Cool detection threshold significantly increased (p=0.006) and warm detection threshold significantly decreased (p=0.015) (i.e. greater sensitivity) following MIST exposure in females, but not males (all p>0.05). The MIST did affect eCB/NAE concentrations in plasma, serum or saliva in all participants. Salivary 2-AG correlated with both plasma and serum 2-AG levels (r = 0.227, p = 0.005 and r_s = 0.463, p < 0.0001, respectively). OEA levels in saliva correlated positively with plasma and serum (r_s = 0.225, p = 0.005 and r_s = 0.213, p = 0.008, respectively). Plasma and serum levels of 2-AG, AEA, PEA and OEA correlated positively (r_s = 0.463, p < 0.0001, r_s 0.456, p < 0.0001, r_s = 0.182 p = 0.024, and r_s = 0.119, p = 0.013). Acute psychological stress resulted in sex differences in somatosensory sensitivity. There were no effects of stress on cold or heat pain thresholds, heat pain tolerance or eCB /NAE levels. Further investigation into the relationship between blood and saliva eCB /NAE levels is warranted to determine the potential use of saliva as a non-invasive alternative to venous blood samples.

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Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.02

Topic: D.02. Somatosensation – Pain

Support: R21 NS112886
1R34NS111654
P30 DA048742-01A1

Title: Dopamine receptor 1 (Drd1) expression identifies a subset of superficial dorsal horn interneurons

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Abstract: Persistent pain is mediated in part by descending modulation of spinal processing of sensory information. Descending serotonergic and noradrenergic inputs have been studied extensively; however, the descending dopaminergic modulation remains poorly characterized. It is well established that the dopaminergic inputs to spinal cord originate in the A11 nucleus of the hypothalamus, but how these inputs are integrated within the dorsal horn circuits engaged during persistent pain remains largely unknown. Although the spinal cord contains both D1-like (D1,

D5) and D2-like (D2, D3, D4) families of dopamine receptors, the D1-like receptors have been implicated in the maintenance of persistent pain. Using mice that express the fluorescent reporter tdTomato under the control of the dopamine receptor 1 (Drd1) promoter, we identified a Drd1-expressing subpopulation of interneurons in lamina II of the dorsal horn (DH). Most tdTomato-labeled neurons expressed high levels of Drd1 mRNA based on RNAscope analysis. Immunohistochemical analysis showed colocalization of tdTomato with the DH markers PKCgamma and calretinin, which label interneurons known to mediate nerve injury-induced hypersensitivity. Preliminary single nucleus RNAseq analysis (snRNAseq) indicated that the majority of DH Drd1-expressing neurons are excitatory. Ongoing classification based on firing pattern characteristics using current clamp recordings from transverse lumbar spinal cord slices in Drd1-tdTomato expressing mice suggests that the majority of Drd1-expressing neurons have tonic and phasic firing patterns. To identify where Drd1 neurons synapse in the dorsal horn, we are using the enhanced GFP reconstitution across synaptic partners (eGRASP) approach. This entails intraspinal injection of both a presynaptic and postsynaptic eGRASP virus, each containing a fragment of GFP, and visualizing their synaptic connection using confocal microscopy. Based on these preliminary analyses and published literature, we propose that excitatory Drd1-expressing DH neurons mediate descending dopaminergic modulation of circuits that maintain inflammatory and neuropathic persistent pain.

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Poster

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Program #/Poster #: 048.03

Topic: D.02. Somatosensation – Pain

Support: NCCIH/NIH Grant R00AT009466

Title: Comparison of pain reductions induced by gentle brushing and deep pressure, and their associations with childhood trauma

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Abstract: Light stroking and deep pressure are commonly embedded in social touch interactions as well as in manual therapies, and lead to pleasant relaxation and pain relief. However, the mechanisms by which they relieve pain are poorly understood. The pleasantness of gentle stroking relies in part on activation of C-tactile (CT) afferents in hairy skin that are tuned to gentle stroking at velocities around 1-10cm/s, while deep pressure appears to rely on A-fibers. While both forms of touch have demonstrated pain relief, these effects have not been directly

compared. Furthermore, while recent studies have demonstrated reduced CT touch pleasantness in individuals with trauma history, the effect of trauma on touch-induced pain relief has not been studied. We modified the widely studied Conditioned Pain Modulation (CPM) paradigm, in which one pain stimulus reduces perception of a subsequent pain stimulus, to study the effect of gentle brushing and deep pressure on heat pain perception, within and between spinal segments. Our preliminary findings from 18 adults (all female; ages 19-57) demonstrate touch-induced pain reductions from both deep pressure and gentle brushing. Despite lower pleasantness ratings, deep pressure reduced pain more effectively than CT brushing. No pain reduction was observed from the control tapping stimulus. Preliminary correlation analyses confirm that higher levels of childhood trauma are associated with reduced CT touch pleasantness, but no reductions in touch-induced pain relief were observed. These results suggest that CT touch pleasantness and CT pain relief may rely on distinct neural mechanisms.

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Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 048.04

Topic: D.02. Somatosensation – Pain

Title: Disentangling network dynamics of pain expectation using multi-site intracranial recordings

Authors: *J. SAAL[1], A. KHAMBHATI[2], J. C. MOTZKIN[3], B. JOSEPH[2], A. SHAUGHNESSY[2], J. PROSKY[2], P. A. STARR[2], E. F. CHANG[2], P. SHIRVALKAR[4]; [1]Neurosci. Grad. Program, [2]Neurolog. Surgery, [3]Anesthesia, [4]Neurol. and Anesthesiol., Univ. of California, San Francisco, San Francisco, CA

Abstract: Rationale: Expectations play a pivotal role in the perception of pain, presumably through a top-down pain-modulatory circuit. Though prior neuroimaging studies in humans suggest that specific pain-relevant regions mediate the effects of expectation on pain processing, the precise neurophysiologic basis underlying dynamic encoding of expectations and pain is not known. Here we use rare multi-site intracranial recordings in an individual with chronic pain to investigate the temporal dynamics of top-down pain-modulatory circuits in a pain-expectancy task to characterize the network interactions that contribute to expectation-related variability in pain.
Methods: One participant was implanted with 10 electrodes consisting of 106 unique cortical and subcortical contacts sampling from key pain-related brain structures during a stereo-electroencephalography trial. The task involved the presentation of low or high visual pain-predictive cues for 15 seconds, followed by 15 seconds of thermal stimulation at one of two previously calibrated temperatures. The participant completed ten conditioning trials during which the cue was reliably followed by low- or high-intensity thermal stimulation (i.e.,

concordant) and 40 experimental trials in which half of the cue-temperature pairs were discordant (i.e., low pain cue followed by high pain, and vice versa). Ratings of pain intensity were recorded after each trial and brain activity was recorded throughout.
Results: General linear modeling revealed significant pain-percept modulation by the cue (p=.04). To explore how neural activity reflected the cue and thermal stimuli, spectral power, and connectivity metrics were computed. Using an unsupervised method, tensor component analysis, we found key pain-related areas in which spectral power changes tracked the task structure. We further investigated network nodes and interactions that dissociated expectation from thermal stimuli. During the thermal stimulus period, the preceding cue type was associated with activity in established pain-modulatory circuits, including the periaqueductal gray and anterior cingulate cortex. The temperature of the thermal stimulus was represented in relevant sensory areas such as the sensory cortex.
Conclusions: Utilizing rare intracranial recordings in an individual with chronic pain, we identified neural activity dissociating representations of expectancy from stimulus processing. These results represent progress toward understanding how top-down circuits can affect the perception of pain and may inform the development of novel therapies to treat pain conditions such as deep-brain stimulation.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.05

Topic: D.02. Somatosensation – Pain

Support: R37 NS098660
F31 DE030677

Title: Direct functional nociceptive input from the trigeminal dorsal horn to pain-modulating neurons in the rostral ventromedial medulla

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Abstract: A key link in the descending pain-modulating system, the rostral ventromedial medulla (RVM) projects to the dorsal horn, where it can suppress or amplify nociceptive transmission. Two physiologically defined classes of RVM neurons, “OFF-cells” and “ON-cells,” respectively inhibit and enhance nociceptive transmission. Both cell classes respond to noxious stimulation: ON-cells are activated and OFF-cell firing is suppressed when an animal

withdraws from a noxious stimulus. We recently showed that noxious information is relayed to the RVM through the parabrachial complex, a major target of ascending projections from the superficial dorsal horn (Roeder et al., *Pain*, 2016). However, there is anatomical evidence for a more direct route from the dorsal horn to the RVM: neurons at the transition from trigeminal subnucleus interpolaris to caudalis (ViVc) project to the RVM, and at least some of these projecting neurons are nociceptive (Sugiyo, et al., *J. Comp Neurol*., 2005). The goal of the present experiments was to determine whether sensory inputs to the RVM from ViVc recruit pain-modulating neurons, ON-cells and OFF-cells, in the RVM. We used a combination of optogenetic activation or inhibition of ViVc terminals in the RVM with extracellular single-unit recording from identified RVM neurons. AAV9-Syn-ChR2-eYFP or AAV9-CAG-ArchT-eGFP (Penn Vector Core) were nanoinjected into the ViVc of male and female Sprague-Dawley rats, or into adjacent trigeminal complex as a placement control. For the recording session, animals were maintained in a lightly anesthetized state, and neuronal recordings were made using an optoelectrode. An RVM neuron was isolated and classified as an ON- or OFF-cell using a noxious mechanical stimulus applied first to the paw, and then to the whisker pad. Optogenetic activation of ViVc terminals in RVM produced a significant increase in ON-cell activity and a significant decrease of OFF-cell firing. ("NEUTRAL-cells," RVM neurons without a known role in pain modulation, showed no change in activity during activation of ViVc terminals.) Optogenetic inhibition of ViVc terminals attenuated noxious-evoked responses of both ON- and OFF-cells. When vector was injected in areas adjacent to the ViVc, terminal activation did not lead to a change in RVM cell activity. Over the last decades, the functional outputs from the RVM have been well defined, whereas inputs to the RVM are only now being elucidated. The present data document a direct, functional input from the trigeminal dorsal horn to identified pain-modulating neurons in the RVM, and demonstrate that this input is functionally related to nociceptive modulation.

Disclosures: C.C. De Preter: None. **M.M. Heinricher:** None.

Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.06

Topic: D.02. Somatosensation – Pain

Support: Rita Allen Foundation
NIH Grant R00DA034648

Title: Investigating the role of the locus coeruleus in periaqueductal gray-mediated antinociception

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Abstract: Activity in the ventrolateral periaqueductal gray (vlPAG) has been shown to be antinociceptive and is considered to be a major component in the expression of opioid drug-induced analgesia. Canonically, opioids disinhibit vlPAG activity, which modulates neuronal responses in the rostroventral medulla (RVM) and ultimately gates spinal outflow of nociceptive information via serotonergic and opioidergic mechanisms in the spinal dorsal horn. Fundamental pharmacological studies, however, indicate that spinal noradrenergic signaling is consistently implicated in opioid antinociception, suggesting that there is a gap in our knowledge of how the vlPAG may control the release of antinociceptive neuromodulators in the dorsal horn through different output regions. Although the locus coeruleus (LC) has been shown to modulate nociception, its role in and recruitment by the canonical descending circuitry is unclear. Using genetically-encoded and anatomically-targeted viral tools, immunohistochemistry, and acute pain behavior assays in conjunction with intrathecal pharmacology in male and female mice, we show that activity in the vlPAG is necessary for the expression of systemic morphine analgesia at low doses, and that both systemic morphine-induced and PAG-driven antinociception require opioidergic and noradrenergic signaling in the spinal cord. Additionally, we find that vlPAG projection neurons send branching axons to both the RVM and LC, which may account for the LC activation we observed in both opioid-induced and PAG-driven antinociception. These findings reveal a previously underappreciated architecture of the descending pain modulatory circuitry and advance our understanding of the circuit mechanisms by which morphine produces analgesia.

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Poster

048. Pain Central Mechanisms

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Topic: D.02. Somatosensation – Pain

Support: Canadian Foundation for Innovation 36548
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Ontario Ministry of Innovation ER16-12-060

Title: Social facilitation of pain involves cholecystokinin neurons in the periaqueductal grey

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Abstract: Cues from the environment, including cues from social partners, can indicate the potential for pain and injury. Interaction with a social partner expressing overt pain behaviors - such as caressing a limb, vocalizations, facial expressions or olfactory cues - has been shown to enhance the pain experience of the observing individual in both humans and rodents. Yet we do

not know the neurochemical basis for how social cues from the environment enhance pain. Environmental conditions that signal threat to safety such as an aggressor have been shown to release cholecystokinin (CCK), a short peptide neurotransmitter that activates metabotropic receptors CCKA and CCKB. Interestingly, CCK has been shown to enhance pain behavior when administered in the medulla. Recently, we showed that proglumide, a CCK antagonist, blocks socially enhanced pain of an observer mouse (C57BL/6) interacting with a same sex cagemate in pain. We saw enhanced c-fos expression, a marker for neural activation, in the periaqueductal grey (PAG). Here, we show characteristics of the PAG and its involvement in socially enhanced pain. We found CCK expressing neurons in the dorsomedial and lateral columns, but not the dorsolateral columns, of the PAG using CCK-IRES-Cre:Ai14 (tdTomato) reporter mice. We show overlap with c-fos and CCK expression in the dorsomedial PAG in mice that observed cagemates in pain. General and CCK-Cre-specific neural silencing, using virus expressing hM4Di activated with clozapine-N-oxide, in the dorsal PAG blocked increased pain behavior after social interaction, indicating a role for CCK-expressing neurons in socially enhanced pain. Both primary neurons and interneurons expressing CCK have been shown to play an important role in several behaviors in various brain regions, painting a complex picture of neural activity for this neurotransmitter. To determine the cell type of CCK expressing neurons of the dorsomedial PAG, we performed RNAscope staining. We also show neural tracing to and from the dorsal PAG to determine the connectivity of these neurons. Our findings point to a role for CCK expressing neurons in pain enhancement due to social context, building a more robust understanding of the neural mechanisms underlying environmental and social-communicative modulation of pain.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.08

Topic: D.02. Somatosensation – Pain

Support: NIH 1R01AR074274-01

Title: Predicted value of temporal summation of pain for pre and post total knee replacement pain.

Authors: *C. BONIN PINTO[1], P. BRANCO[1], J. PEREZ[1], O. CONG[1], L. ARENDT-NIELSEN[3], K. KJÆR PETERSEN[4], D. W. MANNING[5], L. SULEIMAN[5], K. HARDT[5], T. SCHNITZER[2], A. APKARIAN[1];
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Abstract: Patients with severe painful knee osteoarthritis (KOA) often present widespread hyperalgesia, facilitated temporal summation of pain (TSP), and impaired conditioned pain modulation (CPM). Studies have found that preoperative facilitated TSP and impaired CPM can predict chronic postoperative pain after total knee replacement (TKR) but less is known about pain mechanistic profiles after TKR. In this study, forty KOA patients were assessed before and 3 months after TKR. Knee pain intensity was assessed by mean pain in the last 4 weeks using a numeric rating scale (NRS) and pain during daily activities using The Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain subscale. QST was performed using a controlled cuff pressure algometer. The cuff was placed on the gastrocnemius muscle of both legs and was used to assess pain detection thresholds (PDT), pain tolerance thresholds (PTT), TS, and CPM. Wind-up ratio (WUR) was also measured using a pinprick. Linear regression was performed to evaluate the associations between preoperative QST and pain (VAS and WOMAC) pre and post TKR. Next, subjects were sub-classified in facilitated or not facilitated TS/WUR and impaired/low or normal CPM using median split (TS = 1.6; WUR = 2.1 and CPM = 0.4). To predict pre and post TKR differences, a mixed linear model regression was performed with pain scores (VAS and WOMAC) as dependent variables and, somatosensory profiles, time, and pre-TKR pain levels as independent variables. TS pre TKR was significantly correlated with increased pain (VAS) pre TKR (β= .335, adjR^2 = 0.061, $p < .05$) but not post TKR (β= .0471, adjR^2 = 0.153, p = 0.069). Moreover, temporal summation measured using WUR predicted post TKR pain scores after 3 months (β= -0.124, adjR^2 = 0.219, p = 0.036; corrected for pre TKR pain). CPM was not significantly associated with pre or post TKR pain scores. Moreover, changes in pain scores - VAS and WOMAC were significantly different depending on the somatosensory profiles. OA patients with preoperative high TS presented less pain relief (VAS) over time when compared with low TS subjects (β = 0.927, $p < 0.001$) additionally, participants with low CPM showed less decrease in WOMAC pain score when compared with high CPM patients. (β = 3.717, $p < 0.05$). This exploratory study showed that pre-operative temporal summation (TS and/or WUR) could be considered as a bio-marker of post TRK pain relief, while CPM seems to be more related with functional outcomes as measured by WOMAC pain subscale rather than pain scores itself. This preliminary study will be confirmed in a larger KOA population.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.09

Topic: D.02. Somatosensation – Pain

Support: Rita Allen Foundation
NIH R00DA034648

Title: Prefrontal Input to The Periaqueductal Gray Controls Placebo Analgesia

Authors: *G. LIVRIZZI[1], D. A. JOHNSON[3], X. MA[2], S. T. LUBEJKO[3], J. PATEL[3], S. P. MCCLAIN[2], C. A. JOHNSON[2], C. WEISS[4], M. R. BANGHART[4];
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Abstract: Placebo analgesia is associated with activity in a highly interconnected neural network distributed throughout the brain. However, this is based on a large body of functional imaging work that primarily relies on correlative measures of neural activity and neurochemical signaling obtained in human subjects, making it challenging to ascribe causal roles to the underlying neural pathways. To address this gap, we developed a mouse model of placebo analgesia and used chemogenetic loss-of-function manipulations to identify the underlying neural circuits. We specifically addressed the role of the descending pain modulatory system (DPMS) and its upstream cortical circuits. We found that chemogenetic inactivation of a subpopulation of excitatory neurons in the ventrolateral periaqueductal gray (vlPAG) abolishes both morphine analgesia and morphine-conditioned placebo analgesia. Placebo, but not morphine analgesia, depends on vlPAG-projecting neurons in the medial prefrontal cortex (mPFC)and anterior cingulate cortex (ACC), but not the anterior insula (AI). For placebo to occur, mPFC-to-vlPAG neurons must be active during conditioning, consistent with a critical role for activity-dependent plasticity in this pathway. Our findings suggest that the vlPAG implements cognitive pain modulation by integrating top-down commands, at least some of which are conveyed directly from the cortex.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.10

Topic: D.02. Somatosensation – Pain

Support: Buoniconti Fund
Miami Project to Cure Paralysis

Title: Preliminary assessment of chronic pain attenuation in rats with intrathecal transplants of hiPSC-derived adrenal chromaffin cells

Authors: *J. SAGEN[1], K. D. ABU-BONSRAH[2], M. DOTTORI[2], M. HERNANDEZ[1], S. JERGOVA[1];
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Abstract: Chronic pain can dramatically reduce quality of life and participation in work and recreational activities. Cell transplantation is a potentially transformative approach in chronic pain management, as cells may provide sustained and continually renewable sources of pain-modulating substances, thereby reducing or eliminating the need for repeated systemic analgesics administration and their attendant undesirable side effects. In addition, pharmacologic substances that are labile, have short half-lives, and/or do not readily pass the blood-brain barrier can be delivered directly to spinal sites via transplanted cells. Early work indicated that adrenal medullary tissue or its isolated chromaffin cells transplants in the spinal subarachnoid space showed efficacy in various preclinical chronic pain models and in small clinical studies using human donor adrenal glands. However, clinical feasibility of this approach was limited by low availability of human organ donors to obtain sufficient chromaffin cells which are post-mitotic, and immunological concerns with xenogeneic sources. With the recent advent of induced pluripotent stem cell (iPSC) technologies and development of methods to generate chromaffin-like cells (hCCs) from these, the door has now been re-opened to develop human chromaffin cell lines that can be ultimately derived non-invasively from a patient's own tissues such as blood or skin. The goal of these initial studies was to evaluate the potential for human iPSCs to generate hCCs for pain reduction in rat preclinical models. To accomplish this, hiPSCs (WiCell 007i; human foreskin) were differentiated to hCCs using a series of factors including CHIR, BMP2, BMP4, and dexamethasone, according to Abu-Bonsrah et al. (2018) protocols. Resultant cultures were confirmed for production of chromaffin cell markers and catecholamines, and were transplanted into the rat spinal subarachnoid space via intrathecal catheters or embedded in Matrigel at various doses. Recipient rats were immunosuppressed daily and received intrathecal hCCs or control cells following emergence of chronic pain behaviors in spinal cord injury (SCI; using clip compression), peripheral neuropathic pain using chronic constriction sciatic nerve injury (CCI), or low back pain using intravertebral disc injury (IVD). Results showed attenuation of tactile, cold, and heat hypersensitivity and pain-related disability in respective models. Robust clusters of viable hCCs were identified adherent to the rat dorsal spinal surface after implantation. These preliminary findings suggest that hCCs may provide an ideal cell transplantation source for management of chronic pain.

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Poster

048. Pain Central Mechanisms

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Topic: D.02. Somatosensation – Pain

Support: NIH Grant U18 EB029257

Title: Dual Frequency Spinal Cord Stimulation (dfSCS) for Treatment of Chronic Pain

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Abstract: Since its inception in the late 1960s, tonic, regular spinal cord stimulation (SCS) has been used to treat chronic pain for tens of thousands of patients each year. A clear opportunity remains to improve efficacy, as only ~60% of recipients experience reductions in pain scores due to SCS of more than 50%. Recent studies suggest that non-regular temporal patterns of stimulation may improve SCS efficacy [1]. Here, we used a model-based approach to design an optimized pattern of SCS - termed dual frequency SCS (dfSCS) - and compared dfSCS to single frequency controls in both an animal model of chronic pain and through neural recordings in the dorsal horn. We optimized dfSCS using a biophysical model of the dorsal horn pain circuit by delivering two different frequencies of tonic SCS between 0-70 Hz to separate populations of dorsal column axons arising from center and surround sensory receptive fields. A final pattern of 54 Hz targeting surround and 2 Hz targeting center receptive fields was selected for its ability to reduce model wide dynamic range (WDR) neuron firing rate relative to controls and its robustness to the distribution of center-surround axons stimulated and to the loss of GABAergic and glycinergic inhibition that occurs during pain progression. After identifying our dfSCS pattern ("ideal dfSCS"), we implanted spared nerve injured (SNI) Sprague-Dawley rats with 4-contact paddle electrodes at the T12-T13 vertebral juncture. We tested efficacy by measuring mechanical hypersensitivity using paw withdrawal thresholds during sham stimulation, ideal dfSCS, a dfSCS pair not predicted to improve pain , and single frequency controls at the higher and lower dfSCS components . We also tested whether stimulation using the higher dfSCS rate through all contacts (54 Hz, similar to the typical clinical frequency of 50 Hz) or using dfSCS was able to alleviate spontaneous pain from SNI and induce place preference in a 3-chamber conditioned place preference protocol. Finally, we compared the counts of WDR or nociceptive specific (NS) neurons in the dorsal horn responsive to dfSCS relative to single frequency controls. Similar numbers of neurons responded to dfSCS as controls, although higher cell counts are required to discern changes in firing rate among the responding neurons. Overall, dfSCS is a promising technique for improving clinical outcomes for SCS for chronic pain.
References: [1] Edhi, Muhammad M., et. al. "Time dynamic pulse modulation of spinal cord stimulation reduces mechanical hypersensitivity and spontaneous pain in rats." *Scientific Reports.* 10.1 (2020): 1-10.

Disclosures: K.M. Lambert: None. **T. Zhang:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on licensed patents on temporal patterns of spinal cord stimulation and receives royalty distributions from Duke University. **J. Gilbert:** None. **M. Moffitt:** None. **W.M. Grill:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Inventor on licensed patents on temporal patterns of spinal cord stimulation and receives royalty distributions from Duke University.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.12

Topic: D.02. Somatosensation – Pain

Title: Scalp EEG gamma band neural activity reflects pain intensity in humans

Authors: D. WANG[1], X. ZHANG[1], S. MOOSA[1], M. ISHAQUE[1], P. FINAN[2], M. QUIGG[3], W. J. ELIAS[1], *C.-C. LIU[1];
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Abstract: Can pain intensity be objectively measured? While previous neurophysiological studies have demonstrated a positive correlation between pain intensity and magnitude of painful cutaneous laser-induced gamma band neural activity, one may argue that the increased gamma band neural activity is linked to the laser intensity rather than the pain intensity. When keeping the same laser intensity, it remains unknown whether the pain-related gamma band neural activity will decrease when individual's ability to perceive pain is suppressed. To fill this knowledge gap, we inhibited an individual's perception of pain by activating the endogenous pain inhibitory pathways using an iced water (0-2°C) conditioned pain modulation (CPM) approach in healthy controls. Using scalp EEG and brief painful heat pulse generated by a cutaneous laser stimulator (Nd:YAP infrared laser, pulse duration 4ms, beam diameter 5mm, and wavelength 1340 nm), we tested the hypothesis that reduced pain intensity following the CPM will associate with a significant decrease in the pain-related gamma band neural activity. Ten healthy subjects (22 ± 3.7 yrs, 5 Female) signed the informed consent and were enrolled to the present study. We observed that laser pain intensity was significantly reduced following the CPM ($p = 0.001$). For overall, painful laser induced gamma band neural activity was also significantly decreased in all scalp EEG channels ($p < 0.0001$). Post-hoc pairwise comparisons revealed that contralateral sensorimotor C4, midline central Cz, midline frontal Fz, midline posterior Pz and contralateral temporal T7 showed significant gamma reductions following the iced water induced CPM ($p < 0.05$ with FDR adjustments). Pain intensity coding appears to engage a wide range of brain structures including the somatosensory, prefrontal, cingulate, parietal and temporal areas. Our results complement with the notion that scalp EEG gamma band activity reflects pain intensity evoked by brief painful cutaneous laser stimulation in humans.

Disclosures: D. Wang: None. **X. Zhang:** None. **S. Moosa:** None. **M. Ishaque:** None. **P. Finan:** None. **M. Quigg:** None. **W.J. Elias:** None. **C. Liu:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.13

Topic: D.02. Somatosensation – Pain

Title: Selective nociceptive fiber assessment for temporal summation of pain using repetitive painful cutaneous laser stimulation

Authors: *D. WANG[1], X. ZHANG[1], S. YE[1], D. KUMAR[1], S. MOOSA[1], M. ISHAQUE[1], P. FINAN[2], M. QUIGG[3], W. ELIAS[1], **C.-C. LIU**[1];
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Abstract: Elevated temporal summation of pain (TSP) may indicate increased gain of the nociceptive system termed central sensitization, which has been associated with higher risks of chronic pain development. Contact thermode heat stimulation is one of the most common paradigms for TSP assessment in clinical studies of patients and healthy populations. However, the use of contact thermode inevitably activates the low-threshold non-nociceptive mechanoreceptors which may influence the specificity of the neural activities observed in the supraspinal level. In addition, TSP assessed using chronic contact thermode is temporally less accurate if subjects are not fully engaged to the study. To improve the TSP assessment, we utilized a cutaneous Nd: YAP infrared laser for selectively activating nociceptive specific Aδ- and C-fibers without co-activating the low-threshold non-nociceptive mechanoreceptors for temporal summation of pain assessment. Furthermore, additional supraspinal nociceptive information processing knowledge may be gained if these nociceptive pathways are evaluated, separately. For the Aδ-fiber TSP assessment, cutaneous laser was adjusted to primarily activating the Aδ-fibers and evoked 3/4 out of 10 brief pin-prick painful sensations. For the C-fiber TSP assessment, cutaneous laser was set to elicit slightly warm non-painful sensation, indicating C-fiber activations. For both Aδ- and C-fiber TSP assessments, cutaneous laser stimulations were delivered repetitively (i.e. $\geq$ 0.5Hz) to the dermatome areas of the dorsal hand and ventral forearm during Aδ-fiber and C-fiber TSP assessments, respectively. Using a 0-10 visual analog scale, we observed a significantly greater pain intensity report for the last than the first stimulus within a stimulation train for both Aδ- and C-fiber stimulations ($p < 0.03$, $p = 0.0002$ for Aδ- and C-fiber, respectively; One-way ANOVA, FDR adjustment; N = 6, 21.3 ± 1.0yrs, 3 Female), indicating the effectiveness of the TSP induction. The present study represents a first attempt for evaluating TSP by selectively activating nociceptive fibers in humans. These new approaches may enable future studies to selectively investigate distinct nociceptive circuitries, which may reveal unique neural signatures underlying the experience of pain.

Disclosures: D. Wang: None. **X. Zhang:** None. **S. Ye:** None. **D. Kumar:** None. **S. Moosa:** None. **M. Ishaque:** None. **P. Finan:** None. **M. Quigg:** None. **W. Elias:** None. **C. Liu:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 048.14

Topic: D.02. Somatosensation – Pain

Support: Rheumatology Research Foundation Innovative Research Award
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NIH R01AG063975

NIH R33AT009310
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Title: Modulation Effects of Remote-Delivered Mind-body Intervention on thalamocortical circuitry in patients with Knee Osteoarthritis

Authors: *J. KONG[1], C. WANG[2];
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Abstract: Aim: Knee Osteoarthritis (KOA) is a major public health problem. Previous studies have shown that in-person Tai Chi mind-body intervention can improve symptoms associated with KOA; thus, it has been recommended in the guidelines for KOA intervention. Recent studies conducted during the COVID-19 pandemic suggest that remote mind-body intervention is a promising and scalable strategy. However, few studies have investigated the neurobiological effects of remote Tai Chi for KOA. This study aims to investigate how remote Tai Chi modulates the thalamocortical circuitry by investigating the brain resting state functional connectivity (rsFC) of thalamus subregions associated sensation and movement.
Methods: KOA patients were randomized to either a Tai Chi or Wellness Education group and attended 12 weeks of biweekly remote sessions (NCT04678999). The clinical and brain imaging assessments were performed at baseline and after 12-week intervention. We applied CONN toolbox for seed based rsFC analysis. Three thalamus subregions were included as seeds based on parcellation of the thalamic nuclei of AAL3. Seed 1: bilateral motor thalamus subregion; seed 2: somatosensory thalamus subregion; seed 3: medial dorsal thalamus, a subregion associated with the emotional / affective component of pain. A threshold of $p < 0.005$ voxel-wise and $p < 0.05$ False Discovery Rate corrected at cluster level was applied. Small volume correction was applied for pre-defined regions of interest (limbic system and prefrontal cortex).
Results: 31 participants completed the study. At 12 weeks, a significantly greater improvement (as indicated by the Western Ontario and McMaster Universities (WOMAC) pain subscale scores) was found in the remote Tai Chi group compared to wellness education.
Imaging analysis showed these brain imaging outcomes in the Tai Chi group compared to wellness education. 1) motor thalamus subregion rsFC increased at the right cerebellum (lateral hemisphere) and decreased at the bilateral dorsal anterior cingulate cortex (ACC) and right amygdala / hippocampus; 2) somatosensory thalamus subregion rsFC increased at the right cerebellum (medial and lateral hemisphere) and decreased at the right lateral prefrontal cortex, bilateral dorsal ACC / mid-cingulate cortex, and right amygdala / hippocampus; 3) medial dorsal thalamus rsFC increased at the bilateral cerebellum (medial and lateral hemisphere) and decreased at bilateral amygdala / hippocampus / uncus.
Conclusions: Our results suggest that 12-week remote Tai Chi can increase thalamus-cerebellum rsFC and decrease thalamus - limbic rsFC. Our findings endorse the potential of remote Tai Chi for pain management.

Disclosures: J. Kong: None. **C. Wang:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.15

Topic: D.02. Somatosensation – Pain

Title: Spontaneous Neural Oscillations Are Altered In Fibromyalgia: Associations With Clinical Symptoms And Medication Treatment

Authors: D. R. WHITE[1], L. A. HOLCOMB[2], M. MARVIN[4], J. P. HAPPER[1], A. M. CORREAS[5], T. A. CRONAN[3], *K. MARINKOVIC[1];
[2]Psychology, [3]Psychology Dept., [1]San Diego State Univ., San Diego, CA; [4]The VA San Diego Healthcare Syst., San Diego, CA; [5]Complutense Univ. of Madrid, Madrid, Spain

Abstract: Fibromyalgia (FM) is a chronic condition primarily characterized by widespread musculoskeletal pain which is commonly accompanied with fatigue, insomnia, and other comorbidities. FM does not present clearly identifiable pathologies which makes diagnosis and treatment assessment challenging, and further compounded by the lack of effective pharmacotherapy. Extant evidence from studies using electro- or magnetoencephalography (EEG/MEG) indicates that thalamocortical dysrhythmia (TCD) may underlie the centralized pain that characterizes FM and some other chronic pain syndromes. The present study aims to examine the neural underpinnings of FM and their association with clinical symptoms. In addition, the results were considered as a function of pharmacological treatment, given the evidence that drugs that increase inhibitory signaling may be effective for some people with FM. Thirty-four volunteers (mean age ± *SD* = 49.5 ± 13.6 years, 91% female) participated in this study and included people with FM diagnoses (N=16) who were stable on gabapentinoids (FM-GB), or other medications (FM-O), which mostly included SSRIs or SNRIs, opioids, or NSAIDs. Compared to demographically matched control (CNT) participants (N=18), FM participants reported higher scores on all pain-related variables, as well as depression and anxiety, but the groups did not differ in cognitive capacity.Continuous EEG data were recorded during the eyes open and closed wakeful resting paradigm. After artifact removal, data were analyzed with a multitapered Fast Fourier Transform to extract power in theta (4-7 Hz), alpha (8-12 Hz), and beta (15-20 Hz) frequency bands. To examine changes in alpha band in greater detail, alpha ratio (AR) was calculated by dividing power in the slow alpha band (7–9 Hz) by fast alpha band (9–11 Hz), with greater AR indicative of oscillatory slowing. Alpha peak frequency (APF) was determined as the dominant frequency within the alpha band. Age was covaried in all analyses. Group differences were detected for APF and AR. FM-O displayed slower APF and higher AR than both CNT and FM-GB, which correlated with pain-related activity patterns. The observed downshift of dominant spontaneous oscillations is consistent with TCD as a mechanism that underlies centralized pain. In contrast, FM-GB demonstrated faster APFs than FM-O and CNT, confirming that gabapentinoids may ameliorate FM symptoms by enhancing neural inhibition. In sum, alpha slowing can serve as a mechanistic biomarker signature of TCD, the core neuropathology characterizing FM. In the clinical context of use, these EEG indices can assist with diagnostic and treatment-monitoring purposes.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.16

Topic: D.02. Somatosensation – Pain

Support: NIH Grant R01NS106301
New York Stem Cell Foundation
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Title: Impaired feedforward inhibition of corticopontine neurons drives placebo analgesia

Authors: *C. CHEN[1], J. K. NIEHAUS[1], K. L. HUANG[1], A. L. BARNETTE[1], A. SHUSTER[3], L. WANG[4], A. LEMIRE[4], V. MENON[5], K. RITOLA[2], A. HANTMAN[1], H. ZENG[6], M. J. SCHNITZER[7], G. SCHERRER[1];
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Abstract: Pain is a multidimensional experience with sensory-discriminative, affective-motivational and cognitive-evaluative components (Melzack and Casey 1968). Although numerous neurobiological mechanisms underlying the sensory and affective dimensions of pain have been resolved, our understanding of the cognitive modulation of pain remains limited. Here, we identify circuit, cellular and synaptic mechanisms that mediate placebo analgesia, the biomedical phenomenon in which a positive expectation suffices to reduce pain. We first developed a 7-day placebo analgesia conditioning (PAC) assay that generates a pain relief expectation in mice and permits evaluation of placebo analgesia. Activity-dependent genetic tagging during PAC revealed that rostral anterior cingulate cortex neurons projecting to the pontine nuclei (rACC→Pn neurons) could contribute to placebo analgesia. Calcium imaging in freely moving mice trained to expect pain relief revealed a correlated increase in the activity and synchrony of rACC→Pn neurons during pain relief expectations. Whole-cell patch-clamp recording indicated that PAC increases the burst firing, AMPA/NMDA ratio and synaptic plasticity of rACC→Pn neurons, while impairing the efficiency and timing of their feedforward inhibition. Photoinhibition of the rACC-Pn pathway reversed PAC-induced analgesia. Single-cell transcriptomics suggested that 54% of Pn neurons express *Oprd1* (encoding the delta opioid receptor). Additionally, photoinhibition of *Oprd1*$^{+}$ Pn neurons abolished PAC-induced analgesia. These findings advance our current understanding of placebo analgesia and cognitive modulation of pain, from the merely brain-area level to the circuit, cellular and synaptic levels, bridge the gap with our more advanced understanding of the sensory and affective dimensions of pain, and open the possibility of using this pathway to promote positive outcomes during pain management.

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Poster

048. Pain Central Mechanisms

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Topic: D.02. Somatosensation – Pain

Support: DFG Grant SFB1158

Title: Primary somatosensory cortex bidirectionally modulates sensory gain and nociceptive behaviour in a layer-specific manner

Authors: *R. FOLKARD[1], K. ZIEGLER[1], J. BURGHART[1], A. GONZALEZ[1], E. ISAIAS-CAMACHO[1], J. MARTIN-CORTECERO[1], S. ANTHARVEDI-GODA[1], S. KAUSHALYA[3], L. TAN[2], R. KUNER[2], T. KUNER[4], R. A. MEASE[1], A. GROH[1];
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Abstract: The primary somatosensory cortex (S1) is the hub for body sensation originating from both innocuous and noxious body signals, yet its exact role in normal versus pain sensation is much debated. While there is mounting evidence for layer-specific contributions of S1 circuits to sensory gain modulation, their causal consequences for the subjective dimensions of the sensory experience remain elusive. We found that activity in corticothalamic neurons in layer 6 (L6) of mouse S1 hindlimb cortex (S1HL) can lead to aversive hypersensitivity and can drive spontaneous nocifensive behavior. Layer 6 downstream effects recorded with linear multi channel silicon probes revealed enhanced thalamic somatosensory responses in the ventro-posterior thalamus, and in parallel, strong suppression of layer 5 (L5) responses in S1, pointing towards an anti-nociceptive function of L5 output. Indeed, activation of L5 neurons reduced sensory sensitivity and normalized inflammatory allodynia and thus modulated nociception in the opposite direction as compared to L6. These results reveal a layer-specific and bidirectional role for S1 in modulating subjective sensory experiences.

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Poster

048. Pain Central Mechanisms

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Topic: D.02. Somatosensation – Pain

Support: NIH P40OD010996
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Title: The cingulate motor areas and insula receive spinothalamic input from different spinal laminae

Authors: *R. P. DUM[1], A. C. BOSTAN[1], P. L. STRICK[2];
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Abstract: The spinothalamic system has three major cortical targets: granular insular cortex (Ig, 41%); the second somatosensory cortex (29%); and the cingulate motor areas (CMAs, 24%)(Dum et al., J. Neurosci., 2009). We used retrograde transneuronal transport of rabies virus to identify the spinal neurons that project to sites within Ig and the CMAs.We injected portions of Ig (n = 1) and the caudal CMAs (i.e., dorsal and ventral CMAs; n = 1) with the N2C strain of rabies virus. We set the survival time in these animals to allow retrograde transport of the virus to the thalamic neurons that innervate the injection sites, and then retrograde transneuronal transport to the spinal neurons that innervate the infected thalamic neurons.Surprisingly, we found that both cortical regions receive a prominent spinothalamic input from upper cervical segments (C2-C4). The ratio of spinothalamic neurons from the upper versus the lower cervical (C5-T1) segments is 2.3:1 for Ig and 2.2:1 for the CMAs. All of the spinothalamic input from C5-T1 originates from the contralateral cord, whereas 20% of the input from C2-C4 originates from the ipsilateral cord. The majority of spinothalamic neurons from C2-C4 to Ig (65%) and CMAs (98%) are located in the ventral horn and deeper laminae (4-6) of the dorsal horn. The prominence of the spinal input from C2-C4 re-emphasizes the importance of this component of the spinothalamic system (Hodge and Apkarian, 1990).In contrast, Ig and CMAs display marked differences in the origins of their input from C5-T1. Ig receives most (>80%) of its spinothalamic projections from superficial laminae (primarily lamina 1) of the dorsal horn. Lamina 1 neurons are generally thought to be involved in the processing of specific types of nociceptive information. Thus, our results are consistent with Craig's (2006) view that “pain sensation” is mediated by a spino-thalamocortical projection from lamina 1 to Ig. In contrast, the CMAs receive most (79%) of their spinothalamic input from the deep laminae (4-6) of the dorsal horn. Neurons in deeper laminae typically have large receptive fields and process a wide variety of information including noxious and innocuous afferent input as well as activity in spinal reflex and motor circuits. This broad spectrum of responses may provide information about external and internal conditions that could then be used to influence the process of action selection by the CMAs.

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Poster

048. Pain Central Mechanisms

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Program #/Poster #: 048.19

Topic: D.02. Somatosensation – Pain

Support: Tryp Therapeutics, Kelowna CA
Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, MI

Title: Psilocybin attenuates mechanical allodynia and thermal hyperalgesia in a rodent model of formalin-induced chronic pain

Authors: *N. KOLBMAN[1,2,3], B. H. SILVERSTEIN[1,3], T. LIU[1], P. GUZZO[6], J. GILLIGAN[6], G. A. MASHOUR[1,2,3,4], G. VANINI[1,3,4], D. PAL[1,3,4,5];
[1]Anesthesiol., [2]Pharmacol., [3]Ctr. for Consciousness Sci., [4]Neurosci. Grad. Program, [5]Mol. & Integrative Physiol., Univ. of Michigan, Ann Arbor, MI; [6]Tryp Therapeut., Kelowna, BC, Canada

Abstract: There is a renewed interest in the therapeutic potential of psychedelics, including psilocybin, in treating mental health disorders. However, there are no data on the efficacy of psilocybin in alleviating chronic pain. In the present study, we investigated the effect of intravenous psilocybin on mechanical allodynia and thermal hyperalgesia in a rat model of formalin (FML)-induced chronic pain. Under isoflurane anesthesia, adult male Sprague Dawley rats (300-350 g) were implanted with a jugular vein catheter to administer 10 mg/kg psilocybin or 0.9 % saline. Mechanical allodynia was assessed using the von Frey assay (VFA) and was expressed as threshold in grams (g). Thermal hyperalgesia was measured as paw withdrawal latency in seconds (s) using the hot plate assay (HPA) (52.5°C). After post-surgical recovery (7-10 days), baseline responses to mechanical (VFA) and thermal stimuli (HPA) were measured. 24 h after baseline measurements, rats received a subcutaneous FML injection (5%, 50µL) into one of the hind paws (Day 0). 2 h after the FML injection (Day 0), responses to the mechanical and thermal stimuli were measured. 24 h after FML injection, rats received an intravenous bolus of 10 mg/kg psilocybin (N=3) or 0.9% saline (N=3), and 3-4 h later, responses to the mechanical and thermal stimuli were measured (Day 1). Rats were tested every other day during week 1, and then weekly for next 3 weeks. The data are reported as mean ± standard deviation. FML injection induced thermal hyperalgesia (withdrawal latency after FML = 6.13 ± 1.03 s vs. baseline = 7.10 ± 1.88 s) and bilateral mechanical allodynia in the hind paws of all rats (withdrawal threshold in FML-injected paw = 5.13 ± 2.31 g vs. baseline = 13.10 ± 1.79 g; withdrawal threshold in contralateral paw = 6.27 ± 1.83 g vs. baseline = 14.17 ± 1.05 g). Intravenous psilocybin (Day 1) attenuated thermal hyperalgesia (withdrawal latency after Saline = 4.60 ± 0.70 s vs. Psilocybin = 7.52 ± 1.50 s) and bilateral mechanical allodynia (withdrawal threshold in FML-injected paw after Saline = 5.33 ± 3.11 g vs. Psilocybin = 13.67 ± 1.53 g; withdrawal threshold in contralateral paw after Saline = 4.13 ± 0.46 g vs. Psilocybin = 12.00 ± 1.73 g). Psilocybin-treated rats continued to show attenuation of FML-induced thermal hyperalgesia (withdrawal latency after Saline = 4.61 ± 1.78 s vs. Psilocybin = 6.92 ± 1.37 s) and mechanical allodynia in the

contralateral paw (withdrawal threshold after Saline = 5.81 ± 1.00 g vs. Psilocybin = 8.23 ± 3.03 g) until the final day of testing (Day 28). These data show that a single intravenous bolus of 10 mg/kg psilocybin can attenuate mechanical allodynia and thermal hyperalgesia in a rat model of FML-induced chronic pain.

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Poster

048. Pain Central Mechanisms

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Title: Oxytocin promotes prefrontal ensemble activity via the PVN-PFC pathway to regulate pain

Authors: Y. LIU[1,7], A. LI[1,7], C. BAIR-MARSHALL[2,3,4,5], H. XU[1,7], ***H. JEE**[1,7], Q. ZHANG[1,7], A. LEFEVRE[8], Z. S. CHEN[3,4,6], V. GRINEVICH[8], R. C. FROEMKE[2,3,4,5], J. WANG[1,7,3,4]; [1]Dept. of Anesthesiology, Perioperative Care and Pain Med., [2]Skirball Inst. for Biomolecular Med., [3]Dept. of Neurosci. and Physiol., [4]Neurosci. Inst., [5]Dept. of Otolaryngology, [6]Dept. of Psychiatry, New York Univ. Grossman Sch. of Med., New York, NY; [7]Interdisciplinary Pain Res. Program, New York Univ. Langone Hlth., New York, NY; [8]Dept. of Neuropeptide Res. in Psychiatry, Central Inst. of Mental Health, Univ. of Heidelberg, Mannheim, Germany

Abstract: Neural ensembles of the prefrontal cortex (PFC) can provide top-down regulation of sensory-affective experiences such as pain. Bottom-up modulation of sensory coding in the PFC, however, remains poorly understood. Here we examined how oxytocin signaling from the hypothalamus regulates nociceptive coding in the PFC. *In vivo* time-lapse endoscopic calcium imaging in freely-behaving rats showed that oxytocin selectively enhanced population activity in the prelimbic PFC in response to nociceptive inputs. This population response resulted from a reduction of evoked GABAergic inhibition, as demonstrated by *in vitro* whole-cell patch-clamp

recordings, and manifested as elevated functional connectivity involving pain-responsive neurons in our graph-theoretic analysis. Direct inputs from oxytocin-releasing neurons in the paraventricular nucleus of the hypothalamus (PVN) are crucial to maintaining this prefrontal nociceptive ensemble. Activation of the prelimbic PFC by intracranial delivery of oxytocin or by direct optogenetic stimulation of oxytocinergic PVN projections reduced acute pain, as well as persistent inflammatory pain. These results suggest that oxytocinergic signaling in the PVN-PFC circuit constitutes a key mechanism to regulate cortical sensory processing.

Disclosures: **Y. Liu:** None. **A. Li:** None. **C. Bair-Marshall:** None. **H. Xu:** None. **H. Jee:** None. **Q. Zhang:** None. **A. Lefevre:** None. **Z.S. Chen:** None. **V. Grinevich:** None. **R.C. Froemke:** None. **J. Wang:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.21

Topic: D.02. Somatosensation – Pain

Support: R01-GM115384
CBET-1835000
R01-NS121776
GM115384
NS121776

Title: A closed-loop multi-region brain-machine interface for analgesic delivery in rodents

Authors: ***S. GUANGHAO**[1], F. ZENG[2], M. MCCARTIN[1], Q. ZHANG[1], H. XU[1], Y. LIU[3], Z. CHEN[1], J. WANG[1];
[1]New York Univ., New York Univ., New York, NY; [2]Nan Chang Univ., Nan Chang Univ., Nan Chang, China; [3]Hsiang-Ya Sch. of Med., Central South Univ., Chang Sha, China

Abstract: Effective treatments for chronic pain remain limited. Conceptually, a closed-loop neural interface that combines sensory signal detection with therapeutic delivery can provide timely and effective pain relief. The development of such systems is challenging due to the difficulties of accurate pain detection and ultrafast analgesic delivery. Pain has sensory and affective components and is primarily encoded by neural activity in the primary somatosensory cortex (S1) and anterior cingulate cortex (ACC). Meanwhile, studies have shown that stimulation of the prefrontal cortex (PFC) produces decreased pain control. Here, we designed and tested a brain-computer interface (BMI) that combines an automated pain detection arm based on simultaneous recording of local field potential (LFP) signals from S1 and ACC, and a ontogenetic stimulus of the PFC in the treatment arm of free moving rats. Our multi-region neural interface accurately detects and treats acutely evoked pain and spontaneous chronic pain. This neural interface was activated rapidly, and the effect remained stable over time. Given the

clinical feasibility of LFP records and DBS, our findings suggest that BMI is a promising approach to pain management.

Disclosures: S. Guanghao: None. **F. Zeng:** None. **M. McCartin:** None. **Q. Zhang:** None. **H. Xu:** None. **Y. Liu:** None. **Z. Chen:** None. **J. Wang:** None.

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.22

Topic: D.02. Somatosensation – Pain

Title: NYX-2925 is a novel NMDAR PAM which augments NMDAR-mediated activity in hypo-functioning circuits

Authors: *C. J. KELLY[1], K. LEADERBRAND[2], A. L. BARTH[2];
[1]Aptinyx Inc., Evanston, IL; [2]Aptinyx Inc., Chicago, IL

Abstract: NYX-2925 is a novel small molecule positive allosteric modulator (PAM) of the N-methyl-D-aspartate receptor (NMDAR) that is in clinical development for the treatment of the chronic, nociplastic pain condition fibromyalgia (e.g., see NCT04147858). In rodent models of chronic pain, oral dosing of NYX-2925 alleviated pain, as did direct injection of drug into the medial prefrontal cortex (mPFC). In contrast, injection of NYX-2925 into the spinal cord had no effect. In chronic pain states, the mPFC is thought to be hypoactive, whereas glutamatergic activity is increased in the dorsal horn of the spinal cord. Therefore, we hypothesized that NYX-2925 alleviates chronic pain by increasing activity within hypoactive circuits and does not exacerbate high NMDAR-mediated activity within hyperactive regions like the spinal cord. The multi-ligand system of the NMDAR makes it a uniquely complex drug target. NMDARs have two primary co-ligand binding sites and a plethora of modulatory domains, including the novel allosteric binding site of NYX-2925. Many of these binding domains have allosteric interactions with one another and can impact the binding and activity of ligands at other sites. Drugs targeting the NMDAR, therefore, may have different effects depending on the concentrations of other ligands present. This series of exploratory studies looked at the effects of NYX-2925 on NMDAR-mediated current at varying concentrations of glutamate and glycine, the two primary endogenous co-ligands of the NMDAR. We assessed the effects of NYX-2925 on NMDAR currents in NMDAR2A and NMDAR2B-containing HEK cells using the IonWorks Barracuda (IWB) high-throughput patch system. NYX-2925 increased NMDAR-mediated current when both glutamate and glycine were present at low-to-moderate levels. In contrast, NYX-2925 did not increase NMDAR-mediated current at saturating concentrations of glutamate and glycine. These findings lend support to the hypothesis that NYX-2925 selectively increases NMDAR-mediated activity only at hypoactive synapses. This novel therapeutic mechanism has the potential to augment abnormal NMDAR activity and restore normal circuit function in conditions like fibromyalgia without the risk of adverse effects due to NMDAR overactivation.

Disclosures: **C.J. Kelly:** A. Employment/Salary (full or part-time):; Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **K. Leaderbrand:** A. Employment/Salary (full or part-time):; Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc. **A.L. Barth:** A. Employment/Salary (full or part-time):; Aptinyx Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Aptinyx Inc..

Poster

048. Pain Central Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 048.23

Topic: D.02. Somatosensation – Pain

Support: NIH Grant 5R21DA046184
NIH Grant 1R21NS110371
Fellowship: BSD International Student Fellowship
Fellowship: Greene Scholarship
Fellowship: Wooley Scholarship

Title: Cholinergic modulation of the descending pain circuitry

Authors: *S. SULLERE[1], A. L. KUNCZT[1], D. S. MCGEHEE[2];
[1]Univ. of Chicago, Chicago, IL; [2]Anesthesia, Univ. of Chicago Committee On Neurobio., Chicago, IL

Abstract: Acetylcholine (ACh) and its receptors modulate neuronal activity to enable organisms adapt to various evolutionarily conserved and ethologically relevant stimuli. Although pharmacological agents that activate various cholinergic receptors can relieve pain, the impact of endogenous cholinergic circuits in acute and chronic pain conditions has not been investigated. We examined acute pain relief by cholinergic inputs to the descending pain pathway at the level of the ventrolateral periaqueductal gray (vlPAG) and have identified pedunculopontine tegmental nucleus (PPTg) as a key mediator of cholinergic analgesia. Under chronic pain conditions, selectively stimulating cholinergic projections from PPTg to vlPAG relieved somatic and affective pain symptoms. Interestingly, chronic pain conditions are associated with decreased cholinergic tone in vlPAG, as assayed with the fluorescent ACh sensor - ACh3.0. By combining optical stimulation and pharmacology, we determined that α7 nicotinic ACh Receptors (nAChRs) are the key cholinergic receptor mediating these analgesic effects. Finally, using slice electrophysiology and in-vivo calcium imaging with GCaMP6, we identified maladaptive hyper-excitability of α7 nAChR-expressing vlPAG neurons during chronic painful conditions. This hyper-excitability is reversed by systemic treatment of α7 nAChRs agonists through a Peroxisome Proliferator-Activated Receptor (PPARα) mediated intracellular signaling

mechanism. These results implicate cholinergic mechanisms in acute and chronic pain-induced alterations in neuronal excitability and will help identify novel targets for analgesia.

Disclosures: S. Sullere: None. **A.L. Kunczt:** None. **D.S. McGehee:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.01

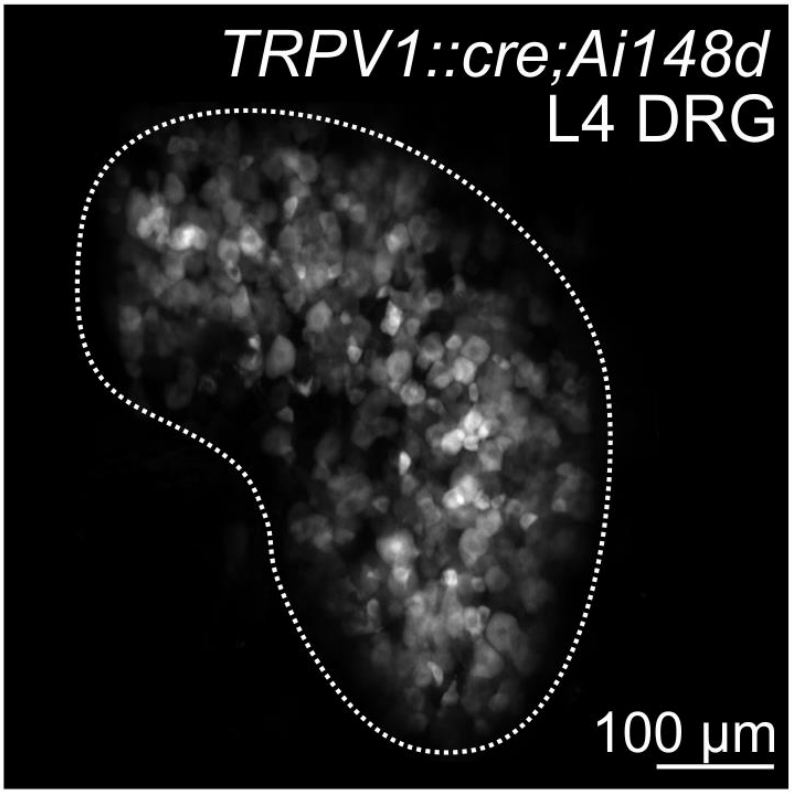
Topic: D.03. Somatosensation – Touch

Support: HFSP (LT000359/2018)
ERC-2015-CoG-682422
DFG (FOR 2143, SFB1315)
Hemholtz Society

Title: Encoding of cutaneous non-painful temperature by mouse primary sensory afferent neurons

Authors: C. WHITMIRE[1,2], ***P. BOKINIEC**[1,2], J. F. POULET[1,2];
[1]Max Delbrück Ctr. for Mol. Med. in the Helmholtz Assn. (MDC), Berlin, Germany; [2]Neurosci. Res. Ctr., Charité-Universitätsmedizin, Berlin, Germany

Abstract: The first step of thermal perception during haptic exploration is the transduction of surface temperature by primary sensory afferent neurons. However, little is known about encoding of non-painful temperatures by populations of afferent neurons. Different models suggest that temperature could be encoded by graded changes in afferent response amplitude, or by recruitment of distinct subpopulations with different sensory thresholds (Wang et al. 2018). Here we address this by performing *in vivo* multiplane two-photon calcium imaging in an anaesthetized mouse preparation that allows optical access to an entire lumbar dorsal root ganglion (DRG). Thermal stimuli were delivered to the hindpaw via a Peltier element in constant contact with the hindpaw from 22 to 42°C, and tactile stimuli were delivered via a piezoelectric actuator. Using transgenic mice expressing a calcium indicator in all thermal afferent neurons (TRPV1-cre x GCaMP6), we show that, while few cells were spontaneously active, afferent neurons show a sparse but robust thermal representation of non-painful temperature, with more cool than warm responsive neurons per DRG. Thermally responsive cells were typically small diameter, consistent with the hypothesis that non-painful thermal can be encoded by C-fibers. Preliminary analysis suggests that thermal afferents encode warm and cool in a graded manner. Taken together, these results present a comprehensive overview of non-painful thermal encoding by afferent neurons innervating the mouse glabrous skin.
References: Wang, F. et al. Sensory Afferents Use Different Coding Strategies for Heat and Cold. Cell Reports 23, 2001-2013 (2018).



Disclosures: **C. Whitmire:** None. **P. Bokiniec:** None. **J.F. Poulet:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.02

Topic: D.03. Somatosensation – Touch

Support: ERC-2015-CoG-682422
FOR 2143, SFB1315
Helmholtz Society

Title: Characterization of the mouse spinothalamic tract

Authors: *I. SAUVE[1], J.-S. JOUHANNEAU[1,2], P. BOKINIEC[1,2], J. F. A. POULET[1,2], N. ZAMPIERI[1];
[1]Max Delbruck Ctr., Max Delbrück Ctr., Berlin, Germany; [2]Neurosci. Res. Center, Charité-Universitätsmedizin, Berlin, Germany

Abstract: The ability to detect and process different sensory stimuli is crucial for generating appropriate behavioral responses. Somatosensory neurons are responsible for the detection of a

wide variety of sensory information including temperature, body position, touch and pain. While extensive data exists about the anatomical, molecular, and functional organization of primary afferent somatosensory neurons, the characterization of neurons that transmit somatosensory information from the spinal cord to the brain is still incomplete. The spinothalamic tract is the major ascending pathway of the spinal cord that transmits temperature, pain and crude touch to the thalamus, however its anatomical and molecular nature remain unclear. Here, we characterize spinothalamic neurons in mice that target two different thalamic nuclei thought to process thermal information, the ventral posterolateral nucleus (VPL) and the posterior triangular nucleus (POt). By using injections of retrograde tracers, we identified two anatomically distinct clusters of spinothalamic neurons in the dorsal spinal cord of adult mice, supporting the existence of parallel input pathways to VPL and POt. Moreover, our data show that the majority of spinothalamic neurons originate from lamina I/II of the dorsal horn, layers associated with thermal and pain processing. We show that the majority of spinothalamic neurons preferentially target POt. Together, these data pave the way to an understanding of the molecular and functional characteristics of ascending neurons carrying thermal information, with the ultimate goal of revealing the circuit logic of warm and cold sensation.

Disclosures: I. Sauve: None. **J. Jouhanneau:** None. **P. Bokiniec:** None. **J.F.A. Poulet:** None. **N. Zampieri:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.03

Topic: D.03. Somatosensation – Touch

Support: HFSP (LT000359/2018)
European Research Council (ERC-2015-CoG-682422)
Deutsche Forschungsgemeinschaft (FOR 2143, SFB1315)
Helmholtz Society

Title: Distinct cellular encoding of warm and cool in the thalamus

Authors: T. M. LEVA[1,2], ***C. J. WHITMIRE**[1,2], C. MEMLER[1,2], J. F. A. POULET[1,2];
[1]Max Delbrück Ctr. for Mol. Med. in the Helmholtz Assn., Berlin, Germany; [2]Neurosci. Res. Ctr., Charité-Universitätsmedizin, Berlin, Germany

Abstract: Temperature is a fundamental sensory system and key component of somatosensory perception. Unlike almost all other sensory modalities, however, the location and cellular encoding of temperature in the thalamus is unclear. One possibility is that thalamic neurons are tightly tuned to warm or cool acting as a functionally and anatomical distinct labelled line-like pathway, while another is that single neurons encode both warm and cool in a more mixed encoding scheme. To address this, we used high density multielectrode probes (Neuropixels,

IMEC) to map cellular activity across multiple somatosensory thalamic nuclei during thermal and tactile stimulation of the mouse forepaw. Guided by retrograde labelling from the cortical representation of temperature, we targeted 4 thalamic regions: rostral and caudal ventral posterolateral nuclei (rVPL, cVPL) and posterior medial and triangular nuclei (Pom, PoT). Closely reflecting functional cortical data, we observed that the tuning of a thalamic neuron is correlated to its rostro-caudal location. Cool only neurons were positioned more rostrally, while cool and warm cells were more caudal. Intriguingly, we found that the dynamics of warm and cool encoding are distinct, even in the same neurons, with cool responses being temporally precise with a rapid onset and faster decay, and evoked responses to warm being temporally delayed and more sustained. Inhibitory neurons that innervate the thalamus (thalamic reticular nucleus, zona incerta) showed sharp and temporally precise thermally driven responses. Taken together, we provide a comprehensive map of thermal encoding in the thalamus that suggest distinct, but overlapping, encoding of warm and cool that is dependent on thalamic location.

Disclosures: **T.M. Leva:** None. **C.J. Whitmire:** None. **C. Memler:** None. **J.F.A. Poulet:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.04

Topic: D.03. Somatosensation – Touch

Support: European Research Council (ERC-2015-CoG-682422)
Deutsche Forschungsgemeinschaft (FOR 2143, SFB1315)
Helmholtz Society
Biotechnology and Biological Sciences Research Council (BBSRC, UK)
Experimental Psychology Society (EPS, UK)

Title: Integration of thermo-tactile inputs in the primary somatosensory cortex of awake and anesthetized mice

Authors: P. SCHNEPEL[1,2], ***I. EZQUERRA-ROMANO**[1,2,3], P. HAGGARD[3], J. F. A. POULET[1,2];
[1]Max Delbrück Ctr. for Mol. Med. in the Helmholtz Assn. (MDC), Robert-Rössle Strasse 10, 13092, Berlin, Germany; [2]Neurosci. Res. Center, Charité-Universitätsmedizin, Charitéplatz 1, 10117, Berlin, Germany; [3]Inst. of Cognitive Neuroscience, Univ. Col. London, Alexandra House, 17 Queen Square, WC1N 3AZ, London, United Kingdom

Abstract: A fundamental function of the brain is to integrate information from multiple sensory pathways to generate a single coherent percept. While primary sensory cortical regions have been proposed to play a role in multisensory integration, little is known about the cellular mechanisms. Principles of multisensory integration such as the law of inverse effectiveness and

spatio-temporal congruency have been examined in the superior colliculus but less is known in the cortex. To address these questions, here we investigate thermo-tactile integration in primary somatosensory forepaw cortex (fS1). Using multi-site extracellular electrodes (Neuropixels), we recorded from fS1 neurons of awake and anaesthetized mice during non-painful thermal and mechanical stimulation of the forepaw. Consistent with previous studies in our lab, we found neurons responsive to touch or cool alone as well as neurons responsive to both touch and cool. Our results indicate that multisensory, thermo-tactile stimulation recruits previously silent neurons in S1 and changes the cellular response dynamics compared to unimodal stimulation. We go on to address core principles of multisensory integration in fS1.

Disclosures: P. Schnepel: None. **I. Ezquerra-Romano:** None. **P. Haggard:** None. **J.F.A. Poulet:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.05

Topic: D.03. Somatosensation – Touch

Support: CHRONOS project
Swiss National Science Foundation through the National Centre of Competence in Research (NCCR) Robotics
the Bertarelli Foundation

Title: Presence of a thermal phantom sensation in transradial amputee patients

Authors: *S. SHOKUR[1,3], F. IBERITE[3], F. MOROSATO[4], S. GALLO[2], E. GRUPPIONI[5], S. MICERA[6,3];
[1]EPFL, EPFL, Genève, Switzerland; [2]Lab. of Cognitive Neuroscience, Brain Mind Institute, Fac. of Life Sci., EPFL, Lausanne, Switzerland; [3]Scuola Superiore Sant'Anna, Pisa, Italy; [4]INAIL, Budrio, Italy; [5]INAIL, Budio, Italy; [6]Ecole Polytechnique Federale De Lausanne, Swiss Federal Inst. of Technol., Lausanne, Switzerland

Abstract: A majority of amputee subjects experience some sort of phantom sensation. Non-painful somatic phantom sensation manifests in tingling, itching, or pressure sensation in the missing limb. The so-called Phantom Hand Map (PHM) describes specific spots on the residual arm that elicit a projected tactile sensation on the phantom limb when stimulated [1]. Several studies have proposed to provide tactile feedback via the PHM [2]. Here we report the presence of *projected thermal sensations* elicited by the localized thermal stimulation of specific spots on the residual arm. We term this map the Phantom Thermal Map (PTM) and exploit it to convey thermal feedback. Seventeen unilateral transradial amputee patients were included in the study. The tests started with the investigation of projected tactile sensations. The thermal tests were done via a custom thermode called the MetaTouch (Metaphysiks, Lausanne), which allowed

precise thermal stimulation in a non-painful thermal range (20-40°C). We found the presence of PHM in 16 out of 17 participants. Among these individuals, we observed evidence of a PTM in 12 of them. The PTM manifested in several points (up to 10 in some participants) with clear somatotopic and thermal description: e.g., 'I feel you are cooling down my left index finger.' The subjects could discriminate between three thermal modalities (baseline skin, 5°C above or below skin temperature). A control experiment using a chemesthetic agent (menthol cream) showed that the PTM was present even when tactile feedback was not provided. We next tested whether we could convey the sensation of the contact with different materials (e.g., copper, glass, plexiglass). Here, the metatouch placed on the PTM reproduced the signature skin thermal drop observed during the contact with these materials at room temperature. In a test comparing the MetaTouch-mediated sensation on the PTM and the sensation produced by touching physical objects with the intact arm, 5 out 6 participants reported that the stimuli matched in terms of position and thermal sensation. Our study opens the perspective for thermal restoration in amputee patients, with the possibility to create an intuitive and phenomenologically close to natural sensation while using a non-invasive approach.

1. Björkman A, Wijk U, Antfolk C, Björkman-Burtscher I, Rosén B. Sensory qualities of the phantom hand map in the residual forearm of amputees. J Rehabil Med. 2016;48(4):365-70.
2. Schofield JS, Evans KR, Carey JP, Hebert JS. Applications of sensory feedback in motorized upper extremity prosthesis: A review. Expert Rev Med Devices. 2014;11(5):499-511.

Disclosures: **S. Shokur:** None. **F. Iberite:** None. **F. Morosato:** None. **S. Gallo:** None. **E. Gruppioni:** None. **S. Micera:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); SensArs.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.06

Title: WITHDRAWN

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.07

Topic: D.03. Somatosensation – Touch

Support: Funding: NIH NINDS 1R01NS124222 - 01

Title: Selectivity of SiC Ultramicroelectrodes in Recording Cutaneous Afferent Signals

Authors: *M. A. GONZÁLEZ-GONZÁLEZ[1], A. GHAZAVI[2], S. F. COGAN[3], M. I. ROMERO-ORTEGA[1];
[1]Biomed. Engin., Univ. of Houston, Houston, TX; [3]Bioengineering, [2]The Univ. of Texas at Dallas, Richardson, TX

Abstract: Selectivity of SiC Ultramicroeletrodes in Recording Cutaneous Afferent Signals.M.A. GONZALEZ-GONZALEZ [1], A. GAZHAVI [2], S.F. COGAN [1], M.I. ROMERO-ORTEGA [21]Biomedical Engineering, University of Houston, Houston TX; [2]Bioengineering, The University of Texas at Dallas, Richardson TX. Limb amputation affects over 2 million people in the US and this number is estimated to increase 185,000 per year for upper extremity loss. While limb prosthesis has evolved from simple hooks to robotic hands with human-like degrees of freedom, giving subjects naturalistic control and 'feeling' remains a formidable challenge as it requires connecting the robotic prosthesis to the nervous system of the user. Several strategies have been proposed to accomplish this including: targeted muscular reinnervation (TMR), muscle and neural interfacing. The former can be accomplished with extraneural cuff of FLAT electrodes, indwelling LIFE, TIME electrodes, or with regenerative sieve or REMI electrodes. A common limitation to intraneural interfaces and regenerative interfaces is the relatively stiff and large size of the electrodes ranging from 2.2×10^4 μm^2 to 4×10^6 μm^2 array size and 200-600 μm electrode pitch, which if implanted in the nerve leads to insertion trauma and exacerbated external body response. We previously reported the design and fabrication of sixteen-channels ultramicroelectrode arrays (UMEAS) based on amorphous silicon carbide (a-SiC) intended for intraneural recording. Our design has eight shanks with two electrodes each, cross-sectional dimensions of 23 μm by 10 μm, and ultramicroelectrode contacts with a GSA of 200 μm^2 (8×25 μm^2). Here we report the use of the UMEA as intraneural interfaces. The electrodes were implanted in the adult rat sciatic nerve and mechanoceptive cutaneous signals evoked by Von Frey filaments (n=3-7 measurements per filament). The evoked neural signals were recorded acutely using a Plexon Inc system at 40kHz sampling rate. Individual action potentials were consistently detected, and specific waveforms from light touch and deep pressure evoked sensation, identified. ANOVA was used as statistical analysis to evaluate the effect of frequency, amplitude, and latency of the signals as a function of light touch and deep pressure. The results demonstrate that small and flexible UMEA microelectrodes can be used reliably to map of different sensory modalities from the peripheral nerves, and thus offer viable alternative as peripheral neural interfaces to record movement intent and to convey naturalistic sensations in amputees. Funding: NIH NINDS 1R01NS124222 - 01

Disclosures: M.A. González-González: None. **A. Ghazavi:** None. **S.F. Cogan:** None. **M.I. Romero-Ortega:** 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.; Funding: NIH NINDS 1R01NS124222 - 01.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.08

Topic: D.03. Somatosensation – Touch

Support: NINDS R01NS086859
NIH T32HL007909

Title: A thermometer circuit for hot temperature adjusts Drosophila behavior to persistent heat

Authors: M. H. ALPERT, A. PARA, H. GIL, ***M. GALLIO**;
Northwestern Univ., Evanston, IL

Abstract: Small poikilotherms such as the fruit fly *Drosophila* depend on absolute temperature measurements to identify external conditions that are above (hot) or below (cold) their preferred range and to react accordingly. Hot and cold temperatures have a different impact on fly activity and sleep, but the circuits and mechanisms that adjust behavior to specific thermal conditions are not well understood. Here, we use patch-clamp electrophysiology to show that a specific class of internal thermosensory neurons function as a thermometer active in the hot range. iTRNs exhibit sustained firing rates that scale with absolute temperature -but only for temperatures above the fly's preferred ~25°C (i.e. "hot" temperature). We identify iTRN axons in the fly brain connectome and demonstrate that they target a single class of circadian neurons. These circadian cells receive excitatory drive from iTRNs and respond robustly to hot stimuli, but their responses do not exclusively rely on iTRNs. Instead, they receive independent drive from thermosensory neurons of the fly antenna via a new class of second-order projection neurons. Finally, we show that silencing iTRN targets blocks the restructuring of daytime "siesta" sleep which normally occurs in response to persistent heat. Our previous work described a distinct thermometer circuit for cold temperature. Together, the results demonstrate that the fly nervous system separately encodes and relays absolute hot and cold temperature information, show how patterns of sleep and activity can be adapted to specific temperature conditions, and illustrate how persistent drive from sensory pathways can impact behavior on extended temporal scales

Disclosures: M.H. Alpert: None. **A. Para:** None. **H. Gil:** None. **M. Gallio:** None.

Poster

049. Encoding of Thermal Information in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 049.09

Topic: D.03. Somatosensation – Touch

Support: NIH/NINDS R01NS094659
NIH/NINDS R01NS069679
NSF Graduate Student Research Fellowship

Title: Learning enhances encoding of time and temporal surprise in primary sensory cortex

Authors: R. J. RABINOVICH[1], D. D. KATO[1], ***R. M. BRUNO**[2];
[1]Neurosci., Columbia Univ., New York, NY; [2]Physiology, Anat. & Genet., Univ. of Oxford, Oxford, United Kingdom

Abstract: Primary sensory cortex has long been believed to play a straightforward role in the initial processing of sensory information. Yet, the superficial layers of cortex overall are sparsely active, even during sensory stimulation; moreover, cortical activity is influenced by other modalities, task context, reward, and behavioral state. Using two-photon microscopy of calcium activity, we longitudinally tracked the activity of layer 2/3 neurons in mouse primary somatosensory cortex across learning. Our study demonstrates that reinforcement learning dramatically alters neural representations in superficial layers as mice gain task-relevant experience. Learning an object detection task recruits previously unresponsive neurons, enlarging the neuronal population sensitive to touch and behavioral choice. In contrast, cortical responses decrease upon repeated exposure to unrewarded stimuli. Most notably, training improved population encoding of the passage of time, and unexpected deviations in trial timing elicited even stronger responses than touch did. Thus, the superficial layers of sensory cortex exhibit a high degree of learning-dependent plasticity and are strongly modulated by non-sensory but behaviorally-relevant features, such as timing and surprise. Our results raise the possibility that primary sensory cortex may model sequences of sensory and behavioral events.

Disclosures: R.J. Rabinovich: None. **D.D. Kato:** None. **R.M. Bruno:** None.

Poster

050. Touch Encoding in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 050.01

Topic: D.03. Somatosensation – Touch

Support: ARC Grant DP200100630

Title: Perceived tactile intensity at a fixed primary afferent spike rate varies with the temporal pattern of spikes

Authors: *D. SHARMA[1,2], K. K. W. NG[3], I. BIRZNIEKS[1,2], R. M. VICKERY[1,2];
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Abstract: The perceived intensity of a vibrotactile stimulus is thought to depend on single neuron firing rates (rate coding) and the number of active afferents (population coding). Unaddressed until now is whether the temporal relation of individual spikes also conveys information about tactile intensity. We used cutaneous electro-tactile stimulation to investigate how the temporal structure of a fixed number of spikes in a 1-second train influenced the perception of intensity. Four mean spike rates spanning flutter and vibratory hum range (36 Hz, 60 Hz; 120 Hz, 180 Hz) were tested, with spikes grouped into regular, or bursts of 2-6 spikes

spaced 3 ms apart. To link a putative neural code to perception, perceived intensity was assessed in sixteen human participants (aged 20 - 45, 4 females) using the psychophysical paradigm of magnitude estimation. Compound sensory nerve action potentials were recorded to assess any stimulus variation in afferent recruitment. The temporal structuring of a fixed number of spikes into periodic bursts of multiple spikes altered perceived intensity as a function of burst spike count. The largest increase was seen at 36 Hz, where the bursts of 6 spikes were rated 2.1x stronger than the regularly spaced spikes (95% CI: 1.9 - 2.3, n = 16). The true increase is likely larger as temporal structuring of spikes into bursts had some negative effect on afferent recruitment. We conclude that the perceived intensity can be modulated by changing temporal features of afferent discharge without changing single neuron firing rates or recruitment of additional afferents. The finding suggests a new strategy for communicating information about contact pressure events to amputees using neural interfaces in afferent nerves. The range of intensity, conveying information about varying contact forces, could simply be achieved by varying timing of spikes in activated non-specific primary afferents using electric pulses of fixed width and amplitude, without requiring alterations in stimulation current that risks eliciting pain or paresthesia, or base mean pulse frequency that simultaneously alters perceived frequency.

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Poster

050. Touch Encoding in the Somatosensory System

Location: SDCC Halls B-H

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Program #/Poster #: 050.02

Topic: D.03. Somatosensation – Touch

Support: CIHR Foundation Grant 167276
CIHR (CGS D)
The Hospital for Sick Children (Restracomp)

Title: Encoding of vibrotactile stimuli by mechanoreceptors in rodent glabrous skin

Authors: *L. MEDLOCK[1,2], D. AL-BASHA[1,2], C. DEDEK[1,2], S. RATTÉ[2], S. A. PRESCOTT[2,1];
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Abstract: Our understanding of somatosensory processing in rodents comes primarily from the whisker system. The coding properties of low-threshold mechanoreceptors (LTMRs) in rodent glabrous skin have not been quantitatively assessed to nearly the same degree as in other species. Classic tactile studies in cats and primates have established that an LTMR's response to touch is influenced by stimulus intensity (rate coding) and frequency (temporal coding). Rate and temporal coding are influenced by the probability of a spike occurring on each cycle (i.e. spike reliability) and the timing of spikes relative to the stimulus cycle (i.e. spike precision), respectively. Our goal in this study was to examine the reliability and precision of LTMR

responses to tactile stimulation because changes in these cellular-level properties can impact spike synchrony and information transmission at the network level. Through *in vivo* extracellular recordings in rodents, we measured the reliability and precision of LTMR responses to sinusoidal vibrotactile stimuli between 2 and 300 Hz. We first subclassified LTMRs as rapid adapting (RA) or slow adapting (SA) based on their response to sustained pressure. Heterogeneity in the response of RAs to vibration further revealed a spectrum of frequency preferences across this LTMR subtype. Furthermore, although stimulus frequency differentially affected spike reliability across different LTMRs, increasing frequency universally increased spike precision. We confirmed that poor precision at lower frequencies was due to co-variation of the sinusoidal stimulus waveform with frequency; abrupt-onset pulses delivered at low frequency evoked precisely timed spikes. Finally, to explore the mechanisms supporting the unique tuning properties of rodent LTMRs, we fit generalized linear models to experimental data. Differences in the fitted model parameters demonstrate that a narrow integration time window and shorter refractory period give RAs the ability to respond reliably and precisely to higher frequency stimulation. These results strengthen our understanding of somatosensory processing in rodents, and the resulting models allow us to dissect the coding properties of different LTMR subtypes.

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Poster

050. Touch Encoding in the Somatosensory System

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Hock E. Tan and Lisa Yang Center for Autism Research at Harvard University
Edward R. and Anne G. Lefler Center for Neurodegenerative Disorders

Title: The encoding of touch by somatotopically aligned dorsal column subdivisions

Authors: *J. TURECEK, B. P. LEHNERT, D. D. GINTY;
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Abstract: The somatosensory system decodes a range of tactile stimuli to generate a coherent sense of touch. Discriminative touch of the body depends on signals conveyed from peripheral mechanoreceptors to the brain via the spinal cord dorsal column and its brainstem target the dorsal column nuclei (DCN). Models of somatosensation emphasize that fast-conducting low-threshold mechanoreceptors (LTMRs) innervating the skin drive the DCN. However, post-synaptic dorsal column neurons (PSDCs) within the spinal cord dorsal horn also collect

mechanoreceptor signals and form a second major input to the DCN. The significance of PSDCs and their contributions to the coding of touch have remained unclear since their discovery. Here, we show that direct LTMR inputs to the DCN convey vibrotactile stimuli with high temporal precision, whereas PSDCs primarily encode touch onset and the intensity of sustained contact into the high force range. LTMR and PSDC signals topographically re-align in the DCN to preserve precise spatial detail. Different DCN neuron subtypes have specialized responses that are generated by unique combinations of LTMR and PSDC inputs. Thus, signals encoding different tactile features are conveyed by subdivisions of the dorsal column pathway and differentially converge in the DCN to generate unique ascending signals suited to distinct sensory processing streams.

Disclosures: **J. Turecek:** None. **B.P. Lehnert:** None. **D.D. Ginty:** None.

Poster

050. Touch Encoding in the Somatosensory System

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Program #/Poster #: 050.04

Topic: D.03. Somatosensation – Touch

Support: NS077986

Title: Investigating Brainstem Encoding of Object Location within Peri-head Distance

Authors: *W. XIAO[1,3], K. SEVERSON[1], V. PREVOSTO[1], J. LU[1], S. CHOI[1], F. WANG[2,1]; [1]McGovern Inst. for Brain Res., [2]Dept. of Brain and Cognitive Sci., MIT, Cambridge, MA; [3]Neurobio., Duke Univ., Durham, NC

Abstract: The rodent whisker system is a tractable and accessible model to study neural representations of object location within an organism's peri-head space. Rodents show high performance in whisker-based object localization tasks in the laboratory. Neurons in the primary somatosensory cortex (S1) are tuned to specific object distances within peri-head space detected by the whiskers, yet it is unknown whether this representation of peri-head distance emerges at an earlier stage. The brainstem principal trigeminal nucleus (PrV) is the first processing stage where information across different types of mechanoreceptive afferents and multi-whisker inputs are integrated. Furthermore, PrV gates whisker-mediated tactile input to S1. Here we performed in vivo electrophysiological recordings of PrV neuronal responses to a wall stimulus that passes with varying distances from the face in awake, behaving mice. In general, the firing rates of PrV neurons with single-whisker and multi-whisker receptive fields are inversely correlated with wall distance. We performed analyses relating PrV neural activity with whisker mechanical and kinematic variables as the whiskers interacted with the passing wall. Behaviorally, mice showed an active component of whisker retraction in response to the passing wall with a distance-dependent relationship, suggesting sensation of wall distance modulates the activity of whisker retraction circuits. These results have implications for understanding how brainstem

sensorimotor circuits transform incoming sensory input into meaningful representations that guide behavior.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: Whitehall Foundation

Title: Two complementary codes in the superior colliculus differentiate external from self-generated tactile features

Authors: *S. CHINTA, S. R. PLUTA;
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Abstract: Spatial awareness is often the expected result of our actions, while at other times it emerges from unexpected changes in the scene. To navigate the landscape, animals must differentiate between the spatial cues generated by their actions from signals that originate externally. To reveal the neural basis of this ability, we examined the midbrain superior colliculus (SC), which contains multiple egocentric maps of sensorimotor space. By simulating whisker-guided navigation through a dynamic landscape, we discovered a transient neural response that selectively emerged for unexpected, externally generated tactile motion. This transient response only emerged when external motion engaged different whiskers, arguing that sensorimotor expectations are specific to a somatotopic location. When external movement engaged the same whiskers, neurons shifted their spike timing to match self-generated tactile features. Thus, spatial representations based on the timing of self-generated cues may surpass the acuity of the whisker array. In conclusion, the SC contains complementary rate and temporal codes to differentiate external from self-generated tactile features.

Disclosures: **S. Chinta:** None. **S.R. Pluta:** None.

Poster

050. Touch Encoding in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: NSF Grant - Award #2117997

Title: Neural encoding of proprioception of the limbs in the mouse primary somatosensory and motor cortices

Authors: *M. LIPTON, M. DADARLAT;
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Abstract: Rodents rely on proprioceptive information from the periphery to guide and coordinate precise forelimb and hindlimb movements, a process called sensorimotor integration. The mouse primary somatosensory (S1) and primary motor (M1) cortices are known to be necessary for adapting motor commands to new sensory environments, and recent work suggests neurons in the forelimb area of S1 encode proprioceptive information about contralateral forelimb movement. However, we do not know how proprioception of all four limbs is represented across multiple brain regions. To address this question and to isolate pure somatosensory responses (proprioception and touch) from motor commands that would be present in awake animals, we recorded neural responses to passive movement of ipsilateral and contralateral limbs in eight mice under anesthesia. Using stereotaxic coordinates to locate S1 and M1 forelimb and hindlimb areas, we performed unilateral two-photon imaging over these two regions simultaneously in mice expressing GCaMP6s, a highly sensitive fluorescent indicator of neuronal activity. A brushing motion was used to provide cutaneous and proprioceptive stimulation to each limb (blocks of five trials per limb were repeated across three cycles). Altogether, we recorded the activity of 12,895 neurons, of which 2,053 neurons (16%) were significantly modulated by passive movement of at least one limb ($p < 0.02$, Wilcoxon rank-sum test on single trial responses vs. baseline). Of significantly modulated neurons, 48% responded to movement of the contralateral hindlimb, 15% to the ipsilateral hindlimb, 30% to the contralateral forelimb, and 7% to the ipsilateral forelimb. A subset of neurons (9%) was significantly modulated by more than one type of limb movement, most often ipsilateral and contralateral hindlimb movement. In terms of response amplitude, neurons that were significantly modulated by contralateral movements had larger responses than those modulated by ipsilateral movements (hindlimb: $dF = 0.90 \pm 0.01$ SEM contralateral vs. $dF = 0.79 \pm 0.01$ SEM ipsilateral, $p = 5.1 \times 10^{-39}$; forelimb: $dF = 0.78 \pm 0.01$ SEM contralateral vs. $dF = 0.74 \pm 0.01$ SEM ipsilateral, $p = 0.012$). In summary, we found evidence of proprioceptive signals related to both ipsilateral and contralateral forelimbs and hindlimbs across primary somatosensory and motor cortices of the mouse. The distributed nature of these responses, across cortical regions and limbs, could be an indication of how proprioception guides the formation of motor commands within the mouse cortex.

Disclosures: **M. Lipton:** None. **M. Dadarlat:** None.

Poster

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Topic: D.03. Somatosensation – Touch

Support: 2020M3A9E410384313
2018R1A5A2025964

Title: Characterizing neural selectivity in sensory systems using a data-driven interpretable feature finding method

Authors: *S.-Y. PARK[1], Y. KIM[2], S. KIM[2], C.-E. KIM[1];
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Abstract: Determining what a neuron responds to is a common approach to understand how cortical neurons process information, i.e., the first step to understand the computational mechanism of information processing. Although there has been a commonly used method to investigate neural selectivity, mischaracterization often occurs due to the convention of using the researcher-predefined features. Despite the importance of this problem, the reason that causes mischaracterization has not been investigated systematically and the problem remains unreserved. Here, we introduce a possible scenario of mischaracterizing neural selectivity with illustrative examples and suggest a data-driven interpretable feature finding (DIFF) method for reliably finding all interpretable features from noisy neural data, solving the mischaracterization problem.We propose the DIFF method that can find all feasible features based on given stimuli by fitting neural response vector in multidimensional feature space. It suggests possible features with a normalized value, which reflects the p-value of linear regression analysis. To validate the superiority of our method, we applied it to the simulated data with a wide range of conditions. Furthermore, to investigate the usefulness of our method, we applied it to an experimental data, in vivo two-photon Ca2+ imaging data of primary somatosensory cortex neurons while providing peripheral stimulation.As a result of applying our method to the simulated data, we confirmed that our method outperforms the MLR-based benchmark method in identifying ground-truth features throughout a wide range of conditions. In the experimental data, we discovered that some researcher-predefined features were also derived by our data-driven method, providing credibility to our method, while other previously unknown features were derived, thereby providing insights for novel features. Among known-features, brush texture, forcpes texture, and noxiousness features are in high rank, while brush stroke, forceps press + forceps pinch, forceps stroke + forceps pinch features are suggested as high relevant features.In this study, we develop a data-driven method for characterizing neural selectivity. We demonstrate that our method can be used for finding all relevant features with interpretability. These results show that a data-driven determination of candidate features can be the crux when characterizing neural selectivity.

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Poster

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Topic: D.03. Somatosensation – Touch

Support: NIH Grant RF1NS113278-01
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Title: Distinguish hemodynamic responses at the white matter tract from the laminar-specific gray matter fMRI signal with high spatiotemporal fMRI

Authors: *S. CHOI[1], B. B. BISWAL[2], B. R. ROSEN[1], X. YU[1];
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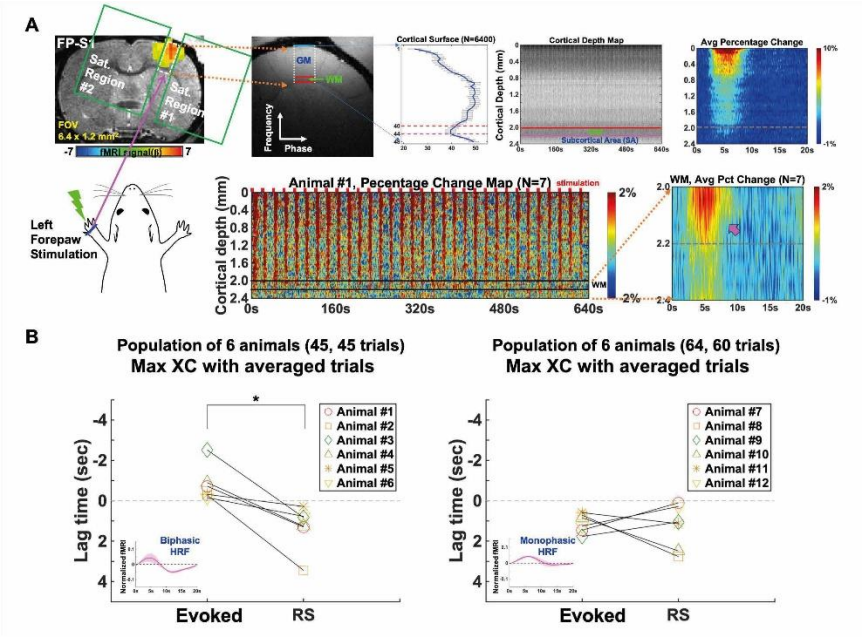
Abstract: Recently, neuronal activity-coupled laminar dynamic features have been investigated using BOLD-fMRI in gray matter (GM). In particular, using ultra high-field MRI, laminar fMRI studies have demonstrated feed-forward/feed-back and top-down/bottom-up neuronal circuitry with sufficient sensitivity and specificity given the laminar vascular dynamic changes. In contrast to GM fMRI, white matter (WM) fMRI signals have not been well-understood due to the low vascular density and extravascular effect from GM adjacent to WM resulting in a low signal-to-noise ratio. Due to the limited spatiotemporal resolution of existing fMRI methods, distinguishing fMRI responses of WM from adjacent GM remains a challenge. Previously, a gradient echo sequence-based line-scanning fMRI (LS) method has been developed to better elucidate laminar fMRI signals with unprecedented high spatiotemporal resolution (50 μm, 50 ms), presenting distinct fMRI onset times within the cortical layers of GM in rodents. Here, we extended the previous LS method to reliably identify WM-fMRI signals (Fig. A) and investigate brain-state dependent temporal dynamic features between the WM and GM in anesthetized rats. First, we calculated the WM-specific cross-correlation lag time to the laminar fMRI signal across the cortical layers of GM in the primary forepaw somatosensory cortex. Interestingly, distinct WM hemodynamic response function (HRF) forms were identified across animals, presenting a biphasic HRF with earlier lag time but a monophasic HRF with later lag time in the evoked fMRI study (Fig. B). In contrast, resting-state WM hemodynamic responses only showed delayed lag times across animals (Fig. B). These results demonstrated two distinct HRF forms indicating brain state-dependent dynamic blood transit times between GM and WM vasculature. Also, altered cerebral blood volume and flow dynamics in the WM will need to be further specified to explain the distinct temporal dynamics of evoked fMRI signals. Furthermore, the WM-LS mapping may also shed a light on understanding the progression of WM-related diseases.



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Poster

050. Touch Encoding in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 050.09

Topic: D.03. Somatosensation – Touch

Title: Temporal codes in somatosensory cortex

Authors: *T. CALLIER[1], M. A. HARVEY[2], S. J. BENSMAIA[1];
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Abstract: The contribution of millisecond-scale spike timing to information transmission in the central nervous system (CNS) remains controversial. Though neuronal responses have been shown to exhibit high-precision spike timing, and this timing has been demonstrated to carry stimulus information, its relevance for perception has been difficult to establish. To investigate temporal coding in the CNS, we leverage the well documented millisecond-precision spike timing along the somatosensory neuraxis, including somatosensory cortex (SC). Indeed, vibrations delivered to the skin or textured surfaces scanned across the skin elicit spiking patterns in SC that are repeatable at a millisecond time scale and are highly informative about vibration frequency or texture identity. The challenge in establishing that spike timing shapes the evoked percept is that the heterogeneous rate responses in SC are also highly informative and could in principle encode the relevant stimulus information. Across the neuraxis, responses are phase-

locked to a sinusoidal vibration and thus carry information about frequency in their interspike interval distribution. This temporal patterning is critical to encode frequency in the somatosensory nerves, but its role in SC is still controversial because rate is also highly informative about frequency. To disentangle the roles of rate and timing in frequency coding, we trained monkeys to discriminate the frequency of vibrations delivered to the skin while we recorded SC responses. We also varied stimulus amplitude so that SC firing rates - and the perceived stimulus intensity - was decorrelated with the behaviorally relevant stimulus parameter - frequency. We found, under these experimental conditions, that a rate code could not account for the ability to discriminate frequency given the amplitude confound. However, the temporal pattern of responses - reflected in the interstimulus interval distribution - could. Next, we trained monkeys to discriminate the frequency of intracortical microstimulation (ICMS) of SC, with concomitant and uninformative variations in amplitude. We found that the animals could do the task, demonstrating that frequency has an impact on the evoked percept that is distinct from amplitude. We demonstrate that the phase-locking of the neural responses to the pulses mediates the impact of frequency on perception. We conclude from these experiments that spike timing in SC plays a key role in shaping tactile perception, implying that neural mechanisms downstream of SC can extract information from temporal spiking patterns.

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Poster

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NIH K01 NS114191-01A1
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Title: The role of two inhibitory interneuron subtypes on tactile feature representation within the somatosensory cortex

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Abstract: The hands and paws are unique organs that allow some mammals to both perceive and manipulate objects in the world around them. This behavior requires tactile features arriving at the sensory organs to be represented within neurons of somatotopically-aligned regions of the primary somatosensory cortex (SI). Tactile vibration frequency is represented in SI via elevated neural firing rates coinciding with the arrival of tactile stimuli containing a particular range of the feature space (i.e., frequency tuning). Previous studies assessed how tactile vibrations are

encoded within excitatory and inhibitory neurons of the forepaw region in mouse SI and found that inhibitory GABAergic neurons have broad frequency tunings, when compared to excitatory neurons, and have strong noise-correlations with other inhibitory and excitatory neurons that are also frequency tuned. However, inhibitory neurons in the cortex have vast heterogeneity and it remains unknown whether feature selectivity is common to all or is unique to some genetic subtypes. Here we present a combination of *in vivo* two-photon calcium imaging experiments with controlled forepaw vibro-tactile stimulation in awake mice to determine the vibro-tactile frequency tuning of two prominent subtypes: the parvalbumin (PV) and somatostatin (SOM) expressing interneurons. We applied a dual-labeling approach using PV-Cre-tdTomato or SOM-Cre-tdTomato mice in combination with a virally expressed calcium indicator (AAV1-hSyn-gCaMP7c) to track frequency tuned responses across the SI neuronal population. Our preliminary findings suggest that subsets of PV inhibitory interneurons showed selective tuning for vibration frequency. The stimulus-evoked activity from PV neurons was also correlated with similar activity in some of the neighboring excitatory and inhibitory neurons. On-going experiments will further examine the vibrotactile tuning in SOM neurons and compare the tuning properties and functional correlations across different cell types. The identification of these feature-specific microcircuits will improve our understanding of how tactile features are encoded and selectively processed in the brain.

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Poster

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Chair Alain Rossier - IRP

Title: Vip interneuron selectivity dynamics in the mouse primary somatosensory cortex during sensory discrimination learning

Authors: *T. BAWA, R. CHÉREAU, A. HOLTMAAT;
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Abstract: During sensory learning, cortical networks are remodelled to retrieve and encode new information. During texture discrimination, many pyramidal neurons (PYRs) in layer 2/3 of the mouse primary somatosensory cortex (S1) adapt their stimulus selectivity. A subpopulation even reshape their selectivity contingent on the texture's associated reward value during reversal learning. Vasoactive intestinal peptide-expressing (VIP) interneuron (IN)-mediated disinhibition of PYRs has previously been identified as an important circuit mechanism for reward-mediated

gain control of PYRs during sensory discrimination tasks. This class of INs was also found to gate cortical plasticity and to be activated during tactile behaviour. However, it is unclear how VIP INs activity and stimulus selectivity evolves during the learning process. To investigate this, we used *in vivo* 2PLSM calcium imaging to longitudinally monitor responses of VIP INs in S1 during a reward-based whisker-mediated texture discrimination-learning task. We found that VIP INs were activated or suppressed upon sensory touch, and a large proportion of them exhibited a significant change in their stimulus selectivity with learning. Activity of a large fraction of the VIP INs reflected the behavioural outcome associated with the stimulus. Interestingly, VIP IN activity was modulated by trial history, specifically during the initial learning phase and then again during reversal learning, but not during naïve or expert phases. This modulation was specific to textures that resulted in a reward but only if they followed on trials in which no reward was obtained. The effect was not directly caused by a difference in licking behaviour. Together, our work suggests that VIP INs in S1 convey context-related information to the cortex, which may include a transitory teaching signal that forecasts the completion of the learning phase. These dynamics may contribute to sensory perception-based shaping of PYR response selectivity and their encoding of texture information.

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Poster

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Program #/Poster #: 050.12

Topic: D.03. Somatosensation – Touch

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JSPS Grant 20K15926

Title: Visual deprivation hinders somatosensory processing during rhythmic movement.

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Abstract: Organisms integrate and predict periodical rhythms based on information from multiple sensory systems, such as the auditory, visual, somatosensory information. In this study, we investigated how temporal processing of somatosensory perception in rhythmic phenomena is affected by inputs from different sensory systems. Specifically, we investigated 1) how somatosensation produced by periodic motion is processed in the primary somatosensory cortex (S1) and 2) how the processing of this somatosensation in S1 is altered when visual input is deprived. An electrode array was implanted into the S1 of the right hemisphere, covering subregions for forelimbs and hindlimbs. The tips of the electrodes were lowered to cortical layers

2/3. After recovery from the surgery, the animal was fixed on a circular treadmill. While the rat walked on the treadmill, LFPs were continuously recorded. The recorded LFPs were aligned to the event when the forelimb or hindlimb touched the surface of the floor, defined as the forelimb or hindlimb stimulus onset, respectively. The onset-triggered LFP averages revealed that in both forelimb and hindlimb stimulus onsets, negative field potentials were observed 200 ms before the stimuli. The same behavioral task was performed under complete darkness (0 lux). The onset-triggered LFP averages in dark condition showed the same negative potential; however, the waveform relatively peaked about 100 ms before the touch onset. Since there were no differences in speed of locomotion between the two environments, this lag in the appearance of negative potentials implies that visual information affects the temporal processing of somatosensory information. To test the effect of visual deprivation on somatosensory perception, we placed two different types of materials on the floor of the treadmill, and LFPs were recorded from rats walking on the two different materials. Then, we sought to predict the floor material from the recorded LFPs using a convolutional neural network (CNN). The results showed that in bright environments, the CNN had an average classification accuracy of about 75%, significantly greater than the 50% chance level, whereas in dark environments, the CNN was unable to predict the floor material from the LFPs. These results indicate that deprivation of visual information causes lag in processing of somatosensory information in S1 during rhythmic limb movements. Furthermore, a CNN-based classification indicates that the information of the floor material in the LFP is reduced in dark environments. Further investigation will reveal the causal relationship between the temporal lag in information processing and perception.

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Poster

050. Touch Encoding in the Somatosensory System

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Topic: D.03. Somatosensation – Touch

Support: KAKENHI (18H0522 and 17H00742)

Title: Distinct cortical connectivity profiles during tactile temporal order judgment

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Abstract: If we are asked to judge the temporal order of two successive tactile stimuli delivered one to each hand, we often make inverted judgments when we cross our hands. This

phenomenon, termed the crossing effect, indicates that our brain utilizes not only the somatosensory information but the posture-related spatial information in the perception of touch and its temporal order. In the present study, we examined the time-frequency profile of the brain network associated with the crossing of the hands and making an inverted judgment. To this end, we recorded Magnetoencephalographic data while participants were performing the tactile temporal order judgment (TOJ) task in crossed and uncrossed postures. We extracted the task-related time-frequency profile of cortical communication across the brain regions previously shown to be involved in the task. We discovered that the brain network was mainly channeled to a low-frequency band (5~10 Hz) when the hands were uncrossed; however, it also recruited a higher frequency band (12~18 Hz) when the hands were crossed. Moreover, a separate examination of the network of the participants with frequent inverted judgments (reversers) and those with few inverted judgments (nonreversers) revealed that the nonreversers mainly relied on their higher frequency band, whereas reversers utilized both. Also, within reversers, when they succeeded at making a correct judgment, they had stronger cortical interactions in the higher band compared to when they made an inverted judgment. Last but not least, we observed that the network in the uncrossed condition was lateralized to the hemisphere contralateral to the hand being stimulated first (i.e., the left hemisphere when the order of stimulation was from right to left hand, and the right hemisphere when the order was from left to right hand). However, this was not the case when the hands were crossed (there was a lesser degree of lateralization slightly to the left hemisphere). Overall, our results show that there are two distinct frequency modes of interactions, one in the high-theta band (5~10 Hz, peaked at 6Hz) and another in the low-beta band (12~18 Hz, peaked at 16 Hz) during the tactile TOJ task. A lateralized network channeled to the high-theta band is sufficient to judge the order as long as when the hands are uncrossed. The low-beta mode is crucial only when the hands are crossed, and its stronger recruitment leads to superior performance.

Disclosures: **A. Moharramipour:** None. **T. Takahashi:** None. **S. Kitazawa:** None.

Poster

050. Touch Encoding in the Somatosensory System

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 050.14

Topic: D.03. Somatosensation – Touch

Support: DARPA HAPTIX
NIH Grant T32EB004314

Title: Tactile percept formation and integration for object feature encoding with electrical stimulation

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Abstract: Touch is a sense that is often taken for granted, but it is critical to use and manipulate objects, feel ownership of one's body, and make meaningful connections with others. Neural interfaces help restore touch to upper extremity amputees, changing how they grab objects and interact with others around them. Flat interface nerve electrodes (FINEs and cFINEs) have been successfully implanted in amputees, providing these participants with somatosensory feedback that feels as though it is their own hand and arm. Prior work using FINEs has revealed the relationship between stimulation paradigms and sensation at a single point of perception, such as the index fingertip, at a time. However, touch requires the integration of multiple sensations across the finger and hand for applications such as object recognition (stereognosis) and improved manual dexterity. The purpose of this study is therefore to understand how electrical stimulation of multiple perception points, or percepts, is integrated and perceived by the brain. Preliminary work focused on using computational modelling to understand the degree of overlap between axon populations responsible for different percepts. To understand how changing timing between multiple percepts alters the final sensation, stimulus pulses were grouped into two pulse bursts and the timing between these two pulses, or interpulse interval, was varied. This burst stimulation pattern was repeated with nine electrode contacts from three different human participants to inform timing when interleaving multiple percepts, as well as anodic versus cathodic phase ratio when using charge balanced stimulation. Preliminary results showed varying the interpulse interval impacted both quality and intensity of sensation, thereby informing interleaving timing when using multi-percept stimulation. Exploratory multi-percept trials have also been conducted with human participants, with initial results revealing trends in location and intensity integration that depend on percept size, location, orientation, and intensity during single percept stimulation. Understanding these integration patterns is vital for understanding how multi-percept stimulation could inform object feature extraction.

Disclosures: L. Roldan: None. **E. Graczyk:** None. **D.J. Tyler:** None.

Poster

050. Touch Encoding in the Somatosensory System

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Support: DARPA HAPTIX N66001-15-C-4014
NIH T32AR007505
VA RR&D Merit ReviewIO1 RX00133401
VA RR&D Center Grant C3819

Title: Pre-perceptual usage of peripheral nerve stimulation occurs at multiple levels of processing

Authors: *N. CHOWDHURY, D. TYLER;
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Abstract: Sensorimotor integration is important, if not required, when using our hands. The integration of the tactile and motor systems is disrupted for individuals living with upper limb amputations because their connection to their fingertips is lost. Tactile substitution is currently achieved through vibrotactile feedback on the residual limb or through direct stimulation of the tactile pathways of the nervous system. Vibrotactile feedback is promising in that it is noninvasive, however, multiple vibrotactile sensations representing multiple fingers cause confusion and high cognitive load during grasping. Direct cortical stimulation addresses this through modality and location matched perceptions, however, the time to process and act upon direct cortical feedback significantly exceeded the time to do the same with naturally produced tactile feedback. Skipping the ascending pathway of tactile feedback from the periphery likely skips many parallel structures in the brain stem meant to integrate tactile feedback with signals from the motor system at a sub or pre-perceptual level before the somatosensory cortex is involved. Stimulation of the upper arm peripheral nerves in multiple subjects' residual limbs produced modal and location matched sensory perceptions in their hands. The artificially generated signals will engage the same peripheral tactile pathways to the pre-perceptual and perceptual structures as natural tactile sensation. Our hypothesis is that pre-perceptual structures will process the electrically generated neural activity as it would naturally generated neural activity. Specifically in this study, we studied simple reaction time to evaluate the minimum time to process peripheral nerve stimulation and form a motor plan in comparison to visual and vibrotactile feedback. We also measured the effect of intensity of peripheral nerve stimulation on the speed of processing of tactile feedback. A backmasking experiment eliminated the perception of a weak stimulus to test the hypothesis that peripheral nerve stimulation interacted with the pre-perceptual pathways throughout the brain. We also measured the effect of peripheral nerve stimulation on the time to correct for visual and/or tactile errors when gripping virtual and real objects. We found peripheral nerve stimulation is processed by the brain in a similar time frame as natural tactile feedback and the effect of stimulation intensity on the rate of feedback processing follows the same trend as natural sensory feedback. When used for basic error correction or when perceptually masked, we conclude that peripheral nerve stimulation engages pre-perceptual pathways of the brain.

Disclosures: N. Chowdhury: None. **D. Tyler:** None.

Poster

050. Touch Encoding in the Somatosensory System

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Program #/Poster #: 050.16

Topic: D.03. Somatosensation – Touch

Support: NRF-2018-Global Ph.D. Fellowship Program
NRF-2021M3E5D2A01019542

Title: Somatosensory cortical representations of assimilation effects by vibrotactile stimulation: an fMRI study

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Abstract: The purpose of this study was to investigate neural activity related to the assimilation effect in the perception of vibrotactile stimuli. Previous behavioral studies have shown that when distinguishing the vibrotactile frequency within one finger, distracting stimulation targeting other types of mechanoreceptors on the other finger results in a bias in the perception of frequency. This phenomenon is one of the assimilation effects, and observed under not only in-hand but also between-hand conditions. Accordingly, we assumed that the assimilation effect of vibrotactile stimulation would be related to cortical processing, rather than peripheral processing. To confirm this hypothesis, functional magnetic resonance imaging experiment was conducted to non-invasively observe the brain responses.Thirty subjects (16 females) participated in the experiment and their fMRI images were obtained using a 3T scanner (Siemens Magnetom Prisma, Germany). We employed the behavioral experiment paradigm used in other previous studies. In a Non-Distractor condition (ND), a target frequency stimulus (30Hz) and a comparison frequency stimulus were sequentially given to the target finger at intervals of 1 second. The target finger was the index or middle finger of the left hand. In an Across Finger condition (AF), not only target finger but also another finger within the same hand was stimulated (termed as a distractor finger here). Under the AF control condition, the target frequency was given to the distractor finger simultaneously. Under the AF test condition, a distractor stimulus (200Hz) was given. An Across Hand condition (AH) was the same as the AF condition except that the target and distractor fingers were the index fingers of both hands. All the statistical significance from contrast analysis showed $p < 0.001$ and the extended threshold of activation was $p < 0.05$, which was familywise error corrected. The contrast of stimuli vs baseline analysis revealed significant responses in the S1 and S2 under the AF and AH conditions. Furthermore, a contrast analysis between the test and control showed a significant difference in the activation level of bilateral S2 under both AF and AH conditions. In summary, activations in bilateral S2 were observed by the test-vs-control contrast analysis for both AF and AH conditions. Thus, this result suggests that the S2 may incorporate tactile information from other mechanoreceptors and other somatotopic to support the perception of vibrotactile stimuli. The follow-up studies on functional connectivity between somatosensory cortical regions would clarify individual differences in the assimilation effects.

Disclosures: **J. Kim:** None. **J. Kim:** None. **S. Seo:** None. **S. Kim:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

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Topic: D.05. Auditory & Vestibular Systems

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Title: A network model of complex scene analysis in auditory cortex with multiple inhibitory neuron types

Authors: *J. NOCON[1], H. GRITTON[2], X. HAN[1], K. SEN[1];
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Abstract: Cortical representations underlying complex scene analysis emerge from underlying circuits with a tremendous diversity of cell types. However, cell type-specific contributions to complex scene analysis are not well-understood. Specifically, how are competing dynamic stimuli from different spatial locations represented by cortical circuits and cell types? Recently, we investigated complex scene analysis in mouse ACx using a cocktail party-like paradigm. In these experiments, we presented target sounds in the presence of maskers from different spatial configurations and quantified neural discrimination performance. We found that cortical neurons were spatial configuration-sensitive, with high discrimination performance at specific combinations of target and masker locations ("hotspots"). Further, optogenetically suppressing parvalbumin (PV) neurons in ACx degrades cortical discrimination of dynamic sounds in a cocktail party-like setting via changes in rapid temporal modulations in rate and spike timing over a wide range of time-scales. These results suggest that PV neurons play a critical role in enhancing cortical temporal coding and reducing network noise, thereby improving cortical representations of dynamic stimuli in complex scenes. Here we propose a network model of ACx to explain these recent experimental observations. The model consists of different spatial channels, with excitatory neurons and multiple inhibitory neuron types based on experimental data. Our results suggest that PV neurons mediate "within-channel" inhibition in the cortical network, while a distinct population of inhibitory neurons mediate "cross-channel" surround inhibition. Complex scene analysis is an active sensing process, strongly modulated by behavioral state. Thus, we next extend the model to simulate top-down modulation via other inhibitory neuron populations. Finally, we hypothesize a mapping of the distinct inhibitory neuron populations in the model to distinct inhibitory neuron types in cortex (PV, SOM, and VIP) to generate experimentally testable predictions for cell type-specific responses in passive versus task-engaged conditions.

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Poster

051. Auditory Processing: Perception and Cognition

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Title: Hierarchical packet-based code in inferior colliculus

Authors: *H. ZHANG[1], I. NELKEN[2], T. O. SHARPEE[3];
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Abstract: The auditory system has a substantial subcortical pathway which complicates the understanding of high-level auditory codes. On the other hand, this also indicates the necessity of sequential transformation and extraction of important information at each step from acoustics to cortex. We investigated the neural responses in inferior colliculus of rats to wideband acoustic stimuli (tone clouds). Despite the enriched knowledge about codes of single neurons in this region, little is known about how large population of neurons encode information together and the underlying principles. When we examine the population responses at the level of hundreds of neurons, coordinated activation among neurons is observed. We will refer to a period of coordinated activation as a “packet”. In packets, each neuron has a preferred latency of firing and is mostly binary, especially for the short-latency neurons. We also found that the number of neurons grows exponentially with their preferred latency, suggesting that there is a tree-like organization of neural responses, with exponentially more neurons of long-latency respond subsequently to short-latency ones. In addition, we show that functional differences exist between neurons at different levels of the hierarchy: short-latency neurons have sharper nonlinearity, convey more information per spike, and encode higher proportion of coarse information (information about a coarse version of the stimulus). Moreover, we found that sets of neurons convey more information than the sum of their individual information in the system, and this synergy exists both on the timescale of packets and the timescale of msec. This indicates that the packets constitute fundamental unit of representation of stimulus, which is further supported by the Zipfian statistics of the packets. These results together reveal the neural coding structures and principles in the inferior colliculus, which could contribute to our efforts in understanding neural codes in higher-order auditory regions.

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Poster

051. Auditory Processing: Perception and Cognition

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Program #/Poster #: 051.03

Topic: D.05. Auditory & Vestibular Systems

Support: NIH R01DC018621

Title: The Impact of Acoustic Environment on Noise Invariance in the Zebra Finch Auditory Cortex

Authors: *S. M. MOSELEY[1], C. FERHMAN[1], J. WEISSMAN[2], C. MELIZA[1];
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Abstract: Vocal communication requires the ability to detect a signal of interest within a background of competing sounds with similar acoustics (the "cocktail-party problem"). This ability has been attributed to higher-order auditory neurons that produce selective responses to vocalizations that are invariant to increasing levels of background noise. Noise-invariant neurons have been observed in several nonhuman species, but it is not yet known how they develop. We hypothesize that noise-invariance requires early experience in complex acoustic conditions. Zebra finches (*Taeniopygia guttata)* are an excellent model for studying communication in noise to test this hypothesis. Because zebra finches live in large colonies, not only must adults solve the cocktail-party problem, but young fledglings need to isolate their tutor's song from the colony background in order to learn and copy that song into adulthood. We predict that this early exposure to colony noise instructs the development of noise-invariant neurons in the zebra finch pallium. To test this, we reared birds in either the presence (n=7 birds) or absence (n=7 birds) of colony noise, then performed single-unit recordings throughout the auditory pallium. Responses were collected to conspecific stimuli embedded in synthetic colony noise at varying signal-to-noise ratios. Noise-invariance was quantified at the single-unit level by directly comparing neural responses to these auditory scenes with responses to the original foreground stimuli. Noise-invariance was also quantified within simultaneously recorded populations of 30–50 neurons using a linear decoder. As predicted, neurons in colony-reared birds were more invariant to noise.

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Poster

051. Auditory Processing: Perception and Cognition

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Support: NIH R01D015232
Departmental Funds

Title: Rate and Temporal Coding of Amplitude Modulation Discrimination in Primary Auditory Cortical Neurons of a Behaving Rhesus Macaque

Authors: *K. STEWART, J. S. JOHNSON, D. LU, J. ROBERTS, G. H. RECANZONE; Dept. of Neurobiology, Physiology, and Behavior, Univ. Of California Davis Ctr. for Neurosci., Davis, CA

Abstract: Temporal envelope processing, particularly at low and medium modulation frequencies, is critical for speech comprehension. To better understand how the auditory cortex decodes amplitude modulated (AM) stimuli, we recorded the activity of 57 single neurons in the primary auditory cortex (A1) of an alert rhesus macaque monkey while it performed a two-alternative forced-choice AM frequency discrimination task. This adult, male macaque monkey indicated with a joystick movement whether it perceived the second of two successive stimuli (S2) to be at a higher/faster or lower/slower modulation frequency compared to the first stimulus (S1). All stimuli were broadband noise with 100 percent modulation depth and were presented for 500ms at 65 dB SPL from a speaker positioned 90 degrees from the animal's midline, contralateral to the implanted recording chamber. S1 were either 17 Hz with fifteen different S2 stimuli (8 - 34 Hz) in blocks of 10 trials/S2 alternating with blocks of S1 stimuli at 34 Hz with S2 ranging from 17 - 68 Hz. Typical recording sessions included two blocks for each S1 (counterbalanced). For each S2, we calculated firing rate ("FR", rate code) and vector strength ("VS", temporal code) metrics. We developed models to investigate how rate and temporal coding correlate to psychophysical performance. Onset responses were found to be non-informative for firing rate analysis and to dominate the vector strength in the temporal analysis and were therefore excluded in both models. Each cell's rate modulation transfer function (rMTF) was calculated and FRs less than or greater than S2=S1 were interpreted as "S2 faster" or "S2 slower" based on the direction of the slope of the rMTF. For the temporal code model, each neural response was passed through a filterbank of VS calculations, one for each S2. The modulation frequency resulting in the highest VS was chosen, and the response was interpreted as representing "S2 faster" or "S2 slower" based on that modulation frequency's relationship to the S1 frequency. Comparing the performance of these models to the animal's behavior showed that both models accurately predicted the animal's behavior, particularly when reasonable exclusion criteria were applied to our dataset (for rate, excluding cells with non-linear rMTFs; for temporal, excluding cells with overall low synchrony). These results suggest that at the level of A1, the information required to perform an AM discrimination task is present in both rate and temporal codes.

Disclosures: K. Stewart: None. **J.S. Johnson:** None. **D. Lu:** None. **J. Roberts:** None. **G.H. Recanzone:** None.

Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: DC015543

Title: Dynamic gating of auditory perceptual learning by diverse cortical responses

Authors: *J. TOTH, B. SIDLECK, P. AGARWAL, D. SAEED, D. LEONARD, M. INSANALLY;
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Abstract: The ability to flexibly adapt to changing environments is the hallmark of an adaptive nervous system and is seen in animals across the entire phylogenetic tree. This is in stark contrast to perceptual and cognitive inflexibility which is implicated in many neurological disorders including hearing loss, autism, and schizophrenia. While sensory and frontal cortical areas have long been implicated in flexible behaviors, we lack a fundamental understanding of how information is gated within these circuits to select and update behavioral strategies based on sensory input and context. We trained mice to perform a go/no-go auditory reversal learning task that required animals to adapt their behavioral response to the same set of auditory cues. Specifically, animals were trained to respond to a target tone (11.2 kHz) and to withhold from responding to a nontarget tone (5.6 kHz) for water reward. Once animals learned this phase of the task we then implemented a rule-switch and reversed which tone was rewarded, requiring animals to remap stimulus-reward contingencies. Chemogenetic silencing demonstrated that auditory cortex is required for reversal learning in mice. Using silicon probe recordings, we simultaneously monitored the activity of single-units in the auditory cortex (AC, n=2,516 neurons) and frontal cortex (M2, n=2,993 neurons) during reversal learning. We found that neural response profiles during learning were highly heterogeneous ranging from highly-reliable or ‘classical’ responses to seemingly-random ‘non-classical’ firing. Neural populations in both regions dynamically altered their response profiles during different phases of learning allowing for the emergence of flexible behaviors.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH GOTOL0380A

Title: Sparse representation of sensory context by single neurons in auditory cortex

Authors: *M. LOPEZ ESPEJO[1], S. V. DAVID[2];
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Abstract: Accurate auditory processing requires working memory and integration of information from ongoing stimuli over time. Thus, neuronal circuits in the auditory cortex (AC) must keep

track of how a sound changes over time in a manner useful for downstream neurons and, ultimately, behavior. The magnitude of this memory trace and how it interacts with the representation of ongoing stimuli, as well as how neural, synaptic and circuit mechanisms contribute to it remain open questions. To study this problem we measured the responses of neurons to a brief natural sound probe, presented in different sensory contexts, where context was defined as the natural sound immediately preceding the probe. We used context-dependent differences in response as a proxy to measure the magnitude of memory and integration. Recordings were performed using multi-electrode arrays in the primary and secondary regions of the AC of awake, passively listening ferrets. We found significant contextual effects lasting in some cases past 1 second, with neurons in secondary auditory regions showing stronger and longer-lasting effects. Contextual effects tended to be greater when one of the contexts was silence, and smaller when it was the same sound as the probe. This difference implicates adaptation to the spectro-temporal features of the context as a potential contributor to the effects. Context effects were often limited to a subset of stimuli combinations in a single neuron, but the specific combinations were diverse among neurons in the local population. Thus the local population as a whole provides a sparse representation of the ongoing sensory context. To gain insight into mechanisms supporting context effects, we trained encoding models to predict the activity of individual neurons as a function of stimulus and past population activity. We found that models containing both past activity of neighboring neurons and the neuron itself were better able to account for long lasting contextual modulation. This hints at a combined role of local connectivity and intrinsic properties of the neuron in generating these context and memory representations.

Disclosures: **M. Lopez Espejo:** None. **S.V. David:** None.

Poster

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Support: German Science Foundation (Deutsche Forschungsgemeinschaft, DFG SFB 1436, TP A06) to FWO

Title: Thalamocortical and intracortical contributions to stimulus-evoked and oscillatory activity in rodent primary auditory cortex

Authors: M. JESCHKE[1,2], **F. W. OHL**[3,4,5];
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Abstract: Different parts of the neocortex are connected by an intricate web of interconnections. Among these are so-called intracortical, horizontal connections which travel within cortex and enable connections between cortical circuits over spatial distances of several millimeters. Intracortical, horizontal connections seem ideally suited to contribute to cortical processing by spreading information across cortical space.
This has led to theories in systems neuroscience which are based on the assumption that horizontal connections coordinate neuronal activity across cortical space. Here, we develop an experimental method to study the contribution of horizontal connections to tuning properties and intracortical coordination at the mesoscopic level. The method is based on the analysis of the relative residues of cortical laminar current source density reconstructions. We tested the method by manipulating the contribution of horizontal connections by surgical dissection. Experiments were performed in the tonotopically organized primary auditory cortex field AI of the Mongolian gerbil, and surgical cuts were oriented parallel to the isofrequency contours, enabling us to relate spatial distances of recording sites separated by cortical cuts to spectral distances of best-frequency representations. Our results indicate that horizontal connections contribute to frequency-tuning of mesoscopic cortical patches. Furthermore, we dissociated a type of cortical gamma oscillation based on horizontal connections between mesoscopic patches from gamma oscillations locally generated within mesoscopic patches and found global and local coordination of activity during sensory stimulation to occur in different gamma frequency bands. Together, the data demonstrate that intracortical horizontal connections play an important role in generating cortical feature tuning and coordinate neuronal oscillations.

Disclosures: **M. Jeschke:** None. **F.W. Ohl:** None.

Poster

051. Auditory Processing: Perception and Cognition

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Topic: D.05. Auditory & Vestibular Systems

Title: X2

Authors: ***J. HA**[1], S.-C. BAEK[2], T. CHUNG[1], H. BYUN[1], Y. LIM[3], J. CHUNG[1];
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Abstract: Auditory attention detection (AAD) is used to identify an attended speaker in a multi-speaker environment based on brain activity. As AAD research grows considerably, the expectation for the applicability of AAD in daily life is increasing. To this end, this study developed a cost-effective device for AAD based on electroencephalography (EEG) and evaluated the usefulness of the device by implementing an online AAD outside the laboratory. The present study devised a Cost-Effective, open-source Device for online Auditory attention detection (CEDA), which provides a sound stimulus by a conventional earphone and allows the

acquisition of EEG data synchronized to the sound stimuli to complete dichotic listening tasks with AAD. To test the feasibility of online AAD performance with CEDA in an everyday environment, nine candidates completed the dichotic listening paradigm tasks in a meeting room without soundproofing. In addition, to improve the accuracy of the AAD decoder, exponential moving average (EMA) was applied. The online AAD task was successfully demonstrated using the CEDA. It achieved an average decoder accuracy of up to 72% with nine participants. After applying an exponential moving average (EMA), the average decoder accuracy increased by up to 78%. Significance. This device allows for online AAD implementation with significant decoder performance outside the laboratory, as well as easy accessibility. These results would help expand the applicability of AAD in a daily life.

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Poster

051. Auditory Processing: Perception and Cognition

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Topic: D.05. Auditory & Vestibular Systems

Title: Role of the cholinergic system in early sensorimotor acquisition

Authors: J. LAWLOR BLONDEL[1,2], S. E. ELNOZAHY[3], F. DU[4,5], F. ZHU[1], A. WANG[1], T. RAAM[6], K. V. KUCHIBHOTLA[1,4,7];
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Abstract: During sensorimotor learning, animals link a sensory cue with actions that are separated in time using circuits distributed throughout the brain. Learning thus requires neural mechanisms that can operate across a wide spatiotemporal scale and promote learning-related plasticity. Neuromodulatory systems—with their broad projections and multiple timescales of activity—fulfill these criteria and could serve as a potent mechanism to link the different sensory and motor components. Yet, it remains unknown the extent to which this proposed model of plasticity occurs in real-time during behavioral learning. Acquisition of sensorimotor learning in a go/no-go task is much faster and more stereotyped than previously considered (*Kuchibhotla et al., 2019*). We trained mice to respond to one tone for a water reward (S+) and withhold from responding to another (S-). We interleaved reinforced trials with those where reinforcement was

absent ("probe"). Early in learning, animals discriminated between S+ and S- in probe but not reinforced trials. This unmasked a rapid *acquisition*phase of learning followed by a slower phase for reinforcement, termed '*expression*'. What role does neuromodulation play in task acquisition? Here, we test the hypothesis that cholinergic neuromodulation provides a 'teaching signal' that drives primary auditory cortex (A1), and links stimuli with reinforcement. We exploit our behavioral approach and combine this with longitudinal two-photon calcium imaging of cholinergic activity in A1 during discrimination learning. We report both robust stimulus-evoked cholinergic activity to both S+ and S- and stable licking-related activity throughout learning at the level of the axon segment. While this activity mildly habituates in a passive control, in behaving animals the S+ and S- stimulus-evoked activity is enhanced (S+: duration, S-: amplitude and duration) during early learning. Additionally, we test the hypothesis that cholinergic neuromodulation impacts the rate of task acquisition. We bilaterally expressed ChR2 in cholinergic neurons within the basal forebrain of ChAT-cre mice and activated these neurons on both S+ and S- trials throughout learning. Test animals acquired the task faster than control groups as measured in probe trials. These results suggest that phasic bursts of acetylcholine, projecting widely to cortical regions, directly impact the rate of discrimination learning.

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Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.10

Topic: D.05. Auditory & Vestibular Systems

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Title: Auditory cortex in awake, freely-moving rats tracks time in trial

Authors: *A. POLTEROVICH[1], M. M. JANKOWSKI[3], J. NIEDIEK[2], A. KAZAKOV[4], I. NELKEN[5];
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Abstract: Auditory cortex plays an important role in the computations underlying sound localization. Here we study the neural activity in the auditory cortex of freely-moving rats that perform a self-initiated sound localization and identification task. To this end we constructed the Rat Interactive Foraging Facility (RIFF). It consists of a large circular arena with 6 interaction areas (IAs) that have a water port, a food port and two loudspeakers. Rat behavior is monitored

online using video tracking and nose-poke identification. Neural responses are recorded using a logger on the head of the animal. In the task studied here, auditory cues consisted of 6 different modified human words, each associated with one IA. When a rat reached the center of the arena, one of the sounds was presented once every 2 seconds from its associated IA, and the rat had to reach the correct IA within 20 seconds in order to collect a reward. Control tasks included pure localization and pure discrimination tasks for the trained rats.The rats learned all tasks rapidly with minimal guidance. They performed best when both the localization and discrimination cues were available, but were able to collect rewards also when either of the cues was missing. Sound-driven neuronal responses were largely as previously described in anesthetized animals, although responses to the same sound presented in active and passive conditions could differ. In addition to the sound-driven responses, we observed large, reproducible slow modulations in firing rates that typically lasted a few seconds (much longer than sound driven responses) and that were locked to self initiated behavioral events before and after sound presentation. These firing rate modulations were often larger than the responses to sounds. The slow modulations were partially correlated with non-auditory, behaviorally-related variables such as speed of motion and head turn direction, but were best explained in many neurons as a slowly-varying function of the time within trial. We conclude that most spiking activity in the auditory cortex during sound-guided behavior tracks the time course of the task rather than the sounds.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

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Title: Modulation of the prefrontal cortex alters auditory processing and impairs perception in a challenging listening environment

Authors: *A. L. SCHORMANS[1], K. WIECZERZAK[1], H. MANDER[2], A. POLAT[1], S. H. HAYES[1], B. L. ALLMAN[1];
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Abstract: Processing and perceiving acoustic information under various listening conditions (e.g., a quiet classroom vs. a crowded conference centre) is known to involve a network of brain regions along the ventral auditory pathway (e.g., the primary and belt regions of the auditory cortex) as well as the prefrontal cortex. At present, however, the causal roles of higher-order brain regions, such as the prefrontal cortex, in auditory processing and perception are not fully

understood. To overcome this gap in knowledge, we used a rat model to directly manipulate activity in the medial prefrontal cortex (mPFC) and examined the causal effects on auditory processing and perception in both quiet and challenging listening conditions. More specifically, cohorts of rats with chronically-implanted bilateral cannulae in their mPFC were infused with muscimol (a GABA-A receptor agonist) or aCSF, and underwent either (1) passive listening electrophysiological recordings to assess spontaneous oscillatory activity, 40-Hz auditory steady state responses (ASSR) and frequency mis-match responses (MMR), or (2) an operant-based amplitude modulation (AM) detection task in the presence of different listening environments. Electrophysiological recordings showed that silencing the mPFC caused a decrease in gamma oscillations in both the auditory cortex and mPFC, whereas sound-evoked responses showed a differential effect in these brain regions, characterized by an enhanced response in only the auditory cortex. Despite the increased sound-evoked response within the auditory cortex, the evoked power and inter-trial coherence of the 40-Hz ASSR was unaffected by the inactivation of the mPFC. Furthermore, we found that silencing the mPFC resulted in a reduction in the magnitude of the MMR within the auditory cortex, such that there was no longer a difference between stimuli when they were presented as a standard versus a deviant. Perceptually, we found that inactivation of the mPFC decreased task performance by slightly worsening the AM detection threshold (i.e., the smallest degree of modulation that was detected). When the same procedure was performed in the presence of a challenging listening environment, performance on the AM detection paradigm further eroded, such that rats were no longer able to accurately identify a significantly modulated acoustic stimulus. Collectively, the present work provides evidence of a causal role of the prefrontal cortex in the processing of simple acoustic stimuli as well as its critical importance in supporting accurate auditory perception in challenging listening environments.

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Poster

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Title: From instinct to insight: Neural basis of learning new sounds to improve an innate social behavior

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Abstract: In nature, animals learn to modify their innate behaviors to better adapt to the environment, transitioning their actions from pre-existing stereotypes to novel, more adaptive ones. Nowhere is this more important than in parenting behaviors, when learning new infant-associated cues could benefit offspring survival. Here we explored the neural basis for such transitions by investigating how freely moving female mice learn to override the way they innately search for pups by relying on a new sound that predicts where pups will be found. Naïve virgins were trained in a T-maze to enter one of the two arms cued by an amplitude-modulated (5Hz) band-pass (30~50kHz) noise and rewarded with pups, which were then retrieved back to the nest in the main stem. All mice (N=9) were highly motivated to perform the task from the start of the training, and initially used an innate spatial memory-based strategy of searching the arm where a pup was presented in the prior trial. Within 3 to 7 days, all animals learned (70% correct) to use the sound to locate pups. We recorded single-unit/multi-unit spiking in auditory cortices (AC, N~1200) and medial prefrontal cortices (mPFC, N~600) during learning. Neural responses in AC before animals made choices encoded the upcoming choice to move toward the sound on a trial-by-trial basis. 55% of all AC units showed significant differences between trials of correct and wrong sound-based choices ($p < 0.05$, tested for each unit), even from the very early stage of training, suggesting a top-down influence on auditory processing and upcoming choices. Neural responses to the sound at the nest, far away from the sound sources, increased over training ($p < 0.001$, for all animals), implying improved sensitivity to the sound. During learning, mPFC was highly active when animals entered the arms. mPFC units were highly selective to both the arm side and the outcome of their choice. At the population level, mPFC neurons exhibited significantly higher activities when animals entered the wrong arm ($p < 0.001$, for all animals), suggesting mPFC's role in error detection. During the retrieval phase of each trial, mPFC neurons exhibited selectivity to the arm side where pups were presented (61% units, $p < 0.05$, tested for each unit), implying mPFC's role in encoding social-spatial memory. The results suggested mPFC neurons encode social-spatial information relevant to the innate searching strategy and evaluate the efficiency of the innate strategy. In addition, a top-down modulation of auditory responses in AC may modulate animals tendency to probe a novel sound and play an important role in the process of switching from the innate strategy to a more adaptive sound-based strategy.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Title: A Comparison of Source Localization Algorithms for EEG Auditory Evoked Data

Authors: *A. ROGERS[1,2,3], A. THIRAKUL[2], A. SHIELDS[1,2], G. COGAN[2,3,4,5,6];
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Abstract: Electroencephalography (EEG) measures aggregate neural signals on the scalp with excellent temporal resolution. While its inherent spatial resolution is limited, techniques have been developed to estimate the location of neural source activity from measured scalp signals. This source localization is typically composed of a forward model describing how neural activity is presented on the scalp and an inverse model that transforms the measured scalp signals into estimated neural sources. These estimates are particularly challenging for auditory stimuli as they produce neural responses that originate in both hemispheres, yet produce only a single central scalp estimate. Auditory data, therefore, presents a unique challenge for EEG source localization. In this work, we compare source localization techniques for auditory evoked data from EEG by comparing spatial resolution metrics of three linear source localization techniques: Minimum Norm Estimate (MNE), dynamic Statistical Parametric Mapping (dSPM), and standardized Low-Resolution Electromagnetic Tomography (sLORETA). Neuro-electrical activity was recorded in data collected from 19 subjects using a 124 Channel EEG recording system with 4 additional EOG channels, during a passive auditory listening task: 200 trials of 500 or 1000 Hz tone presentation with an ISI of 4 s (+/-500 ms randomly jittered). Each subject also received a T1 structural magnetic resonance image scan for more accurate source localization. Signals analyzed were the N1-P2 evoked response which we expected to localize to the superior temporal gyrus (STG) bilaterally. We quantified the peak localization error (PLE) from the signal maxima, and anatomical PLE (aPLE - PLE from the centroid of the STG). We also estimated the cross-talk function (CTF - an estimate of the influence of other sources on a single source) and point spread function (PSF - spread of sources). Our results show that for both PLE and aPLE, sLORETA had the best results of 0 cm (with the exception of the left hemisphere aPLE, with a value of 5.6 cm), and dSPM outperformed MNE for both aPLE (2.5 cm vs 2.7 cm) and PLE (1.9 cm vs 2.5 cm). For PSF, MNE had the best value (3.7 cm), but all other techniques had similar values (~ 4 cm). For CTF, sLORETA had a value of 3.7 cm, and all other methods had similar results (~ 3.7 cm). Taken together, our results show that for auditory evoked EEG data, sLORETA provides superior abilities to resolve source localization of EEG auditory evoked data. These preliminary results provide an empirical comparison of EEG source localization for bilateral auditory signals and could help guide researcher's decisions for appropriate source localization techniques.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

Support: NIH 5R01DC014989-07

Title: The posterior parietal region as a relay station between auditory and premotor areas in the macaque

Authors: R. AFSAHI[1], J. T. JACOBS[1,2], P. KUSMIEREK[1,3], P. A. WIKMAN[1], P. FORCELLI[2], *J. P. RAUSCHECKER[1];
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Abstract: Dating back to Wernicke (1874), the dorsal-stream model of auditory and speech processing postulates that connections between auditory and motor regions of the brain are crucial for relating motor commands to their sensory outcomes (Rauschecker, 2011). Previous studies with functional MRI in our lab have shown that motor regions are activated when rhesus macaques listen to auditory sequences they had previously learned to produce (Archakov et al., 2020). Additionally, anatomical tracer studies have shown direct connectivity between the superior temporal gyrus and sulcus (STG/STS) and areas of the premotor cortex (PMC; Luppino et al, 2001). However, no previous study has specifically investigated connectivity between auditory cortex and regions of the PMC that physiologically respond to auditory input. To investigate this further, we injected Cholera Toxin B subunit (CTB) tagged with Alexa Fluor (AF) 488 and 594 fluorophores into anterior and posterior regions of the PMC, respectively, in adult rhesus macaques (*Macaca mulatta*). The anterior injection was made at the junction between the F2 and F4 premotor areas, inferior to the spur of the arcuate, while the posterior injection was made at the junction between areas F1, F2, and F4. These locations were chosen based on the activations found in our previous fMRI study (Archakov et al., 2020). The animals were euthanized after appropriate survival times, the brains were blocked and cut in the coronal plane, and immunohistochemical staining was performed. Two different primary antibodies were used: Anti-Cholera Toxin B, which recognizes both tracers, and a polyclonal antibody raised against the AF488 fluorophore, which recognizes only that tracer. The results indicate that both tracers were taken up by axon terminals near the corresponding injection sites and retrogradely transported to cell bodies throughout the cortex. The highest concentration of labeled cells, projecting to the posterior injection site, was found in the posterior parietal region, including area PE, the ventral intraparietal area (VIP), the lateral intraparietal area (LIP), the posterior supramarginal gyrus (PFG), and the operculum (7op). Additional label from both injections was found in the supplementary motor area (SMA), the anterior cingulate cortex (ACC), and in F2. While there were no labeled cells in auditory areas, the current dorsal-stream model postulates that posterior parietal areas may serve as a relay station between auditory centers and the PMC. This may include area VIP, which has previously been shown to play a role in auditory processing (Lewis and Van Essen, 2000).

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Poster

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Title: Characterization of local field potential responses in mouse cortex during conscious perception of auditory stimuli using a Go/No-Go task

Authors: *S. H. MCGILL[1], C. W. ZHAO[1], L.-A. SIEU[1], T. NGUYEN[1], Q. PERRENOUD[2], H. BLUMENFELD[1];
[1]Neurol., [2]Neurosci., Yale Univ. Sch. of Med., New Haven, CT

Abstract: The sensory cortices receive and process a near-continuous stream of signals from neurons throughout the body, but only some of the information they produce enters conscious perception. Experiments using human subjects have shown that cortical and subcortical regions exhibit a rapid (within a few hundred milliseconds), ordered series of responses to perceived stimuli but not to identical unperceived stimuli. These and other studies suggest there exists a stereotyped mechanism for conscious perception of individual stimuli. However, it is not yet known if any animal model exhibits analogous changes in neuronal activity. To address this problem, we developed a mouse model of auditory conscious perception. Adult c57bl6 mice were surgically implanted with a steel headplate as well as twisted-pair bipolar electrodes targeting secondary auditory cortex and the frontal association region. Following recovery, the mice were trained to initiate (auditory stimulus present) or withhold (stimulus absent) licking from a spout while head-fixed on a running wheel. Access to the lickport was mechanically restricted to a three second period during each trial, at least one second after presenting the auditory stimulus during relevant trials, to minimize the effects of motor correlates on electrophysiological measurements. After meeting the training completion criteria mice were able to behaviorally identify the presence of an auditory stimulus with overall accuracy of 93% to 98%, and absence of the stimulus with accuracy of 60% to 84%. The mice further exhibited event related potentials on local field recordings from the auditory cortex and frontal association region following stimulus presentation. By comparing these against potentials in the absence of stimuli and at-threshold stimuli, and other cortical and subcortical neurophysiological signals, we can further uncover the mechanisms underlying auditory conscious perception within a mouse model.

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Poster

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Title: A behavioral paradigm for studying auditory-guided motor learning in mice

Authors: *G. W. ZEMPOLICH, D. M. SCHNEIDER;
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Abstract: Many behaviors - from playing the violin to learning a new language - rely on auditory feedback. During these auditory-guided actions, we form predictions for the sounds our movements should produce, and if expectation and experience differ, adjust our subsequent behavior. The auditory cortex produces activity that appears well suited to support auditory feedback-based motor learning. In humans, monkeys, and mice, auditory cortical responses to self-generated sounds that match expectation are weak, while responses to self-generated sounds that violate expectation are large, consistent with the auditory cortex producing prediction error signals during sound-generating behaviors. It remains unknown whether or how these signals are used to guide learning. To explore this question, we developed a novel auditory-guided behavior in which mice press a lever forward with their forelimb toward a 2mm wide target zone. Mice hear a 16kHz tone when the lever enters the zone and an 8kHz tone if the press exceeds the bounds of the zone. Presses that peak within the zone produce only the entry tone and are rewarded when the lever returns to the starting position. Presses that are too short (producing no tones) or too long (producing both an entry and an exit tone) are unrewarded. Every approximately 30 trials, the zone is relocated without warning and mice must use acoustic feedback to adjust their lever presses to peak at the new location. Over 3 weeks, mice learn to produce precise lever presses that peak within the target zone. Performance errors decrease with training and mice plateau at a level of performance where the variance of peak positions is approximately equal to the size of the target zone. When the target zone is unexpectedly shifted, mice rapidly adjust their lever movements and find the new target zone within several trials. If mice make an error, they direct their press on the next trial toward the target zone, suggesting that mice use real time acoustic feedback to guide behavior. Consistent with this conclusion, when we omit all entry and exit tones on a subset of trials mice fail to find the target zone and performance error increases significantly. We have made preliminary multi-electrode array recordings from the auditory cortex of well-trained mice and find that many neurons respond to both movement and sound. Interestingly, some neurons respond differently to the 16kHz entry tone based on where the target zone is located, suggesting that auditory cortex neurons encode a combination of lever position and sound. These experiments establish a novel auditory-guided motor learning paradigm that may provide insights into how predictions and errors are used to guide behavior.

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Poster

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Title: Revealing latent knowledge in cortical networks during goal-directed learning

Authors: *C. DRIEU, Z. ZHU, K. FULLER, A. WANG, S. ELNOZAHY, Z. WANG, K. KUCHIBHOTLA;
Johns Hopkins Univ., Baltimore, MD

Abstract: Goal-directed learning is canonically considered a slow process with high inter-subject variability. Exploration of the neural mechanisms, therefore, has focused on identifying dynamics concomitant with these gradual improvements. Recent work, however, used performance in reinforced and non-reinforced 'probe' trials to show that goal-directed learning can be dissociated into two behavioral phases: rapid 'acquisition' of task contingencies (measured in probe trials), and slower 'expression' that reveals the learned content (measured in reinforced trials). To what extent is the auditory cortex (AC) involved in either learning phase? To address this, we trained mice to lick to a tone for water reward (S+) and withhold from licking to another tone (S−) to avoid a timeout. Optogenetic inactivation of the AC significantly impaired both acquisition and expression. Surprisingly, this inactivation-induced deficit gradually waned during expression arguing for an ephemeral associative and teaching role for the AC, rather than one focused on task execution. To determine how the two learning phases are implemented by AC networks, we used longitudinal two-photon calcium imaging of the same large population of excitatory neurons (n=8,235 neurons in 8 mice across 15 days) in layer II/III. We isolated learning-related dynamics by comparing mice learning the task (n=5) to those passively listening to the same tones over the same period (n=3). We used unsupervised low-rank tensor decomposition (TCA) to uncover low-dimensional network dynamics at different timescales. While stimulus-related habituation dominated in passive networks, three striking behavior-driven features emerged in learning networks. First, a population of S+ driven neurons rapidly shifted to firing later in the trial, suggesting a role in reward encoding at the timescale of acquisition. Second, a distinct subset of S- responsive neurons gained a late-in-trial behavioral inhibition signal that increased gradually, at the timescale of expression. Third, reward-related signals appeared to be transient, ramping up and then gradually waning at expert levels. Thus, the AC plays a default, but temporary, role in goal-directed learning that is mediated by a network that shifts from being largely stimulus-driven to one that is optimized for behavioral needs.

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Poster

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Title: Auditory motor integration dynamics in the tail of the striatum

Authors: *I. LINARES-GARCIA[1], S. LEVIN[2], T. VAJTAY[3], D. J. MARGOLIS[4];
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Abstract: A critical role of the CNS is to select appropriate actions based on incoming sensory information such as audition. This process, known as sensorimotor integration, allows animals to adapt their behaviors in dynamic environments. However, there is little mechanistic knowledge about how meaningless auditory frequencies are transformed into action-related survival cues.One key area that integrates sensory signals from auditory regions (primary auditory cortex and thalamus) and is believed to transform them into actions is the tail of the striatum (TS). It contains a diverse microcircuitry that includes a multitude of interneurons and two discrete spiny projection neuron (SPN) subpopulations (direct pathway, dSPN; indirect pathway, iSPN) that are essential for action selection. Additionally, striatal circuitry can be restructured to alter SPN output via neuromodulators such as dopamine and acetylcholine. Dopamine is thought to be involved in learning and reinforcement, while acetylcholine is implicated in enhancing learning and striatal plasticity. Thus, it remains poorly elucidated how dSPN and iSPN activity coordinates with these neuromodulators to promote sensorimotor learning.The calcium indicator jGCaMP7f (pGP-AAV-syn-jGCaMP7f-WPRE) and a gradient-index (GRIN) lens were injected and implanted unilaterally in the TS of male and female double transgenic mice, respectively. These mouse lines permit the identification of either the dSPN (Tg(DrD1-cre)EY262 x Ai14 (R26-LSL-tdTomato)) or the iSPN (Tg(Adora2a-cre) KG139Gsat x Ai14 (R26-LSL-tdTomato)) subpopulation via red fluorescence. Further, we designed the Dynamic Auditory Action Selection (DAAS), a novel head-fixed auditory discrimination paradigm, in which mice are presented with two different harmonic frequencies and must associate them with two different actions (pulling or pushing a joystick). Thus, using two-photon microscopy via a GRIN lens and calcium imaging, we recorded the activity of identified dSPN and iSPN subpopulations as mice progressed from naive to expert status.Our preliminary data show that mice are able to learn this complex task during longitudinal 2p recordings, and there appear to be discrete SPN subsets that are tuned to certain paradigm events such as the sound harmonic, joystick movement, and water reward. There is a subpopulation of neurons that bidirectionally encode specific task parameters by decreasing their activity aligned to the sound and increasing their activity to the reward. Finally, we have ongoing experiments to assess acetylcholine and dopamine dynamics across DAAS learning using fiber photometry.

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Poster

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Title: Flexible and stable representation of the auditory information in the cortico-cortical and cortico-collicular circuits

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Biol. sciences, Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Reversal learning task requires animals to learn to reverse the association between sensory stimuli and motor actions. It is a highly cognitive process, involving multiple regions and circuits in the brain. However, it is still unclear which brain circuits reverse the sensorimotor transformation by updating reward contingencies in association with sensory stimuli. Here, we identified that the posterior parietal cortex (PPC), the auditory cortex (AC), and the inferior colliculus (IC) mutually interact with each other and play distinct roles in auditory reversal learning in mice. By performing *in vivo* single-unit recordings and muscimol-induced local inactivation, we found that the PPC and the AC are necessary for inverting their action after changing the stimulus-reward contingency. On the other hand, the IC is critical for gating sensory information to motor actions *via* representing stimulus and choice stably regardless of the reward contingency. Circuit-specific optogenetic inactivation revealed that the PPC-to-AC top-down projection mainly contributes to reversing the behavioral responses during the task, while the AC-to-IC projection is important for mice to transform auditory information into motor action. Taken together, our results demonstrate that the cortico-cortical and the cortico-collicular circuits exert distinct roles in updating auditory information according to the reward contingencies in animals flexibly adjusting their responses to the auditory stimuli. These two parallel circuits must be well-balanced for animals to take optimal action decisions.

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Poster

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Topic: D.05. Auditory & Vestibular Systems

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ONR N00014-19-1-1223
ONR N00014-18-1-2069
ONR N00014-20-1-2709

Title: Prospective electrophysiological correlates of auditory attention in the bottlenose dolphin.

Authors: M. D. SCHALLES[1], J. MULSOW[2], D. HOUSER[2], J. FINNERAN[3], P. TYACK[4], B. SHINN-CUNNINGHAM[1];
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Abstract: In a noisy acoustic environment like San Diego Bay, how does a dolphin attend to an auditory source of interest and ignore boat engine noise, snapping shrimp, and even another dolphins' echo returns? Selective attention requires actively directing focus onto a target while ignoring competing sound sources. In many mammals, endogenous changes in task strategy, including the focus of selective attention, can modulate the magnitude of early cortical responses evoked by sound onsets (auditory evoked potentials or AEPs). Here, we investigated whether task demands alter AEPs in an adult male bottlenose dolphin (*Tursiops truncatus*). Each trial played a rapid sequence of 20 kHz and 28 kHz, 10-ms tones, with a jittered 50-150 ms inter-stimulus interval. The dolphin was trained to whistle to a "target" tone, which was 10 dB more intense (140 dB re 1 μPa SPL) than other tones, and to withhold responses on catch trials (no target). Correct responses earned a fish reward. We employed a 2 x 2 design in which, across blocks (each ~1-2 weeks of training and testing), we varied both the target frequency (20 kHz or 28 kHz) and the frequency context (whether 20 kHz or 28 kHz occurred frequently). Specifically, in each block, 80% of the tones were a standard of one frequency (either 20 kHz or 28 kHz) and the other 20% were the other frequency (deviants). We predicted larger AEP responses to tone deviants whose frequency was infrequent (reflecting an exogenous mismatch negativity or MMN response). We further expected the MMN to be larger when the deviant frequency matched that of the target, reflecting endogenous effects. Preliminary results from an electrode site on the midline (~20 cm posterior to the blowhole) suggest an increased MMN when the target and deviant frequencies match for both 20- and 28-kHz conditions. More specifically, the difference in the standard and deviant peak-to-peak amplitude between N1-P2 AEP components was larger from about 50-100 ms post-stimulus onset for tones of the target frequency. This MMN was smaller (28 kHz) or nonexistent (20 kHz) for tones not matching the target. This suggests that dolphins exhibit top-down effects on auditory processing, particularly when goal-directed task demands interact with stimulus statistics and expectations.

Disclosures: M.D. Schalles: None. **J. Mulsow:** None. **D. Houser:** None. **J. Finneran:** None. **P. Tyack:** None. **B. Shinn-Cunningham:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.21

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01D017480

Title: Evaluating the Effect of Vagus Nerve Stimulation on Auditory Learning in a Rat Model of Autism Spectrum Disorder

Authors: *B. M. WILLIAMS[1,2], T. DANAPHONGSE[2], A. REYES[2], S. KROON[1], V. PASAPULA[1], A. JACOB[1], A. MEHENDALE[1], C. T. ENGINEER[1,2];
[1]Neurosci., The Univ. of Texas at Dallas, Richardson, TX; [2]Texas Biomed. Device Ctr., Richardson, TX

Abstract: Individuals with Autism Spectrum Disorder (ASD) often struggle with everyday communication. Specifically, they exhibit impairments in processing receptive and expressive language. These difficulties are thought to arise from the improper development of neural structures along the auditory pathway. The result is a weakened and delayed response to auditory cues. When responses are partially impaired, a cascade of processing errors can occur—resulting in a failure to correctly process sound. Typical function may be partially regained through extensive speech therapy. However, many individuals still report deficits following treatment. To improve outcomes following treatment, an adjunctive therapy is needed. One such therapy is vagus nerve stimulation (VNS). Paired with an auditory cue, VNS has been shown to drive plasticity across the auditory pathway. In rodents, VNS-sound pairing increases neural responses to sound. Using prenatal exposure to valproic acid (VPA), we model the physiological and behavioral deficits associated with ASD — in rats. Prior works have shown that when VPA exposed rats receive VNS-sound paired therapy, the physiological deficit in sound processing is overcome and neural performance is rescued. It has yet to be determined whether VNS-sound pairing could improve a VPA exposed rats' performance in a behavioral task. This study tests the hypothesis that VNS paired with successful trials on a speech discrimination task will improve the performance of VPA exposed rats. Three groups (saline-exposed control, VPA-exposed, VPA-exposed+VNS) of rats were trained on a go, no-go speech sound discrimination task. All three groups received identical surgical and training procedures. Our preliminary results suggest that VNS positively modulates performance on a consonant discrimination task. VPA+VNS rats have improved target sound recognition and fewer nosepokes to non-target sounds than their VPA counterparts. This study could be fundamental in developing clinical strategies to implement VNS paired auditory therapy to improve the auditory processing capabilities of individuals with ASD.

Disclosures: B.M. Williams: None. **T. Danaphongse:** None. **A. Reyes:** None. **S. Kroon:** None. **V. Pasapula:** None. **A. Jacob:** None. **A. Mehendale:** None. **C.T. Engineer:** Other; Married to an Employee of Microtransponder Inc..

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.22

Topic: D.05. Auditory & Vestibular Systems

Title: Visual stimuli-induced neural activity in the auditory region of zebra finch

Authors: *S. NOGUCHI, M. IWASAKI, M. INDA, K. HOTTA, K. OKA;
Keio Univ., Kanagawa, Japan

Abstract: Males of zebra finch(<I>Taeniopygia guttata</I>) sing to females, and females choose their partners based on the social context and quality of the song. This social context includes visual, postural, and motor elements. Previous study revealed that the higher brain region NCL (nidopallium caudolateral) is connected to the visual pathway and received visual information (Hsiao *et al*., 2020). Furthermore, it has been found that auditory cortex L1(Field L subdivision 1) has a connection to the NCL by using retrograded tracers (Stacho *et al*., 2020). Other study has shown that expression of immediate early genes triggered by songs in the higher auditory cortex, NCM (caudomedial nidopallium) and CMM (caudomedial mesopallium), of females is modulated by visual stimuli from courting males (Avey *et al*., 2015). These findings suggest that there is a connection between the visual and auditory cortex. In this study, we investigated whether visual information affects the neural activity of the auditory cortex. We constructed the experimental system to obtain neural activities under free moving conditions and recorded the neural responses of the NCM during visual stimulation. Using the improved cage and the electrodes, we recorded and analyzed the neural activities under free-moving conditions and confirmed the validity of the recorded neural activities. We played back multiple songs and compared the relationship between neural activities and each song's acoustic features under restricted and free-moving conditions. The results suggest that the selectivity of acoustic features is different under restricted and free-moving conditions. Furthermore, to investigate whether visual information affects the neural activity of the auditory cortex, we recorded the neural responses of the NCM during presentation of video footage of a male bird singing to a female bird. In order to test the influences of visual and auditory information, we presented three stimuli; video only, song only, and video and song simultaneously. We compare the firing rates during each presentation of stimulations. The result suggest that the average firing rate was higher when video and song stimuli were present than when the one they were absent. This suggests that there are some neurons in the auditory cortex that respond to both visual and sound information.

Disclosures: S. Noguchi: None. **M. Iwasaki:** None. **M. Inda:** None. **K. Hotta:** None. **K. Oka:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.23

Topic: D.05. Auditory & Vestibular Systems

Title: Movement-related activity in auditory cortex encodes behavioral state and expectation

Authors: *W. ZHOU, D. M. SCHNEIDER;
New York Univ., New York, NY

Abstract: To properly process sensory stimuli generated by one's own movements, the brain may use copies of movement-related signals (e.g. corollary discharge) to modulate sensory responses to self-generated stimuli. In mouse auditory cortex (ACtx), sound-evoked activity is influenced by movement, expressed as a marked suppression of sound-evoked responses during movement compared to rest. This suppression of sound responses during movement can be shaped by learning to reflect the properties of self-generated sounds. Here, we show that activity in ACtx right before an expected self-generated tone is also modulated by movements and contains information about the upcoming sound. We developed a task in which mice push a lever and hear a predictable self-generated tone. We then recorded in ACtx on the first and last days of training with the sound-generating lever. We found that ACtx neurons changed their activity during movement hundreds of milliseconds prior to the self-generated tone, resulting in an overall ramping-up of baseline firing rates in ACtx. Both regular-spiking (RS) and fast-spiking (FS) units are influenced; and the change is much more strongly observed in deeper layers. Population-level analyses (e.g. PCA) showed that movement-related activity gradually pushed the neural dynamics in ACtx away from that during rest as the mouse started to push the lever. Movement-related activity in ACtx populations was in dimensions that did not completely overlap with sound-coding dimensions, leading to an easily detectable difference between population trajectories for a sound heard during rest and the same sound heard during movement. We found that the increase of pre-tone activity during movement becomes more significant on the last session of training compared to the first, especially among those neurons that are responsive to the expected self-generated tone frequency. This biased selectivity in movement-related activity, along with the stronger suppression of expected compared to unexpected self-generated tone, could both be eliminated by application of Muscimol in ACtx during training. Together, these findings suggest that movement-related activity in ACtx encodes an expected self-generated sound, relies on ACtx activity during learning, and may facilitate the processing of self-generated sounds.

Disclosures: W. Zhou: None. **D.M. Schneider:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.24

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant R01DC009836
NIH Grant P50DC015857
NIH Grant F31 DC018974

Title: Cortical PV neurons regulate the perceptual volume knob: bi-directional changes in loudness perception via reduced or enhanced PV-mediated inhibition in the auditory cortex

Authors: *M. MCGILL[1], C. KREMER[2], K. CLAYTON[3], K. STECYK[3], Y. WATANABE[3], D. SKERLEVA[3], E. SMITH[3], C. RUTAGENGWA[3], S. G. KUJAWA[3], D. B. POLLEY[1]; [1]Harvard Med. Sch., Boston, MA; [2]Harvard Univ., Cambridge, MA; [3]Massachusetts Eye and Ear, Boston, MA

Abstract: Noise exposure that damages cochlear sensory cells and afferent nerve endings is associated with reduced feedforward inhibition from parvalbumin+ (PV) cortical GABAergic neurons, resulting in hyperactive, hyperresponsive, and hypercorrelated spiking in ACtx pyramidal neurons. To better establish how disinhibited neural circuit pathology studied in laboratory animals relates to loudness hypersensitivity and other clinical phenotypes observed in humans with sensorineural hearing loss, we developed a two-alternative forced-choice classification task for head-fixed mice to probe changes in the perception of loudness after controlled cochlear injuries. At baseline (N=17 total) or in sham-exposed control mice (N=6), behavioral classification of soft versus loud varied smoothly across a 40-80 dB SPL range. After noise exposures that caused either a “pure” cochlear neural damage (N=6), or mixed sensorineural pathology (N=5), mice rapidly developed loudness hypersensitivity that manifested as a 9 dB shift in their loudness transition threshold. As expected, bilateral silencing of auditory cortex via optogenetic activation of PV neurons did not affect tone detection probability but had an interesting effect on loudness perception, in that PV activation strongly biased mice to report high-intensity sounds as soft (N=6). Taken together, these data suggest that cortical PV neurons function as a perceptual volume knob; sounds are perceived as louder than normal following acoustic exposures that reduce PV-mediated cortical inhibition but softer than normal when PV neurons are artificially activated via optogenetics. Clinically, these data enrich the growing literature that identifies PV pathology as critical point of dysfunction in auditory perceptual disorders.

Disclosures: **M. McGill:** None. **C. Kremer:** None. **K. Clayton:** None. **K. Stecyk:** None. **Y. Watanabe:** None. **D. Skerleva:** None. **E. Smith:** None. **C. Rutagengwa:** None. **S.G. Kujawa:** None. **D.B. Polley:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.25

Topic: D.05. Auditory & Vestibular Systems

Support: NIH Grant K99DC018600

Title: Transformation of acoustic information to sensory decision variables in parietal cortex

Authors: *J. D. YAO[1], K. O. ZEMLIANOVA[1], D. L. HOCKER[1], C. SAVIN[1], C. M. CONSTANTINOPLE[1], S. CHUNG[2], D. H. SANES[1];
[1]New York Univ., New York, NY; [2]Flatiron Inst., New York, NY

Abstract: The process by which sensory evidence contributes to perceptual choices requires an understanding of its transformation into decision variables. Here, we address this issue by evaluating the neural representation of acoustic information in auditory cortex-recipient parietal cortex while gerbils either performed an auditory discrimination task or while they passively listened to identical acoustic stimuli. Gerbils were required to discriminate between two amplitude modulation (AM) rates, 4- versus 10-Hz, as a function of AM duration (100-2000 msec). Task performance improved with increasing AM duration, and reached an optimum at approximately 800 msec. Decoded activity of simultaneously recorded parietal neurons reflected psychometric sensitivity during task performance. Decoded activity during passive listening was poorer than during task performance, but scaled with increasing AM duration. This suggests that the parietal cortex could accumulate this sensory evidence for the purpose of forming a decision variable. To test whether decision variables emerge within parietal cortex activity, we applied principal component and geometric analyses to the neural responses. Both principal component and geometric analyses revealed the emergence of decision-relevant, linearly separable manifolds on a behaviorally-relevant timescale, but only during task engagement. Finally, using a clustering analysis, we found 3 subpopulations of neurons that may reflect the encoding of separate segments of task performance: stimulus integration and motor preparation or execution. Taken together, our findings demonstrate how the parietal cortex integrates and transforms encoded auditory information to guide sound-driven perceptual decisions.

Disclosures: J.D. Yao: None. **K.O. Zemlianova:** None. **D.L. Hocker:** None. **C. Savin:** None. **C.M. Constantinople:** None. **S. Chung:** None. **D.H. Sanes:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.26

Topic: D.05. Auditory & Vestibular Systems

Support: R01-DC011284

Title: Cingulate cortex facilitates auditory perception under difficult listening conditions

Authors: *K. L. ANBUHL, M. DIEZ CASTRO, N. A. LEE, D. H. SANES;
Ctr. For Neural Sci., New York Univ., New York City, NY

Abstract: Individuals with hearing loss (HL) often exert greater cognitive resources (i.e., listening effort) to understand speech, especially under challenging acoustic conditions. The resulting cognitive fatigue can impede language acquisition and can have long-term negative consequences for quality of life. However, the neural mechanisms that support listening effort are uncertain. Evidence from human studies suggest that the cingulate cortex is engaged under difficult listening conditions and can exert top-down modulation of the auditory cortex (AC). Here, we asked whether the gerbil cingulate cortex (Cg) sends anatomical projections to the AC in the gerbil, and whether it mediates effortful listening.

Retrograde and anterograde virus tracers were injected into AC and Cg, respectively, to determine anatomical connectivity. To assess effortful listening, an amplitude modulation (AM) rate discrimination task was used, and stimulus parameters (AM rate, sound duration) were varied to adjust the difficulty of listening conditions. Using an appetitive Go-Nogo paradigm, gerbils were trained to discriminate between "Go" stimuli consisting of a range of AM rates (4.5-12 Hz, broadband noise carrier, 100% depth) and a "Nogo" AM stimulus (4 Hz). Trials were clustered into 'easy' or 'hard' blocks, where the sound duration was 1s or 0.25s, respectively. AM rate discrimination thresholds were determined from psychometric functions. Once asymptotic performance was reached, gerbils were implanted with bilateral cannulae in Cg. To determine whether Cg is required for task performance, muscimol was infused bilaterally prior to testing to pharmacologically inactivate Cg and compared to saline-infused controls. Recordings are currently being obtained from Cg during task performance to determine whether Cg neurons represent task difficulty.

Viral tracing experiments revealed a strong, descending projection from Cg to AC. Next, we asked whether locally inactivating Cg impairs perceptual performance. We found that Cg inactivation disrupted performance *only for difficult listening conditions*: thresholds for the 1s blocks (i.e., 'easy' blocks) remained the same across saline and muscimol conditions (~5 Hz AM), whereas thresholds for 0.25s blocks were elevated only for muscimol conditions (saline: ~5.5Hz AM; muscimol: ~7Hz AM).

Taken together, the results reveal a descending cortical pathway from Cg to AC that mediates perceptual performance during difficult stimulus conditions. This pathway is a plausible circuit that may be undermined by HL.

Disclosures: K.L. Anbuhl: None. **M. Diez Castro:** None. **N.A. Lee:** None. **D.H. Sanes:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.27

Topic: D.05. Auditory & Vestibular Systems

Support: R01NS128904
R01AG077681

Title: Motivational modulation of sensory representation during auditory prey capture

Authors: M. SHALLOW[1], ***M. WEHR**[2];
[1]Biology, Inst. of Neurosci., Univ. of Oregon, Eugene, OR; [2]Inst. of Neurosci., Eugene, OR

Abstract: Traditionally, studies of sensory encoding have focused on a single brain region or sensory system in isolation using artificial and reduced behavioral tasks. However, producing adaptive natural behavior requires the integration of multiple senses along with information about the organism's current motivational state. Here we approached this problem by studying a complex natural behavior in order to understand how different regions of the brain work in concert. We used an auditory prey capture paradigm, in which mice engage in natural nocturnal hunting behavior to search for and capture crickets in complete darkness using only auditory cues. During auditory prey capture, we found a strong correlation between the activity of auditory cortical neurons and behavioral variables such as cricket speed, suggesting that these neurons are likely responding to acoustic signals from the cricket's movement. We used geometric variables such as speeds and angles to extract behavioral states using autoregressive Hidden Markov Models. Hierarchical clustering of these states and their transition probabilities revealed broad classes such as "search," "detection," and "pursuit." Distinct populations of auditory cortical neurons were active in different states. To determine how motivational state interacts with sensory processing, we optogenetically activated the subthalamic region zona incerta (ZI), which has been implicated in motivational drive to hunt. We acclimated mice to capture prey under both light and dark conditions. After mice acclimated and became skilled at prey capture, we activated ChR2-expressing inhibitory Vgat+ cells in the medial portion of ZI on a subset of prey capture trials. Pilot experiments revealed that activation of ZI, a brain region involved in motivation, drove diverse effects during auditory prey capture. In addition to its role in motivation and complex motor decisions, ZI sends direct inhibitory projections to all of the neocortex and may help modulate sensory representation. In auditory cortex, as well as other cortical regions, these projection cells have been found to preferentially target other inhibitory cells in layer 1. These results indicate a potential locus for the integration of motivational and sensory information during complex tasks, as well as providing a more holistic view on the processing that must occur to generate complex behavior.

Disclosures: M. Shallow: None. **M. Wehr:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

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Program #/Poster #: 051.28

Topic: D.05. Auditory & Vestibular Systems

Support: T32-MH019524
R01-DC018802

Title: Precise movement-based predictions in the mouse auditory cortex

Authors: *N. AUDETTE, W. ZHOU, A. LA CHIOMA, D. SCHNEIDER;
New York Univ. Ctr. For Neural Sci., New York, NY

Abstract: Many of the sensations experienced by an organism are caused by their own actions, and accurately anticipating both the sensory features and timing of self-generated stimuli is crucial to a variety of behaviors. In the auditory cortex, neural responses to self-generated sounds exhibit frequency-specific suppression, suggesting that movement-based predictions may be implemented early in sensory processing. Yet it remains unknown whether this modulation results from a behaviorally specific and temporally precise prediction, nor is it known whether corresponding expectation signals are present locally in the auditory cortex. To address these questions, we trained mice to expect the precisely timed acoustic outcome of a forelimb movement using a closed-loop sound-generating lever. Dense neuronal recordings in the auditory cortex revealed suppression of responses to self-generated sounds that was specific to the expected acoustic features, specific to a precise time within the movement, and specific to the movement that was coupled to sound during training. Predictive suppression was concentrated in L2/3 and L5, where deviations from expectation also recruited a population of prediction-error neurons that was otherwise unresponsive. Recording in the absence of sound revealed abundant movement signals in deep layers that were biased toward neurons tuned to the expected sound, as well as temporal expectation signals that were present throughout the cortex and peaked at the position of expected auditory feedback. Together, these findings reveal that predictive processing in the mouse auditory cortex is consistent with a learned internal model precisely linking a specific action to its acoustic outcome, while identifying distinct populations of neurons that anticipate expected stimuli and differentially process expected versus unexpected outcomes.

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Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

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Topic: D.05. Auditory & Vestibular Systems

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Title: Localizing neural activity underlying sensory predictions of naturalistic tone series with intracranial EEG

Authors: *T. J. BAUMGARTEN[1], R. HARDSTONE[1], A. FLINKER[2], S. DEVORE[2], D. FRIEDMAN[2], P. DUGAN[2], W. K. DOYLE[3], O. DEVINSKY[2], B. J. HE[4];
[1]Neurosci. Inst., [2]Dept. of Neurol., [3]Dept. of Neurosurg., [4]Neurosci. Institute, Dept. Neurology, Dept. Neurosci. and Physiology, Dept. Radiology, New York Univ. Langone Hlth., New York, NY

Abstract: Effective responses to natural stimuli require anticipation of upcoming stimulus changes. Continuous natural stimuli such as speech, music, and natural soundscapes exhibit specific statistical properties—following a 1/f type temporal power spectrum, which allows the prediction of future stimuli based on past stimuli. Since predictions of future sensory input must be based on the integration of past sensory information over time, this process likely requires the coordination of multiple brain areas. Indeed, previous studies showed that both frontal and sensory areas act in concert to orchestrate sensory prediction: frontal areas generate low-frequency activity holding predictive information (i.e., prediction signals), whereas high-frequency activity emerges in sensory areas when incoming sensory information disagrees with current predictions (i.e., prediction error signals). However, these results are largely based on simple and artificial paradigms, where prediction is based on repetition or fixed transition probabilities. While our previous MEG work showed that prediction and history integration for stimuli with natural statistics are carried by slow aperiodic neural activity, it remains unclear where in the brain these processes are located. Here, we made use of the excellent spatial and temporal precision of intracranial EEG (iEEG) to localize neural correlates of prediction, prediction error, and history integration by presenting subjects with auditory tone sequences whose pitch fluctuations follow the power spectral patterns commonly found in natural soundscapes. Nine patients undergoing surgical evaluation with iEEG monitoring listened to tone sequences and predicted the ending of each sequence. We asked in which cortical regions and spectral bands the different prediction-related components operate and how these neural processes interact to predict upcoming stimulus trajectories. We found that neural prediction signals were mostly carried by slow aperiodic activity in both frontal and superior temporal electrodes. Prediction error signals were evident in both high frequency (70-150 Hz) and slow aperiodic activity, with high frequency components restricted to sensory areas and slow components spread around frontal, temporal, and parietal regions. Neural correlates of history integration were mainly found in slow aperiodic activity and was widespread across the cortex. In sum, our results suggest that neural underpinnings of prediction for time-varying stimuli with statistical structures similar to natural stimuli spatially and spectrally extend beyond findings based on more simplistic prediction tasks.

Disclosures: T.J. Baumgarten: None. **R. Hardstone:** None. **A. Flinker:** None. **S. Devore:** None. **D. Friedman:** None. **P. Dugan:** None. **W.K. Doyle:** None. **O. Devinsky:** None. **B.J. He:** None.

Poster

051. Auditory Processing: Perception and Cognition

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 051.30

Topic: D.05. Auditory & Vestibular Systems

Support: NSERC Grant RGPIN-2021-02602

Title: Neural responses to frozen noise snippets in sounds are invariant to temporal irregularities

Authors: *B. HERRMANN;
Baycrest, Rotman Res. Inst., Toronto, ON, Canada

Abstract: Detecting structure in sounds is fundamental to human auditory perception. A prime example of this ability is that listeners can detect repetitions of random white-noise snippets embedded in a longer white noise. Detection of repeating noise snippets is facilitated when noise snippets are embedded at a temporally regular rate that enables neural activity to synchronize with snippet occurrences. The current study investigates the neural mechanisms underlying the sensitivity to repeated noise structure in sounds. We ask whether neural oscillatory activity elicited by periodic snippet presentations continues (i.e., sustains) throughout an omission of a noise snippet and whether the neural response to a noise snippet declines when it is preceded by a snippet omission.

In two electroencephalography (EEG) experiments, participants (18-34 years) listened to ~5-s white noises in which repetitions of a short white-noise snippet were embedded (~0.2 s). Participants judged whether a repetition was present. In Experiment 1, ~5-s white noises contained either six, seven, or eight repeating noise snippets at a constant onset-to-onset interval (0.5 s). In a fourth condition, six snippets were presented in succession (at 0.5 s intervals), followed by an omission, and a snippet in the 8th position. Results showed that rhythmic neural activity did not continue during the omission of a noise snippet, suggesting the absence of oscillatory sustainability. The neural response to the noise snippet following an omission was as large as the response to the noise snippet that was not preceded by an omission, suggesting response invariance once a neural representation of a noise snippet is established. In Experiment 1, the noise snippet following the omission was presented temporally aligned with the preceding periodicity, and it was unclear whether the response invariance was due to an oscillatory process not measured with EEG. Hence, in Experiment 2, six repeated snippets were embedded in the ~5 s white noise, followed by an omission, and a snippet in the 8th position that occurred either early, on-time, or late relative to the preceding stimulus periodicity. Preliminary data suggest that the auditory system is similarly sensitive to early, on-time, and late snippets following the omission.

The results of this study suggest that, once the auditory system establishes a neural representation of repeating structure in sounds, subsequent occurrences elicit a neural response independent of whether the structure is temporally predicted. This invariance may help a listener to recognize newly learned sound structure in different contexts.

Disclosures: B. Herrmann: None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.01

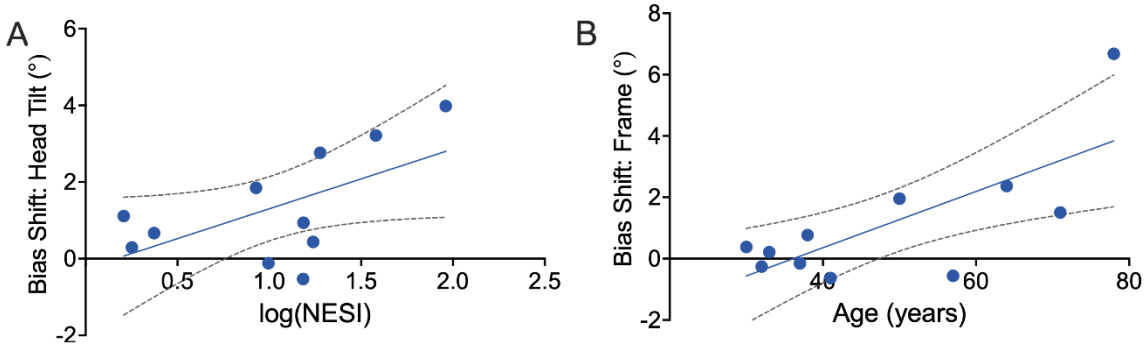
Topic: D.05. Auditory & Vestibular Systems

Support: 1R01AG073157

Title: Effects of Noise Exposure on Verticality Perception with and without Head Tilt

Authors: *A. VELISAR, C. P. AGATHOS, N. M. SHANIDZE;
The Smith-Kettlewell Eye Res. Inst., San Francisco, CA

Abstract: Reduced vestibular function plays a significant role in elevated fall risk (Agrawal et al., 2012). Noise exposure has been shown to damage the vestibular periphery (Stewart et al., 2020), yet is often overlooked as a potential source of vestibular damage and thus a contributing factor in increased fall risk (e.g., Picard et al., 2008; Girard et al., 2014). Visual vertical orientation estimation relies to a large extent on vestibular sensation (Angelaki et al. 2020). At the same time, vestibular aging is thought to contribute to older adults' increased visual dependence for perception and postural control, classically evaluated via subjective visual vertical (SVV) estimation tasks (Alberts et al., 2019).To determine degree of noise exposure, 11 healthy adults (age range: 30-78) completed the Noise Exposure Structured Interview (NESI, Guest et al. 2018). They also performed 3 SVV tasks while seated in darkness viewing stimuli on a large screen through an optical tube and with the head resting in a head and chin rest. They were instructed to align a tilted bar (length=20º, width=0.9º visual angle) to vertical using a keyboard. The task was repeated with a head tilt of ~20º in roll, thus affecting vestibular signal reliability. Finally, to assess visual dependence, the SVV was estimated in the presence of a tilted square frame (diagonal: 28º, tilt: ±18º). 12 trials were repeated for each task and absolute error from true vertical was determined.We found that the shift in SVV with the head tilted versus upright was associated with the degree of noise exposure (A, Pearson r=0.59, p=0.05), while the degree of visual dependence significantly correlated with age (B, r=0.74, p=0.001).Our findings suggest a potential link between the physiological effects of noise exposure on the vestibular periphery and functional outcomes. While there appears to be an association between noise exposure and vestibular contribution to verticality perception, this effect is likely independent from the mechanism contributing to older adults' increased visual dependence.



Disclosures: **A. Velisar:** None. **C.P. Agathos:** None. **N.M. Shanidze:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.02

Topic: D.05. Auditory & Vestibular Systems

Support: NIH/NIDCD R01-DC018287
ONR MURI N000142012163
NASA NCC 9-58

Title: The role of vestibular noise in closed-loop self-orientation control

Authors: K. LOVE[1], M. J. ROSENBERG[2], R. GALVAN-GARZA[3], T. K. CLARK[4], *F. KARMALI[5,1];
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Abstract: Performance in closed-loop tasks like postural control and piloting, where motor outputs are sensed by sensory organs and used to plan new motor commands, is thought to be degraded by sensory and motor noise (i.e., variability) [1]. The way sensory noise affects closed-loop tasks has been studied using theoretical models and experiments, but not by applying modeling to experiments in which sensory noise was measured, to our knowledge. Specifically, models of postural control predict that noise arising in closed-loop control can be amplified by feedback and result in behavioral variability. Experimental studies have examined correlations between behavioral variability (e.g., postural sway) and sensory noise assayed using perceptual thresholds [2], but these correlations do not account for behavioral variability being fed back in to sensory signals. To address this, here we developed closed-loop models of our published experimental results [2]. In the experimental study, subjects sat in the dark on a motorized chair that could be roll tilted using a joystick. Subjects were instructed to use the joystick to align themselves with their perceived upright while they experienced a random disturbance. This study was conducted on a centrifuge which simulated altered gravity environments and subject performance was studied in different G levels. We quantified behavioral precision by the standard deviation of chair position over time. We found better behavioral performance in subjects with lower vestibular perceptual thresholds (i.e., less noisy) and in higher G levels. In the closed-loop model, sensed tilt orientation was used to determine joystick commands. Consistent with decision-making theory, we assumed that subjects would only be able to make a joystick command once they had reliable sensory information - i.e., the sensed tilt exceeded their sensory noise. We also assumed that each subject had an individual “effort” that determined how intensely they reacted to an error. Our model closely predicted human closed-loop responses to disturbances. In particular, it predicted better performance for subjects with lower vestibular perceptual thresholds, supporting the hypothesis that vestibular noise worsens performance

during closed-loop behavior. It also predicted better performance in higher G levels, consistent with experimental data, supporting the hypothesis that high G level increases otolith signal-to-noise ratio, resulting in better performance. [1] Diaz-Artiles A and Karmali F. 2021. Neuroscience, 468: 282-320. [2] Rosenberg, MJF, R Galvan-Garza, TK Clark, DP Sherwood, LR Young, and F Karmali. 2018. J Neurophysiol, 120: 3187-97.

Disclosures: K. Love: None. **M.J. Rosenberg:** None. **R. Galvan-Garza:** None. **T.K. Clark:** None. **F. Karmali:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.03

Topic: D.05. Auditory & Vestibular Systems

Support: NSF Grant No. #1920896

Title: Natural statistics of heading in humans: Implications for modeling heading perception

Authors: *C. B. SINNOTT[1], P. HAUSAMANN[2], P. R. MACNEILAGE[1];
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Abstract: Heading, the direction of linear self-motion of the head relative to the stationary environment, is estimated by the human nervous system using linear acceleration sensed by the otoliths of the vestibular system, and optic flow sensed by the visual system. Prior work has demonstrated significant repulsive biases in visual and vestibular heading azimuth perception, meaning that the heading azimuth angle is perceived to be more eccentric than the presented stimulus. Modeling work suggests that these biases can be explained by efficient coding and Bayesian decoding, both of which are shaped by the natural distribution of heading angles experienced during daily life. Unfortunately, the natural distribution of heading angles during normal, everyday activities have not yet been sufficiently characterized to inform these models. In the current study we present measurements obtained using the Intel RealSense T265, a tracking camera that uses both visual and inertial data to generate robust estimates of 6 degree of freedom position and velocity. Ten clinically normal participants wore this camera on their heads during five hours of unstructured, everyday activity. The camera was connected to a laptop worn in a backpack. Across all participants, heading azimuth and elevation were centered close to straight-ahead with much greater variability in azimuth than elevation. Additionally, azimuth was multimodal with modes at 0° and ±90° degrees while elevation was unimodal. Heading distributions were used in an efficient coding and Bayesian decoding model (Wei and Stocker, 2014) to predict biases in heading perception. Two free parameters of the model (sensory and stimulus noise) were adjusted to optimize the fit between modeled bias and bias observed in previous psychophysical work (Crane, 2014; Cuturi and MacNeilage, 2013). Next we considered the impact of sampling on natural stimulus distributions. Empirical priors differ across

individuals and activities, leading to quantitatively different patterns of bias predicted by the model. Additionally, priors on visual heading should likely be considered in a retinal reference frame. In ongoing work, we are modeling visual heading bias by collecting statistics of heading in eye-based, rather than head-based coordinates via joint measurement of head and eye movements.

Disclosures: C.B. Sinnott: None. **P. Hausamann:** None. **P.R. MacNeilage:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.04

Topic: D.05. Auditory & Vestibular Systems

Support: Lockheed Martin sponsored project

Title: Modeling the effects of galvanic vestibular stimulation on perceived head orientation

Authors: *T. HYATT[1], O. REFY[2], R. C. GALVAN-GARZA[4], M. D. ZIEGLER[4], D. J. WEBER[3];
[1]Mechanical negineering, Carnegie Mellon Univ., PITTSBURGH, PA; [2]Mechanical Engin., [3]Neuroscience, Mechanical Engineering, Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA; [4]Advanced Technol. Labs, Lockheed Martin, Arlington, VA

Abstract: Sensory feedback is a crucial component of motor control, particularly for physically interactive tasks such as grasping and manipulating objects. This is evident during telemanipulation and virtual reality activities, where user experience and motor performance depends critically on the types and quality of surrogate sensory feedback that are provided. Visual and auditory feedback can be displayed with high fidelity, and even haptic feedback can be provided through robotic or vibrotactile displays. However, while the sense of body orientation and motion also play an important role in motor control, vestibular inputs are not typically rendered in VR or teleoperation applications. Galvanic vestibular stimulation (GVS) is a way to modulate signaling in vestibular afferents, evoking simple sensations of body orientation and motion. However, implementing GVS in a virtual reality environment requires modeling the perceptual effects of GVS as a function of the stimulation parameters. Here, we performed experiments in 3 able-bodied subjects to characterize the effects of initial head orientation, GVS amplitude, and stimulation duration on the perceived sensation of head tilt induced during GVS, as measured by the induced angular velocity (IAV) of the head. We delivered 1.0 - 2.5mA DC of GVS using an electrode placed on each of the mastoid processes. Stimulation was applied for 0.25 - 2 seconds while the subjects were seated in a chair at a starting head-orientation of 0 - 20 degrees of tilt offset. In all subjects, GVS produced a consistent head-tilt along the frontal plane, with direction dependent on the polarity of stimulation, and amplitude of IAV dependent on the starting angle and current amplitude. An

increase in either current amplitude or starting angle increased the IAV with current amplitude being the more prominent factor, having a maximum independent IAV of 12.1 degrees per second vs. starting angle's maximum independent IAV of 7.9 degrees per second. When modeling the amplitude of the IAV, we found that the effects of GVS on IAV increased as the starting angle increased. This was shown by the delta in IAV between 0 and 20 degrees of starting angle at a low stimulation (1.5mA) being 1.2 and the delta at a high stimulation (2.5mA) being 4.2. Duration of stimulus was found to influence induced motion as a linear function and was independent of other variables tested. A final fitted function using stimulation current, starting angle, and time as variables was created to estimate the induced angular motion driven by GVS, allowing for virtual feedback of orientation and motion to the operator.

Disclosures: **T. Hyatt:** None. **O. Refy:** None. **R.C. Galvan-Garza:** A. Employment/Salary (full or part-time):; Lockheed Martin. **M.D. Ziegler:** A. Employment/Salary (full or part-time):; Lockheed Martin. **D.J. Weber:** F. Consulting Fees (e.g., advisory boards); Bionic Power, Inc., BlackFynn, Inc., Iota Biosciences, Inc., Neuronoff, Inc., Neuroone, Inc., Reach Neuro, Inc..

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.05

Topic: D.05. Auditory & Vestibular Systems

Support: Région Normandie/FEDER
French Ministry of Higher Education and Research Grant for Emma Milot's PhD

Title: Effects of a ten-day galvanic vestibular stimulation on sleep in seniors

Authors: E. MILOT, S. RÉHEL, N. BESSOT, ***G. QUARCK**;
COMETE INSERM U1075, Univ. of Caen, Caen, France

Abstract: **Background** The vestibular system is responsible for sensing every angular and linear head acceleration, mainly during periods of activity. Previous animal and human experiments have shown biological rhythm disruptions in small rodents exposed to a hypergravity environment, but also in patients with bilateral vestibular loss compared to a control population. There are evidence that vestibular pathologies induce sleep disturbances which can be partly explained by the strong link between the sleep/wake cycle and biological rhythms. This raised the hypothesis of the vestibular afferent influence on sleep. Galvanic Vestibular Stimulation (GVS) is a non invasive, safe and useful tool to stimulate vestibular afferents. The present study targets the impact of a 10-day GVS program on objective sleep parameters in seniors with sleep complaints.**Method** 18 seniors declaring sleep complaints participated in this study. 10 participants (4 women, 6 men aged 64.7 ± 2.79) underwent a 10-morning-scheduled GVS home program over two weeks. 8 seniors (5 women, 3 men aged 63.9 ± 3.5) underwent an health-education course (active control group) via videoconferencing over two weeks. During the GVS

a direct current (1mA) was applied on the mastoid for twenty minutes. Sleep was evaluated by actigraphy. Subjects continuously wore the actigraph on the non-dominant hand, one week before the program (baseline) and one week one day after the program (post-treatment).**Results** Statistical analysis did not reveal significant differences in actigraphy between the 2 conditions (vestibular stimulation, active control) for M10 (individual's most active 10 h), L5 (individual's least active 5 h), WASO (wake after sleep onset), sleep efficiency and sleep latency. The GVS group presented a significative decrease in total sleep time (TST) after the treatment compared to the control group (p=0.025).**Discussion**: The expected results were a sleep latency shortening and a sleep improvement (better efficiency, less fragmentation), we didn't observe what we expected but our first results demonstrate that a "chronic" GVS program induces sleep modification . We also recorded polysomnography for our 2 populations, data treatment is in progress and will allow us to characterize further the sleep microstructure.

Disclosures: E. Milot: None. **S. Réhel:** None. **N. Bessot:** None. **G. Quarck:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.06

Topic: D.05. Auditory & Vestibular Systems

Title: Detailed analysis of compensatory saccades in participants with and without motion sickness

Authors: *K. MOÏN-DARBARI[1,2,3], D. PAROMOV[1], B.-A. BACON[4], M. MAHEU[1,2], F. CHAMPOUX[1,3];
[1]Univ. de Montréal, Montreal, QC, Canada; [2]CRIR-IURDPM, Montreal, QC, Canada; [3]CRIUGM, Montreal, QC, Canada; [4]Carleton Univ., Ottawa, QC, Canada

Abstract: Several studies have investigated the integrity of the vestibulo-occular reflex (VOR) in patients with sensitivity to motion sickness. These studies have shown a lower VOR gain in motion sickness sufferers, suggesting a less effective or damaged VOR, but results are still under debate. It has recently been proposed that studying saccades while performing a gaze stabilization task could reveal subtler dysfunctions of the vestibular system. Twenty-four adult participants (19-40 years old) were recruited and divided into two groups, with and without motion sickness, based on the Motion Sickness Susceptibility Questionnaire. All participants were assessed using both passive (induced by experimenter) and active (self-induced) lateral head impulses while stabilizing their gaze on an earth fixed target. Saccade frequency, amplitude and latency were analyzed via a customized Matlab program (R2020a). No significant differences between groups were found for saccade latency and frequency in either task. However, the group with motion sickness demonstrated a significant increase in saccade amplitude during the active task, but on the left side only. Further studies are needed to understand the origin and implications of this lateralization effect.

Disclosures: K. Moïn-Darbari: None. **D. Paromov:** None. **B. Bacon:** None. **M. Maheu:** None. **F. Champoux:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.07

Topic: D.05. Auditory & Vestibular Systems

Support: NIH/NIDCD Grant DC018287

Title: Vestibular dysfunction in neurofibromatosis type 2

Authors: *S. KING[1], A. MADHANI[1], J. ZHU[1], F. KARMALI[1,2], D. B. WELLING[2], J. JORDAN[3], C. HABURCAKOVA[1], R. F. LEWIS[1,2,3];
[1]Massachusetts Eye and Ear, Boston, MA; [2]Otolaryngology Head & Neck Surgery, [3]Neurol., Harvard Med. Sch., Boston, MA

Abstract: Neurofibromatosis Type 2 (NF2) is a genetic disorder characterized by multiple neurologic tumors, notably vestibular schannomas (VS) which often occur bilaterally and originate on the vestibular nerves. Although vestibular symptoms can be disabling, vestibular function has not been carefully studied in patients with NF2. Furthermore, chemotherapy such as bevacizumab has been shown to reduce VS volume and improve hearing in NF2, but nothing is known about its vestibular effects. We evaluated vestibular function across three modalities (eye movements, motion perception, and balance) as well as clinical disability (dizziness, gait impairment), MRI, and hearing in pre-intervention NF2 patients (n=10) as compared to unilateral VS patients (n=38) and normal controls (NC; n=23). We found that there was reduced vestibular precision (inverse of variability, reflecting central signal-to-noise ratio, SNR) but not vestibular accuracy (amplitude relative to ideal amplitude, reflecting central signal magnitude) and increased clinical disability in NF2 patients. In two of the NF2 patients, we studied the effects of bevacizumab, finding that post-infusion, there was increased vestibular precision and improved clinical outcomes, but no effect on vestibular accuracy. We propose that VS in NF2 patients degrades the central SNR by generating noise on vestibular afferents, while bevacizumab improves SNR by suppressing afferent noise.

Disclosures: S. King: None. **A. Madhani:** None. **J. Zhu:** None. **F. Karmali:** None. **D.B. Welling:** None. **J. Jordan:** None. **C. Haburcakova:** None. **R.F. Lewis:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.08

Topic: D.05. Auditory & Vestibular Systems

Title: Investigating the relation between vestibular reflex and self-motion perception may help distinguish Meniere's disease from vestibular migraine

Authors: *A. CÉDRAS[1,2], A. S. PIERRE[1], M. MAHEU[1,2];
[1]Univ. of Montreal, Montreal, QC, Canada; [2]CRIR-IURDPM, Montreal, QC, Canada

Abstract: Introduction It is known that there is an overlap between the diagnostic criteria of Meniere's disease (MD) and vestibular migraine (VM). This, coupled with the lack of an objective measure to distinguish these two conditions, delays the management of these patients. Recently, a study has shown that although the vestibular reflexes are comparable between the two pathologies, the vestibular perception could be different. This suggests a possible discrepancy between vestibular reflex and perception. However, to date no studies investigated this relationship and how it differs between MD and VM. **Objective** To compare the relation between VOR and vestibular perception following caloric stimulation between healthy participants, participants with MD and participants with VM. **Method** 20 participants divided into 3 groups (Controls: 12; Meniere's: 6; Vestibular migraine: 3) were evaluated. Participants performed a caloric assessment during which the velocity of the slow phase component of the nystagmus was recorded. For each caloric irrigations, participants were asked to indicate the perceived velocity of the induced rotation using a tachometer. A ratio between the maximum angular velocity of rotation of the tachometer and the maximum velocity of nystagmus was calculated. This variable was compared between the 3 groups using a nonparametric test (Kruskall-Wallis) with Bonferroni correction. **Results** Preliminary results show that the dissociation between VOR and vestibular perception is significantly different between participants with vestibular migraine and healthy participants [p=0.005] and between participants with vestibular migraine and those with Meniere's [p=0.030]. **Conclusions** For the first time, these preliminary results not only demonstrate a method for assessing vestibular perception following caloric stimulation, but also demonstrate the possibility of distinguishing Meniere's disease from vestibular migraine.

Disclosures: **A. Cédras:** None. **A.S. Pierre:** None. **M. Maheu:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.09

Topic: D.05. Auditory & Vestibular Systems

Support: NIH DC002390

Title: Biomimetic vestibular stimuli from a prosthesis improve dynamic postural responses to support surface tilts in nonhuman primates

Authors: *O. M. E. LEAVITT, K. E. CULLEN;
Johns Hopkins Univ., Johns Hopkins Med. Institutions, Baltimore, MD

Abstract: Bilateral vestibular loss (BVL) patients remain at an increased risk of falls even following rehabilitation. A promising solution is stimulating vestibular afferents via a prosthesis. Clinical trials have reported improvements in quality of life, but the prosthesis does not effectively restore natural reflexes. We hypothesize that the prosthesis may be made more effective by applying mapping functions between head motion and stimulation pulse rate which replicate the endogenous dynamics of vestibular afferents. Thus, here we tested the effects of applying naturalistic prosthesis mapping functions on dynamic balance in a rhesus monkey model.

We first established benchmark postural response dynamics by applying transient support surface motion to 2 normal animals. Perturbations comprised roll tilts at 3 accelerations (200, 500, 1000 deg/s^2) and 3 velocities (20, 40, 80 deg/s). Head motion was measured by IMU, ground reaction torque by force plate, and kinematics by markerless motion tracking. As previously shown in humans, normal animals tilted opposite the direction of the platform motion to restore an earth-vertical orientation. The amplitude and latency of head motion depended on the angular acceleration of the platform, while peak torque depended on the angular velocity.

We then determined the contribution of the vestibular system to normal postural responses by repeating this study in a BVL animal. Head motion in the BVL animal had shorter latency, greater amplitude, and was in the opposite direction to the normal animal response. Additionally, peak torque was hypometric to normal. Rather than acting in a compensatory manner, this maladaptive response increased body tilt with respect to gravity. In contrast to observations in normal animals, responses in the BVL monkey were dependent on both velocity and acceleration of the platform.

Finally, we tested the effectiveness of naturalistic prosthesis mapping functions for restoring dynamic balance. We repeated the study while delivering pulsatile stimuli mimicking the endogenous dynamics of regular and irregular afferents, a highly dynamic “super-irregular” mapping, and a static mapping currently used in clinical trials. Static and regular mappings only slightly reduced head motion amplitude without changing latency, while the super-irregular mapping produced a shorter-latency response with no change in amplitude. Stimulating with an irregular mapping resulted in the best performance, restoring both the amplitude and latency of head motion responses. Our findings demonstrate that leveraging endogenous vestibular afferent dynamics has the potential to improve postural control outcomes.

Disclosures: O.M.E. Leavitt: None. **K.E. Cullen:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.10

Topic: D.05. Auditory & Vestibular Systems

Title: Vibration thresholds in lower limbs and its contribution to fall risk assessment

Authors: A. S. PIERRE[1], A. M. CÉDRAS[1,2], D. PAROMOV[1], P. GERMAIN[1], K. MOÏN-DARBARI[1,2,3], F. CHAMPOUX[1,3,2], M. MAHEU[1,2];
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Abstract: Introduction: Maintaining postural stability requires the integration of multiple sensory inputsuch as vestibular, somatosensory and visual cues. The function of these sensory systems isknown to deteriorate with normal aging contributing to higher risk of falls in elderly. One way toprevent falls is to screen for sensory deficits to rapidly compensate the deficit. Even thoughseveral methods exist to screen for hearing, visual and vestibular function, the methods to screenfor somatosensory loss are limited. It has been proposed that vibration threshold using a bonevibrator (ankle audiometry) could be a valuable tool in screening for neuropathy. This vibratorbeing widely available in audiology clinics could become an interesting tool to screen forsomatosensory loss in fall risk prevention. However, no previous studies demonstrated thespecificity and sensitivity of this method to distinguish between fallers and none-fallers.
Objective: To assess the difference in lower limbs vibration threshold as measured by ankleaudiometry between fallers and non fallers. Secondarily, this project aims at assessing therelation between hearing, vestibular function and postural control stability.
Method: We recruited participants aged between 65 and 80 years old. Participants weresubdivided in two groups (fallers and non-fallers) based on history of a fall in the past year. Allparticipants performed a hearing test (hearing thresholds), video head impulse test (VOR gain),ankle audiometry (vibration thresholds on big toe, ankle and tibia) and the modified clinical testof sensory integration and balance on a force platform (sway velocity).
Results: Preliminary results reveal that vibration thresholds (ankle audiometry) and swayvelocity are significantly higher in the group of fallers as opposed to none-fallers. Moreover, wemeasured a significant negative correlation between sway velocity and VOR gain in fallersgroup. However, hearing thresholds did not correlate with sway velocity.
Conclusions: These preliminary results support the use of ankle audiometry in fall riskassessment, where higher vibration threshold may be associated with a higher risk of falls.Larger sample size will allow to compute sensitivity and specificity of the test to distinguishbetween faller and none-fallers.

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Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.11

Topic: D.05. Auditory & Vestibular Systems

Support: Région Normandie-00115524-21E06581

Title: Effects of vestibular stimulation on the perception of time and space and their interference

Authors: D.-C. NAVARRO-MORALES, O. KULDAVLETOVA, G. QUARCK, G. CLÉMENT, ***P. DENISE**;
COMETE U1075 Inserm, Univ. Caen, Caen CEDEX, France

Abstract: Introduction Time and space are closely intertwined dimensions in the brain. For example, judgement of stimulus length is affected by its concurrent duration, and vice versa. However, at present, our knowledge about the interdependence between the representation of our environmental space and our perception of time is scarce. Using virtual reality, Riemer et al. (2017) found bidirectional interaction between space and time in static condition but unidirectional interaction during virtual linear displacement: time perception was influenced by the distance travelled but not vice versa. Currently, no experiments have investigated the interference between space and time during real self-motion. Here, we investigated space-time interactions during whole-body rotations.**Methods** Perceived angular amplitude and motion duration were explored in 32 healthy volunteers during whole-body rotations in the dark (amplitude 60° or 120°, duration 2s or 4s). In a time perception task, subjects were instructed to reproduce the perceived duration of the rotation by pressing a button. In a space perception task, subjects were instructed to reproduce the perceived amplitude by pressing a button that induced a rotation in the same direction of the first rotation but at a different velocity. 32 rotations per task were applied. **Results and discussion** During time perception task, larger amplitudes of rotation are perceived as lasting longer. During space perception task, there was an interaction between amplitude and time perception: small rotation amplitude is perceived larger when rotation is longer, while, on the contrary, large rotation amplitude is perceived smaller when rotation is longer. This interaction could be explained by two opposite effects: i) a positive cross modal effect of duration on the perception of amplitude ii) a negative effect of the vestibular perceptual time constant on the perceived amplitude of rotation.**Conclusion** This experiment showed for the first time that, during a whole-body rotation, the judgement of stimulus amplitude is affected by stimulus duration, and vice versa. This bidirectional interaction supports the theory of magnitude (ATOM) which suggests that perceptions of space and time are derived from the same general magnitude system (Walsh, 2003).**References**Riemer, M., Shine, J. P., & Wolbers, T. (2018). On the (a)symmetry between the perception of time and space in large-scale environments. *Hippocampus*, *28*(8), 539-548.Walsh, V. (2003). A theory of magnitude: Common cortical metrics of time, space and quantity. *Trends in Cognitive Sciences*, *7*(11), 483-488.

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Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

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Program #/Poster #: 052.12

Topic: D.05. Auditory & Vestibular Systems

Support: NSF Grant CHS-1901423
University of Minnesota University Research Scholar award

Title: Active head tilt during use of a head-mounted display

Authors: *T. A. STOFFREGEN[1], G. S. BAILEY[2], D. ARRUDA[2], E. GARZA[2];
[1]Kinesiology, Univ. Minnesota, Minneapolis, MN; [2]Kinesiology, Univ. of Minnesota, Minneapolis, MN

Abstract: Motion sickness is common when virtual locomotion (walking, driving, or flying) is presented via head-mounted displays (HMD). Many methods have been proposed to prevent or reduce motion sickness in this setting. One such proposal is for software to tilt the visual scene in the direction of left/right turns. The hypothesis is that a tilted visual scene could help to reduce the extent to which users tilt their own heads (as they normally would in a physical vehicle). The potential value of such an intervention rests on the assumption that people actually tilt their heads during turns in head-mounted virtual reality. Yet no existing research has evaluated actual head tilt among HMD users. Accordingly, an assessment of active head tilt among HMD users was the primary motivation for our study. Our second motivation relates to sex. In HMD-based virtual reality, motion sickness is more common among women than men. In addition, patterns of postural activity that are related to motion sickness differ between women and men. For these reasons, we separately evaluated head tilt in adult male (19) and female (22) participants. In a between-participants design, some participants drove a virtual automobile around a flat track, while others drove the same virtual vehicle around a banked track. Participants controlled the vehicle using a steering wheel and associated foot pedals (accelerator and brake). Each participant completed a single trial, lasting 15 minutes. We evaluated the kinematics of the head and torso during virtual driving. Results revealed that in the roll axis, mean tilt of the head relative to the torso differed between the flat- (M = 2.07 degrees, SD = 3.03 degrees) and banked-track (M = 0.08 degrees, SD = 3.43 degrees) conditions ($F_{1,37} = 4.43$, $p = 0.042$, partial eta-squared = 0.107). However, a significant Condition × Sex interaction, $F_{1,37} = 4.36$, $p = .044$, partial eta-squared = 0.105), revealed that tilt differed between conditions in men ($M_{Flat} = 2.16$ degrees, SD = 2.75 degrees; $M_{Banked} = -1.81$ degrees, SD = 3.41 degrees) but not in women ($M_{Flat} = 2.14$ degrees, SD = 3.35 degrees; $M_{Banked} = 1.98$ degrees, SD = 2.29 degrees). The results confirm that adults can use head tilt while driving a virtual vehicle presented via an HMD, and that they can vary the magnitude and direction of tilt in response to variations in the depicted tilt of virtual environments. However, the latter effect was observed only among male participants. This sex difference may be related to the existence of sex differences in motion sickness in virtual environments, and may indicate that sex should be taken into account in the design of mitigation techniques.

Disclosures: T.A. Stoffregen: None. **G.S. Bailey:** None. **D. Arruda:** None. **E. Garza:** None.

Poster

052. Vestibular Perception, Posture, and Spatial Orientation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 052.13

Topic: D.05. Auditory & Vestibular Systems

Support: French Ministry of Higher Education and Research Grant for E. MILOT PhD
The European Union, the Normandy Region within the framework of the FEDER/FSE 2014-2020 operational program and the State

Title: Effect of a galvanic vestibular stimulation protocol on static balance in older adults

Authors: *E. MILOT, S. REHEL, A. LANGEARD, N. BESSOT, G. QUARCK;
Univ. of Caen, Caen, France

Abstract: Introduction: One-third of people aged 65 years old and older fall at least once a year. The impact of falls leads to loss of independence. The reasons are numerous: advanced age is often accompanied by a decrease in functional capacities characterized by a reduction of muscular function as well as sensory and neurological systems degradation. Decline in muscular mass can impair postural control of seniors. Alterations also concern sensory systems involved in the detection of body displacements, as vestibular system, resulting in an impairment of sensory information integration that might increase postural control deficits. Studies conducted in our lab previously demonstrated that rehabilitation of the muscle of the lower limb using physical activity or electromyostimulation can improve postural control in seniors. The next step is now to investigate the impact of sensory stimulation on postural control in seniors. The aim of the present study is to measure the effect of a 10-day remote chronic galvanic vestibular stimulation (GVS) protocol on static balance in older adults. **Methods:** Eighteen healthy older adults completed this study. Ten participants (4 women; mean age: 64,7 ± 2.79 y.o.) received ten GVS home sessions over two weeks. During the GVS a direct current (1mA) was applied on the mastoid processes for 20 minutes. Eight other participants (5 women; mean age: 63,9 ± 3.5 y.o.) received a health-education course (active control group) via videoconferencing over two weeks. Balance was assessed by a standard force platform before and after the 2-week intervention. Static postural control was assessed in different conditions: eyes opened (EO) and eyes closed (EC), and this was completed by limits of stability (LoS). Positions and the maximum oscillation amplitude of the centre of pressure were collected in both anteroposterior and mediolateral directions. **Results:** Statistical analysis did not reveal significant differences in static balance pre- and post-intervention in any of the three conditions (EO, EC, LoS). We did not observe any effect of chronic GVS on static balance compared to control group. **Conclusion:** The expected results were a decrease in the centre of pressure positions in EO and EC condition, and an increase in amplitude of oscillation of the centre of pressure in LoS condition. To our knowledge, this is the first study evaluating the effect of chronic GVS on postural control and balance in older adults. Also, this study is the first attempt to adapt the GVS to the remote home-based mode. Further data analysis and then modified protocol has to be conducted before determining if chronic GVS is efficient in improving static postural control in seniors.

Disclosures: **E. Milot:** None. **S. Rehel:** None. **A. Langeard:** None. **N. Bessot:** None. **G. Quarck:** None.

Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.01

Topic: D.06. Vision

Support: Kakenhi 20H00521
Kakenhi 21K18267
Kakenhi 21H00211
Kakenhi 21K07262
Kakenhi 21H05060
Kakenhi 21K20303
Kakenhi 17H00891

Title: Spatiotemporal profile of representation of visual categories revealed by concurrent fMRI-EEG deep neural decoding in humans

Authors: N. WATANABE[1], K. MIYOSHI[2], K. JIMURA[3], D. SHIMANE[1], R. KEERATIVITTAYAYUT[1], K. NAKAHARA[1], ***M. TAKEDA**[1];
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Abstract: Deep neural networks (DNNs) have achieved state-of-the-art results for the large-scale image recognition, and they have recently been applied to neural data in a procedure known as neural decoding. In this study, we investigated how a DNN captures the categorical representation of visual objects from concurrent fMRI-EEG. During the experiment, participants (n = 50) performed a visual object classification task (face/object). For the DNN classifier, we constructed a three-dimensional convolutional neural network and a temporal convolutional network for fMRI and EEG, respectively. Hyperparameters were optimized based on a grid search implemented in the train/validation datasets (n = 45), and then generalization performance was assessed using an independent test dataset (n = 5). We used guided gradient-weighted class activation mapping (guided Grad-CAM) to identify brain regions involved in processing class-discriminative information related to the visual categories. For both fMRI and EEG, the generalization performance of categorical classification (face/object) was statistically above chance (chance level of 50%, t-test against zero, false discovery rate $q < 0.05$) when no trials were averaged and performance improved as a function of the number of averaged trials (N =9 trials). In subcategorical classification (e.g., male-face/female-face or natural-object/artificial-object), fMRI did not show significant performance, whereas EEG showed significant performance. Furthermore, combination of both DNNs improved classification performance for both categorization and subcategorization. Guided Grad-CAM analysis revealed that in

categorical classification, the fMRI DNN valued brain-wide regions identified in the univariate analysis. Interestingly, the EEG DNN valued the earlier phase of neural responses for categorization and the later phase of neural responses for subcategorization. These deep learning-based results demonstrate a categorization principle in which visual objects are represented in a spatially and temporally organized manner.

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.02

Topic: D.06. Vision

Support: EOS HUMVISCAT G0E8718N
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Title: Comparison of representational dynamics for different categorical distinctions in object space using multivariate EEG

Authors: *G. LEYS[1], J. B. RITCHIE[2], A. VON LEUPOLDT[3], H. OP DE BEECK[1];
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Abstract: Speed and efficiency are hallmarks of human cognition with the human brain being able to receive sensory input, integrate and process incoming information from different modalities and provide relevant sensory outputs within a range of hundreds of milliseconds. Human visual perception is a prime example of this, with its ability to correctly identify objects already from 100ms onwards. An important brain area involved in object identification and categorization is the occipitotemporal cortex (OTC) where different regions encode for distinct categories, such as faces and bodies. However, besides this category selectivity, OTC organization also seems to suggest a clustering of these object representations along more abstract dimensions, such as animacy. In this study, we aimed to compare the temporal dynamics of the representation of these different dimensions. To do this, we set up an EEG study (n=25) with a carefully constructed visual stimulus set that allowed for investigation of the temporal dynamics of the face/body division, animate/inanimate distinction and animal taxonomy using multivariate pattern analysis (MVPA). All three factors show higher-than-chance level decoding, with highest peak accuracy found for the face/body division around 150ms after stimulus onset. After this peak, we notice a steady decline over time until it no longer reaches significance from 400ms onwards. The same temporal pattern is found for taxonomy, with peak decoding around 150ms and a strong decline for the rest of the trial. Animacy has lower decoding accuracy

overall, but shows more consistency over time. Single stimulus decoding also proved to be successful with peak accuracy around 100ms and staying significant throughout the whole trial. When comparing theoretical models with our EEG data, we see the strongest contribution of the low-level GIST model in the early stages of processing. Afterwards, the neural patterns of our EEG data were mostly explained by the face/body model, and later also by the taxonomy model. We made the same comparison of our EEG data with previously gathered fMRI data and results show an early contribution of V1 processing. Afterwards, V1 correlation drops down, and lateral and ventral OTC processing take over. However, we do see a recurrence of V1 processing which might point towards feedback connections. Our findings are consistent with the hierarchical nature of visual processing with low level features being processed first by lower regions (V1), and higher regions (OTC) encoding more complex object features. All three categorical factors are of importance in OTC for object processing, with similar but not identical temporal dynamics.

Disclosures: G. Leys: None. **J.B. Ritchie:** None. **A. von Leupoldt:** None. **H. Op de Beeck:** None.

Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.03

Topic: D.06. Vision

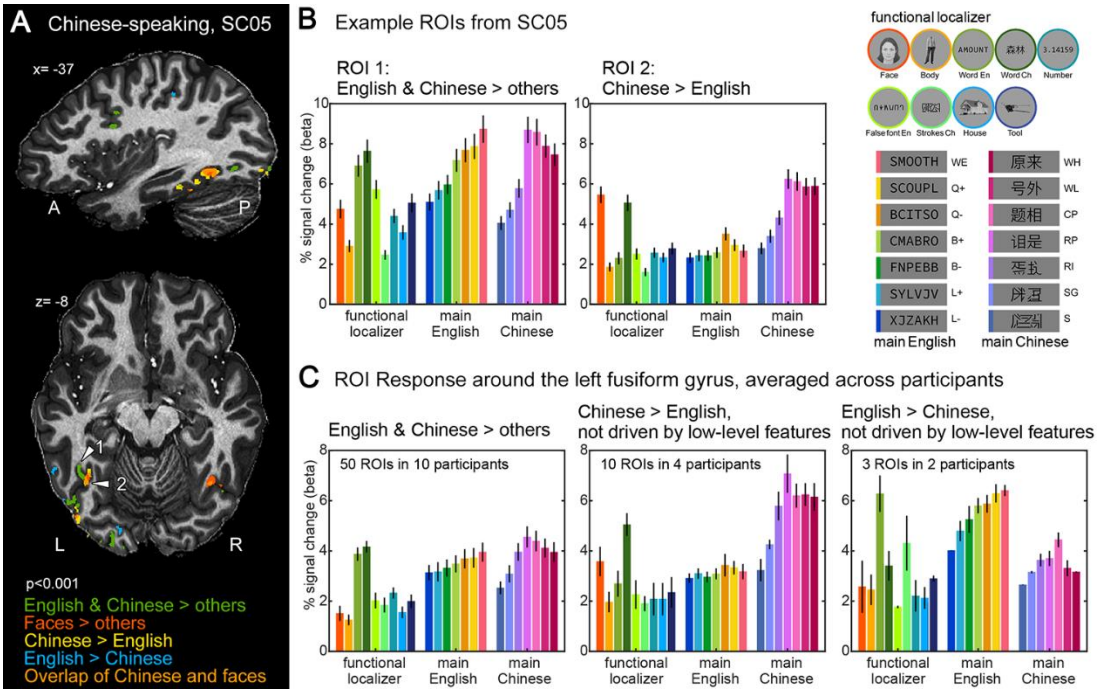
Support: ANR-20-CE37-0002-01 "TOPLEX"
Fondation Bettencourt Schueller

Title: Does the visual word form area split in bilingual readers? A millimeter-scale 7T fMRI study

Authors: *M. ZHAN[1], C. PALLIER[1], S. DEHAENE[1], L. COHEN[2];
[1]UNICOG, NeuroSpin, Gif sur Yvette, France; [2]ICM, ICM, Paris, France

Abstract: During reading acquisition, brain plasticity allows part of the ventral occipito-temporal cortex (VOTC) to specialize for written words. In expert readers, the visual word form area (VWFA) exhibits a posterior-to-anterior gradient of word-similarity effect, showing increased activity to orthographic stimuli whose statistics match those of real words. Here, using high-resolution 7T fMRI, we ask whether this plasticity also affords an even finer specialization in bilingual readers. Do distinct cortical patches implement reading in different languages? Does this depend on whether the languages share the same script? In 21 individual English-French bilinguals, unsmoothed fMRI at 1.2 mm isotropic resolution revealed that the VWFA is actually composed of several small cortical patches highly selective for reading, with a posterior-to-anterior effect of word similarity, but was driven by the two languages in near completely similar manners. In 10 English-Chinese bilinguals, similarly, word-specific patches irrespective of

language again exhibited reading selectivity and word-similarity gradients common to Chinese and English. However, additional patches which did not fully overlap with word-specific patches responded with specificity only to Chinese writing and, surprisingly, to faces. Our results show that the VWFA is composed of small cortical patches finely tuned to the statistics of written scripts, and very different writing systems can indeed tune the visual cortex differently in bilinguals, even leading to ultra-specialization in small cortical patches to a single language.



Disclosures: **M. Zhan:** None. **C. Pallier:** None. **S. Dehaene:** None. **L. Cohen:** None.

Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.04

Topic: D.06. Vision

Support: NIMH Intramural Research Program

Title: Temporal dynamics of facial identity and expression processing from magnetoencephalography

Authors: ***R. KUMAR**, K. BRANNIGAN, L. TEICHMANN, C. I. BAKER, S. JAPEE; NIH, Bethesda, MD

Abstract: Recognition of facial identity and facial expression are both critical for social communication. One model of face perception (Bruce & Young, 1986) proposes that invariant aspects of a face (like identity) and changeable aspects of a face (like expression) are processed by distinct neural pathways (Haxby, Hoffman & Gobbini, 2000). Evidence for this dissociation has come from functional neuroimaging studies, which have implicated the fusiform gyrus in the processing of invariant aspects (Grill-Spector et al., 2004) and the superior temporal sulcus in the processing of changeable aspects of a face (Pitcher et al., 2011). However, the timing of this dissociation has been less studied. Magnetoencephalography (MEG) has recently been combined with machine learning to explore the time course of how we process facial information like age, gender, identity (Dobs et al., 2019), and expression (Dima et al., 2018). However, no study has simultaneously explored the temporal decoding of identity and expression processing from MEG signals. Thus, the present study uses MEG and time-resolved classification methods to examine how facial identity and expression processing unfolds in the human brain. Participants viewed videos of emotional faces from the Karolinska Directed Emotional Faces database (KDEF-dyn; Calvo et al., 2018) that varied along two dimensions (six identities and six expressions) while performing an orthogonal facial motion target detection task. Target trials were later excluded from analysis. MEG data were analyzed using a linear support vector machine classifier (one for each dimension of interest) which used the pattern of MEG sensor activity at each time point during a trial to predict which type of stimulus was presented. The resulting decoding performance reflects the discriminability of the brain activity patterns elicited by each identity and expression. Preliminary results showed successful decoding of both identity and expression. Identity decoding peaked rapidly at around 180 ms after stimulus presentation, while expression decoding rose as the video progressed, peaking around 1 s. Data from additional participants and cross decoding analyses will help further differentiate the time course of identity and expression processing. In addition, source reconstruction analyses will help dissociate the neural substrates that underlie the difference between the processing of the invariant and changeable aspects of a face.

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.05

Topic: D.06. Vision

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MEXT KAKENHI(Grants-in-Aid for Scientific Research) 19K12149
MEXT KAKENHI(Grants-in-Aid for Scientific Research) 22K12189

Title: Are receptive field sizes of area TE neurons related to encoding information about categories of face images?

Authors: *K. SHIOYA[1], S. KATAKAMI[1,2], K. HAYASHI[2], K. MATSUDA[2], N. MATSUMOTO[2], S. AKAHO[2], K. KAWANO[2], M. OKADA[1], Y. SUGASE-MIYAMOTO[2]; [1]Grad. Sch. of Frontier Sci., The Univ. of Tokyo, Kashiwa-shi, Japan; [2]Human Informatics and Interaction Res. Inst., Natl. Inst. of Advanced Industrial Sci. and Technol., Tsukuba city, Japan

Abstract: Our previous study suggested that in area TE of the macaque inferotemporal cortex, neural mechanisms underlying representations about a global category of visual stimuli (monkey vs. human vs. shape images) may differ from those about facial expressions or identities (Sugase-Miyamoto et al., 2014). It is known that receptive field (RF) sizes of visually responsive neurons in area TE vary from neuron to neuron. To examine whether or not there are relationships between RF sizes of face-responsive neurons and their encoding information, neuronal activity was recorded in area TE of two rhesus monkeys (*Macaca mulatta*). The monkeys performed two types of fixation tasks, one for evaluating the amount of information about facial stimuli and the other for determining the RF size within the tested visual field. During the first task, one of sixty colored images (9 monkey faces, 9 human faces, and 2 shapes, each in three different sizes, i.e., 3°x 3°, 6°x 6°, or 12°x 12°) was randomly presented for 400 ms at the center of the CRT-screen. During the second task, one of the 6°x 6° stimuli that evoked strong response during the first task was presented randomly at 15 positions of the screen, as a 3 vertical x 5 horizontal grid, while the monkey fixated at the center of the screen. Based on the responses at the 15 positions in a window 50-250 ms after the stimulus onset, a contour map of the RF of each neuron was obtained by linear interpolation, and the area in the contour map that was at or above 50% of the maximum response was determined as the RF size. Activities of 81 face responsive neurons were recorded and the RF size was measured in 72. The mutual information about monkey vs. human faces and that about facial expression/identity was calculated using the neuronal responses to the stimuli during the first task in a window 50-250 ms after the stimulus onset. The neurons mainly represented the information about the monkey vs. human faces and that about the monkey expression. The information amount was not significantly different across the different sized faces (Wilcoxon rank sum test). The correlation coefficient between the RF sizes and the information amount about the monkey vs. human faces was positive (12°: $r = 0.309$, $p = 0.005$; 6°: $r = 0.287$, $p = 0.015$; 3°: $r = 0.305$, $p = 0.006$), that between the RF sizes and the information amount about the monkey expression was negative (12°: $r = -0.117$, $p = 0.297$; 6°: $r = -0.172$, $p = 0.149$; 3°: $r = -0.092$, $p = 0.412$), and these two correlation coefficients were significantly different (12°: $p = 0.008$, 6°: $p = 0.005$; 3°: $p = 0.004$). These results indicate that area TE neurons with relatively small RFs represented information about monkey expressions regardless of the size of the face images.

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.06

Topic: D.06. Vision

Support: Intramural Research Program at NIMH (ZIA-MH-002909)

Title: Early preference for bodies over faces in emotional expression recognition

Authors: *M. M. SARGEANT[1], K. D. RANA[1], J. TAUBERT[3], L. G. UNGERLEIDER[2], E. MERRIAM[1];
[1]Natl. Inst. of Mental Hlth., [2]Natl. Inst. of Mental Hlth., NIH, Bethesda, MD; [3]Univ. of Queensland, St. Lucia, Australia

Abstract: Evaluating emotional expressions is fundamental to social interactions. While facial expressions, body postures, and biological movements all contain information about emotion, how these separate cues are combined is poorly understood. Given that faces and bodies are thought to be processed independently in the brain, we questioned whether face and body expressions are integrated when perceiving emotion. We conducted a behavioral experiment in which participants evaluated the emotional expression of stimuli created by combining faces and bodies. Face and body combinations were either emotionally congruent, with matching expressions (e.g., fearful body, fearful face), or emotionally incongruent, with mismatched expressions (e.g., fearful body, angry face). To select stimuli for each emotion category (angry, fearful, and neutral), we ran a rating task on the Amazon Mechanical Turk platform. Images that were consistently rated as angry or fearful, and had high rating confidence scores, were used as the emotional images in the main experiment, and images that were rated with low confidence as being either angry or fearful were used as neutral images. Trials began when participants placed the mouse cursor at a fixed point at the bottom of the screen. After a fixation period, a composite stimulus appeared, which participants rated as being fearful or angry by moving their mouse towards the top left or the top right of the screen, respectively. We predicted that the separate sources of information (i.e., face or body) would contribute to the expression judgment at different time points during the trial. To test this prediction, we recorded the speed and trajectory of participants mouse movements. By comparing the deflection of the average mouse trace relative to a straight-line trajectory, we discovered an initial movement towards with the body expression, which changed later in the trial to a bias towards the facial expression. We decoded the contribution of face and body expressions from the mouse position across time. This analysis indicated higher classification accuracy for the body earlier in the trial, but a higher classification accuracy for the face later in the trial. These results suggest that face and body expressions are dynamically weighted during emotion judgments with an earlier bias for body expression, but an overall bias for face expression. These findings also reveal the utility of using dynamic mouse position for making inferences about recognition judgments.

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.07

Topic: D.06. Vision

Support: NIH-NINDS 1R01 NS107727

Title: Fmri-based reconstructions of face images using the active appearance model

Authors: *P. A. KEENE, M. L. DRASCHER, B. A. KUHL;
Psychology, Univ. of Oregon, EUGENE, OR

Abstract: fMRI-based reconstructions of face images using the Active Appearance Model
Paul A. Keene, Maxwell L. Drascher, Brice A. Kuhl
A growing number of fMRI studies have used multivoxel encoding or decoding models to reconstruct visual stimuli from patterns of fMRI activity. Examples of reconstructions range from simple orientation gratings (e.g., Serences et al., 2009; Kamitani & Tong, 2005) to complex, dynamic stimuli such as movies (e.g., Nishimoto et al., 2011). In prior work from our lab, we have shown that face images can also be reliably reconstructed from patterns of fMRI activity by mapping Eigenfaces to fMRI activity patterns (Cowen, Chun, & Kuhl, 2014; Lee & Kuhl, 2016). Indeed, this approach is successful not only for reconstructing visually-perceived faces, but also faces held in memory (Lee & Kuhl, 2016). However, more recent evidence from electrophysiological recordings in monkeys demonstrates that an alternative face model—the Active Appearance Model (AAM)—yields significantly more accurate reconstructions than an Eigenface model (Chang & Tsao, 2017). The AAM also has the added benefit of explicitly dissociating shape and shape-free (appearance) information in face images, which allows for the potential dissociation of brain regions that code for these different sets of dimensions. Here, we compared AAM-based and Eigenface-based reconstructions of face images from human fMRI data. During fMRI scanning, participants (n = 35) completed a continuous recognition memory task with 432 unique face images. To generate the shape information for the AAM model, each face was annotated by hand with 62 landmarks. Ridge regression models were then used to map AAM and Eigenface face components to patterns of fMRI activity patterns in a number of cortical areas. We observed reliable reconstructions across many visual and parietal cortical areas when using either of the face models (AAM or Eigenface). Critically, however, AAM components were better predicted by fMRI activity patterns than were Eigenface components, reflecting a general advantage for the AAM. Additionally, we found that visual/parietal areas varied in their relative preference for shape versus appearance information from the AAM. These findings provide additional evidence of fMRI-based reconstructions of complex visual stimuli (faces), but point to a clear advantage in using the AAM, as compared to Eigenfaces. Finally, we consider potential applications of AAM-based face reconstructions for characterizing the representations of stimuli in visual perception and memory and discuss qualitative differences in face representations across cortical areas.

Disclosures: **P.A. Keene:** None. **M.L. Drascher:** None. **B.A. Kuhl:** None.

Poster

053. Social Vision: Face and Body Representation

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Topic: D.06. Vision

Support: Grossman-Kavli Scholar
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Klingenstein-Simons Fellowship Award in Neuroscience
NIH BRAIN R34 116739

Title: Marmosets can robustly recognize faces defined by 3D geometry alone, invariant to pose, lighting & background changes

Authors: *Y. JEON, H. CHO, E. B. ISSA;
Neurobio. and Behavior, Columbia Univ., New York, NY

Abstract: Face recognition is dependent on both the shape and appearance of faces, the former providing information about geometry and depth, the latter providing color and texture information. Previous face recognition research has often relied on photographs of frontal or profile views of faces, which demand little robustness across viewing conditions while confounding shape and appearance cues. In our study, to minimize the usage of texture cues and hone in on the effect of shape for face discrimination, we created 3D, textureless face meshes and rendered them in greyscale. In addition to measuring human performance on this task, we tested common marmosets, a New World monkey that has garnered attention as a small primate model for neuroscience. We sought to determine whether marmosets have the ability to recognize faces which has not been thoroughly tested but is ultimately critical to the marmoset's utility as a model for studying the neurobiological mechanisms of human face processing.We trained three marmosets to discriminate between two different artificial face identities presented in varying pose, size, position, illumination, and background. On held-out images, marmoset performance was ~70% across different conditions (M1: 80%, M2: 75%, M3: 65%), compared to ~80% for humans. Above chance performance was nontrivial given the challenging nature of the task - various state-of-the-art deep neural networks (DNNs) which excelled at a basic-level object recognition control task (80-90%) performed quite poorly on our face discrimination task (50-60%). Rather than perform near chance, the same DNNs would typically perform near ceiling for face identification from photographs - stimuli that have also been used previously in face recognition researchBesides absolute performance, we are currently interested in whether marmosets demonstrate similar deficits in face processing as humans. One such deficit is the face inversion effect where humans have difficulty discriminating upside-down faces, more so than discriminating upside-down objects. In preliminary experiments, we found that performance for inverted faces was lower by 17% in one marmoset despite high proficiency at upright face discrimination (>80% performance), similar to the 14% performance decrement observed in humans. Our results show that marmosets can indeed perform fine geometry-based face discrimination that is challenging even for humans and machines. Furthermore, the presence of a face inversion effect, points toward the marmoset sharing a common repertoire for face

recognition as humans, endorsing the argument for marmosets as a model for high-level visual neuroscience.

Disclosures: Y. Jeon: None. **H. Cho:** None. **E.B. Issa:** None.

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

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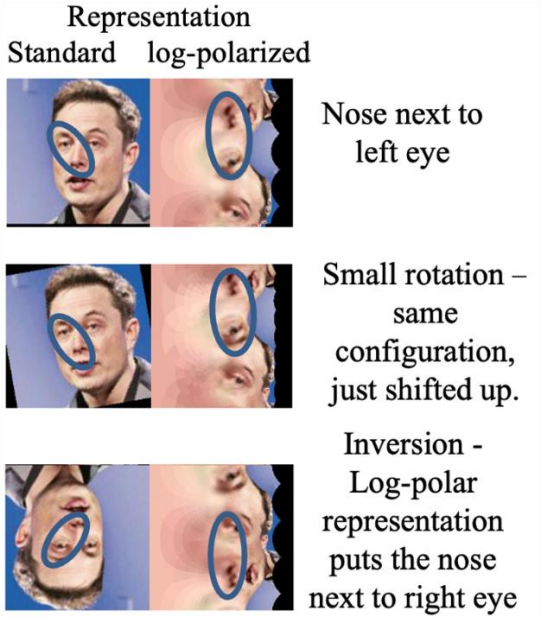
Program #/Poster #: 053.09

Topic: D.06. Vision

Title: An anatomically constrained model of the primate visual system explains the inverted face effect as a function of expertise and suggests it arises at the level of V1

Authors: *G. COTTRELL, M. GAHL, S. KULKARNI, A. RUSSELL;
Computer Sci. and Engin., Univ. of California San Diego, La Jolla, CA

Abstract: Subjects perform poorly at recognizing upside-down faces. This face-inversion effect is in contrast to subjects' performance with inverted objects, which is not as drastically impaired. Experimental results have suggested that a similar effect, though to a lesser degree, may be seen in the inversion of mono-oriented objects, such as cars, where subjects' performance on inverted mono-oriented objects is between that of faces and other objects. We build an anatomically-inspired neurocomputational model to explore this effect. Our model includes a foveated retina and the log-polar mapping from the visual field to V1. This transformation causes changes in scale to appear as horizontal translations, leading to scale equivariance. Rotation is similarly equivariant, leading to vertical translations. When fed into a standard convnet, this provides rotation and scale invariance. It may be surprising that a rotation-invariant network shows any inversion effect at all. This is because there is a crucial topological difference between scale and rotation: Rotational invariance is discontinuous, with V1 ranging from 90 degrees (vertically up) to 270 degrees (vertically down). Hence, when a face is inverted, the configural information in the face is disrupted, while feature information is relatively unaffected. We show that as the model learns more faces, i.e., becomes a face expert, its performance on inverted faces degrades. A similar effect occurs when the model learns car models, but not when it just learns a more basic level representation ("van", "hatchback", etc.). A standard convolutional network shows the same effect, except that its inverted performance degrades to near 0, unlike the log-polar network. This suggests that the inversion effect arises as a result of visual expertise, where configural information becomes relevant as more objects are learned at the subordinate level. Contrary to conventional wisdom, the model suggests that the inversion effect begins at the level of V1, rather than at higher-level visual areas.



Disclosures: **G. Cottrell:** None. **M. Gahl:** None. **S. Kulkarni:** None. **A. Russell:** None.

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 053.10

Title: WITHDRAWN

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 053.11

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HFSP RGP0036/2016
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NVIDIA Corp.

Title: Encoding of dynamic facial expressions in the macaque superior temporal sulcus

Authors: *R. SIEBERT[1,2], M. STETTLER[3,4], N. TAUBERT[3], P. W. DICKE[1], M. A. GIESE[3], P. THIER[1];
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Abstract: Faces of primates are a rich source of information on the identity and emotional state of the other, key for the guidance of viable social interactions. Whereas the neural encoding of facial identity has been extensively studied at the single-cell level, facial expressions have received relatively little interest, arguably because of their grounding in motion as well as figural cues. In order to get a handle on the motion dimension of expressions and its interaction with figural information we took advantage of a highly naturalistic, dynamic rhesus monkey avatar [1,2], ensuring standardized and parametrized stimulus control, while recording from neurons in the macaque superior temporal sulcus (STS). In our experiments, the monkeys watched video clips of the avatar producing facial expressions of fear, threat or affiliative lip-smacking, as well as an artificial "blowing" expression, in different degrees of intensity. Additionally, dynamic point-light expressions, real monkey videos, moving objects, speed-matched optic flow fields and static images of faces and non-face objects were shown.
Among STS neurons which showed a significant response to any of the expressions offered, two major response types could be distinguished. More posteriorly located cells seemed to correspond to "optic flow pattern" neurons, responding similarly to the full dynamic avatar and to impoverished moving point-light expressions lacking figural features. "Dynamic expression" neurons, located more anteriorly in the STS, were characterized by sustained responses to one expression category only, increasing almost linearly with the degree of expressivity, and only small responses to the other expressions or to other dynamic control stimuli, including point-light facial expressions. Most of these neurons also responded more to static faces than to static non-face stimuli but differed only little in their responses to static depictions of expressions.
It seems that neurons in the macaque STS process expression-relevant information in a hierarchical manner. Whereas more posterior neurons extract motion cues, a more anterior population integrates motion and form information in a first step towards categorizing expressions. The dynamic expression cells' continuous increase in firing with the intensity of the expression fits a norm-referenced encoding model, which postulates that neurons are tuned to the amount of distance in face-space between a facial expression shape and a neutral reference pose [3].

References

1. Siebert, R. et al. eNeuro 7(4), 2020: ENEURO.0524-19.2020
2. Taubert, N. et al. eLife 2021;10: e61197
3. Stettler, M. et al. (2020). ICANN 2020 (168-179)

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.12

Topic: D.06. Vision

Support: Intramural NIH funding

Title: Distinct representations for executing and judging grasp movements

Authors: L. ETTENSOHN[1], C. I. BAKER[2], ***M. VAZIRI PASHKAM**[1];
[1]Natl. Inst. of Mental Hlth., Natl. Inst. of Mental Hlth., Bethesda, MD; [2]NIH, NIH, Bethesda, MD

Abstract: Beyond executing grasp movements, we also regularly observe others grasping objects. How well can we discriminate grasp movements? Are we sensitive to all kinematic information in grasp movements when distinguishing them? We gathered kinematic data of hand movements as participants grasped a set of 58 3D-printed objects. To determine if the kinematic data contained enough information for distinguishing different grasp movements, we measured the accuracy of a linear classifier in discriminating grasp movements from the kinematic data. The accuracy of the classifier at the final grasp position was ~85% across objects. Next, using the kinematic data, we created point-light videos to serve as stimuli in a behavioral experiment. We asked participants to perform a match-to-sample task. The sample showed a video of a grasp movement towards one of the 58 objects, and the choices showed videos of two grasp movements, one towards the same object as in the sample and another towards one of the other 57 objects. The accuracy of the human participants was, on average, at ~70%, significantly lower than the classifier's accuracy. To determine if participants rely on the same kinematic information used by the classifier to perform judgments on the behavioral tasks, we used the confusion matrix from the linear classifier as a measure of kinematic similarity and compared it with the confusion matrix from the identification task. The classifier accuracies had low albeit significant correlations with similarity measures from our behavioral experiments. These results suggest that the participants use only a subset of the kinematic information available to them to perform the behavioral judgments and indicate a dissociation between objective grasp movements and subjective judgments of those movements.

Disclosures: L. Ettensohn: None. **C.I. Baker:** None. **M. Vaziri Pashkam:** None.

Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.13

Topic: D.06. Vision

Support: NIH Grant 1R15NS121788

Title: Face masks increase the N170 and P200 amplitudes. An ERP study.

Authors: *N. BRUNET[1,2], A. TALL[2], V. PATRICK[2], P. JAGDALE[2];
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Abstract: Measuring event-related brain potentials (ERPs) from college-aged participants, we investigated different aspects of facial information and whether they have an effect on early ERP components. To that extent, we comprised a five-dimensional stimulus set consisting of 416 images of faces, equally divided by gender (male/female), emotion (happy/angry), age (young/old), race (black/white) and face cover (mask/no mask). Our preliminary results suggest that neither age nor race nor emotion nor gender significantly affects the first 400 ms of the ERP waveform. Interestingly, and counterintuitively, we found that both the N170 and the P200, but not the P100, for electrodes placed over the occipital and temporal areas of the scalp, were significantly larger in response to faces with a face mask compared with faces without a face mask. Wearing face masks in public has become more common, increasing the need to understand how masks impact face perception. By showing how brain activity, most likely from the fusiform gyrus, is strongly modulated by face masks, about 150 to 250 ms after seeing a face, we hope to contribute to that effort.

Disclosures: N. Brunet: None. **A. Tall:** None. **V. Patrick:** None. **P. Jagdale:** None.

Poster

053. Social Vision: Face and Body Representation

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Topic: D.06. Vision

Support: NIH K00/R00 Award: 1K99EY032603
NIH Pioneer Award: DP1HD091957
NSF NSF Science and Technology Center

Title: Computational models of human category selective regions generalize broadly across datasets and cognitive neuroscience experiments

Authors: *A. ABATE, E. MIECZKOWSKI, M. KHOSLA, J. J. DICARLO, N. KANWISHER, N. MURTY;
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Abstract: Deep convolutional neural network (DNN)-based models have emerged as our leading hypotheses of human vision. We recently described DNN-based encoding models of the fusiform face area (FFA), the extrastriate body area (EBA) and the parahippocampal place area (PPA),

that can predict the response to new images with very high accuracy. But do these models also explain the key experimental results from previous studies? Many stimuli in these studies were highly manipulated (e.g. isolated face parts in re-arranged spatial positions), far outside the domain of natural images, and thus could provide strong tests of generalization. Furthermore, these stimuli were designed to test, and taken as evidence for, classic "word model" hypotheses about visual representation (such as "holistic" face processing). Here we asked whether our current best encoding models directly replicate the main findings in prior published papers. To do this, we identified 20 influential papers that localized and reported response magnitudes of the FFA, PPA, and EBA. We tested the key experimental conditions directly on our encoding models of these regions without retraining the model. Our models could recapitulate all the key univariate and multivariate signatures of neural face, body, and scene processing described in those publications, including results previously taken to demonstrate holistic face processing, real-world size effects, sensitivity to animacy, and eccentricity bias. These findings show that our models generalize even outside their training domain. They also provide a computationally precise basis for findings previously described only with word models and show that these phenomena can emerge without any built-in, domain-specific biases or world knowledge apart from what can be gleaned from hierarchical computations. This approach is made possible because of our functional region-of-interest level of computational modeling and paves the way to efficiently test novel hypotheses completely in-silico in future work.

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Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.15

Topic: D.06. Vision

Support: NIMH Intramural Research Program

Title: Examining the processing of facial expression and head orientation in the human brain

Authors: *K. BRANNIGAN[1], R. KUMAR[2], J. TAUBERT[5], C. I. BAKER[3], S. JAPEE[4]; [1]NIMH, Bethesda, MD; [3]Lab. Brain and Cognition, [4]Lab. of Brain & Cognition, [2]NIH, Bethesda, MD; [5]Sch. of Psychology, The Univ. of Queensland, St Lucia, Australia

Abstract: Facial dynamics communicate a considerable amount of social information. For example, the fine movements that create facial expressions convey a person’s emotional state, while other changes, like adjustments of head position or orientation, signal the focus of a person’s attention. A prominent theory of face processing (Bruce & Young, 1986; Haxby, Hoffman, & Gobbini, 2000) posits that dynamic facial information, like expression, is processed primarily by the superior temporal sulcus (STS; Pitcher et al., 2011) while invariant aspects of a

face, like identity, are processed primarily by the fusiform face area (FFA; Grill-Spector et al., 2004). While the role of the STS in processing facial expressions is well characterized, less is known about its sensitivity to other dynamic information such as changes in head orientation. In a recent fMRI-adaptation study in rhesus macaques (Taubert et. al., 2020), we found greater sensitivity to facial expression than head orientation in anterior and middle fundus face patches in the STS and the amygdala, while the reverse was true for the anterior lateral face patch. These findings suggest that the STS fundus plays a major role in processing facial expression, while lateral STS regions are more involved in processing head orientation. In the current study, we used a similar fMRI-adaptation paradigm to examine whether a parallel dissociation between facial expression and head orientation processing exists in humans. Participants were asked to view images of human faces presented in four different block types where: (1) the expression and orientation of the faces changed; (2) only expression changed; (3) only orientation changed; and (4) neither expression nor orientation changed, while performing a fixation change task. Following the adaptation task runs, participants also completed a face localizer task comprising images and videos of static and dynamic faces and objects, to identify face-selective regions in posterior STS and FFA. We found greater fMRI activity in posterior STS during blocks when only expression changed relative to when expression and orientation were held constant. By contrast, activity in FFA was greater during blocks when only orientation changed compared to when both were held constant. These results suggest that while posterior STS is sensitive to facial expressions, the FFA may be sensitive to head orientation. Data from additional participants and further analyses will reveal the exact role of these regions in the processing of these two types of facial dynamics, and will help uncover a functional homology in how dynamic facial information is processed by the two species.

Disclosures: K. Brannigan: None. **R. Kumar:** None. **J. Taubert:** None. **C.I. Baker:** None. **S. Japee:** None.

Poster

053. Social Vision: Face and Body Representation

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Topic: D.06. Vision

Support: German Research Foundation Emmy Noether Program awarded to Caspar M. Schwiedrzik (SCHW1683/2-1)
Outgoing Grant awarded by the Leibniz ScienceCampus Primate Cognition Göttingen awarded to Tarana Nigam

Title: High-level prediction signals cascade through the macaque face-processing network for efficient pattern separation

Authors: *T. NIGAM[1,2,3,4], C. M. SCHWIEDRZIK[1,2,3];
[1]Perception and Plasticity Group, German Primate Ctr., Göttingen, Germany; [2]Neural Circuits

and Cognition Lab., European Neurosci. Inst. Göttingen - A Joint Initiative of the Univ. Med. Ctr. Göttingen and the Max Planck Society, Göttingen, Germany; [3]Leibniz ScienceCampus Primate Cognition, Göttingen, Germany; [4]Intl. Max Planck Res. Sch. for Neurosciences at the Georg August Univ. Göttingen, Göttingen, Germany

Abstract: We live in highly structured environments where temporal events and spatial patterns often repeat. The brain extracts such regularities to make predictions about upcoming instances. Predictions enable efficient neural processing of stimuli and facilitate perception. Predictive processing theories propose that in cortical hierarchies, high-level prediction signals are sent to lower regions via feedback pathways, where they are compared against ascending inputs to compute prediction errors. However, the neural mechanisms underlying propagation of prediction signals and its role in efficient coding remain unclear, especially in higher vision.
In this study, we investigate predictive processing in macaque monkeys using functional magnetic resonance imaging. We leverage the macaque face-processing network, a three-level hierarchy in the ventral visual pathway where face representation becomes more view-invariant as information ascends from stage to stage. We test the role of feedback pathways in sending predictions by investigating how expectations affect neural representations.
We hypothesize that higher areas send predictions, such that the lower-areas inherit the tuning properties of higher areas from which they receive feedback. By conducting representational similarity analysis, we show that after statistical learning of arbitrary face-pair sequences, lower face areas express view invariant representations when stimuli are predictable. Rather than the view-specific tuning properties they show during feedforward processing, these lower areas now exhibit view invariant abstract representations of higher face areas. This cascading down of high-level prediction signals in the entire face-processing network suggests a functional role of feedback connections in signaling predictions, which is in line with predictive processing theories. Further, we find that prediction errors decorrelate face representations, leading to increased pattern separation.
Taken together, we find that learned high-level predictions lead to increased generalization early on in the face processing hierarchy such that lower areas can abstract over irrelevant features, which accelerates invariant recognition. Moreover, prediction errors aid neural discriminability, making extraction of identities more efficient. By showing how the top-down information flow of predictions and previous experience affects face processing, our work contributes to a revision of currently dominant theories that view face perception and generally, object recognition through the lens of pure feedforward architectures.

Disclosures: T. Nigam: None. **C.M. Schwiedrzik:** None.

Poster

053. Social Vision: Face and Body Representation

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Program #/Poster #: 053.17

Topic: D.06. Vision

Support: SBA-2019-4254

Title: Which face is mine? Reactions of face transplant patient to his old and new face

Authors: *E. GÜLBETEKIN[1], S. BAYRAKTAR[2], Ö. ÖZKAN[3], Ö. ÖZKAN[3], H. UYSAL[4], A. U. ŞENOL[5], Ö. H. ÇOLAK[6], A. ŞAVKLIYILDIZ[6];
[2]Psychology, [1]Akdeniz Univ., Antalya, Turkey; [3]Dept. of Plastic and Reconstructive Surgery, Med. Fac., [4]Dept. of Neurology, Med. Fac., [5]Dept. of Radiology, [6]Dept. of Electrical and Electronics Engin., Akdeniz Univ., Konyaalti, Turkey

Abstract: The face is a distinguishing part of the body that represents the identity of its owner. Therefore, self-face is actively represented in the human cognitive and emotional system. Face transplantation is a recent development that makes a spontaneous change in self-face perception. We aimed to examine self-face perception of a face transplant patient in order to find out his behavioral and neural responses to his old and new faces.One face transplant patient and ten male controls participated in the study. 3 stimulus categories were used: Face, body, and scrambled images of faces and bodies. We took phototographs of the faces and bodies of each participant in standard conditions. Subjects' facial photographs which were taken 1-2 years ago were also used. The experiment consisted of two blocks: In the first block a face which belonged to himself (old or new) or another male was presented. The participant pressed 1 for his own face and 2 for the other faces. In the second block a body which was belonged to himself or another male or scrambled image was presented. They pressed 1 for their own body and 2 for other bodies or scrambled images. Stimuli were presented by E-Prime 3.0. Before the presentation of the stimulus, a fixation dot was shown for 2 sec. Facial stimuli were randomly presented for 1000ms. The intertrial interval was 2200 ms. During the experiment, brain signals were recorded via 64 channel BrainVision Actichamp EEG system. Response time and accuracy were also recorded by E-Prime 3. We preprocessed the data and determined P100 responses (100-160ms). ERPs were averaged from 200 ms before to 1000 ms after stimulus onset. We measured the mean amplitudes of the selected electrodes: F7,F8,P1, P2,P3,P4,P5,P6,P7,PO3,PO4,PO7,O1,O2,T7,T8,TP7,TP8,TP9,TP10,FT7,FT8,FT9,FT10. Repeated measures ANOVA was conducted to determine the effects of electrode regions, conditions and hemisphere on P100 responses. A significant effect of electrode F (1.55, 13.99) = 33.744, p = .001, ηp2 = .79 and condition F (2, 18) = 5.51, p = .01, ηp2 = .38. were found. The amplitudes for other faces (M = 6.18, SS = .65) were higher than the amplitudes for their new (M = 4.06, SS = .79) and old faces (M = 5.01, SS = .60) in the control group. On the other hand, the amplitudes of facial transplant patient for other faces and the new face of himself were similar to the control group while the amplitudes for his old face was lower than the amplitudes of the control group. In addition, the patient’s amplitudes in all brain regions were lower than the controls while it was higher in frontal regions during the self-recognition task. The findings indicated that the patient processes his old face differently.

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Poster

053. Social Vision: Face and Body Representation

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David and Lucile Packard Foundation
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Title: Animal-feature encoding in macaque brain and in artificial networks

Authors: Z. ZHANG[1], T. S. HARTMANN[2], R. T. BORN[2], M. S. LIVINGSTONE[3], *C. R. PONCE[3];
[1]Computer Sci., Univ. of California San Diego, San Diego, CA; [2]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [3]Dept. of Neurobio., Harvard Univ., Boston, MA

Abstract: Macaque monkeys are foraging, social animals that spend a significant fraction of their time identifying conspecifics, classifying their actions, and avoiding threats from other animals (Post and Baulu, 1978; Cheney and Seyfarth, 1990; Son, 2004). This suggests that in learning information from the visual world, many neurons of the monkey ventral stream might focus on encoding animal-based features. To test this, we designed experiments to show how neurons respond over entire natural scenes containing animals and other types of objects. Inspired by feature-mapping operations in artificial neural networks (ANNs), we developed heatmaps describing the spiking activity of V1, V4, and IT neurons over full natural scenes (Arcaro et al., 2020). Heatmaps can be compared across cortical areas and also to feature channels in ANNs. We selected dozens of scenes, and each was segmented using independently obtained annotations from neural networks (Chen et al, 2017) and human participants. These segmentations served as masks quantifying the concentration of neuronal and ANN activity within labeled regions. Consistent with the observed behavior of macaques in the wild, we discovered that animal masks identified regions with strong neuronal activity for IT better than they did for V4 and for V4 better than for V1 (AUC values, median ± SE; V1: 0.19 ± 0.01, V4: 0.63 ± 0.06, IT: 0.73 ± 0.08). No such linear trend was found for non-animal masks (e.g., food, books). To determine if this trend could emerge from any system with a hierarchical architecture, we replicated these analyses using ANNs. Most ANNs did not show this animal-focused trend. We studied the few ANNs that did express this pattern, such as CORNet-S (Kubilius et al, 2019), and measured their ability to overcome nuisance changes (distortion robustness), e.g., bias for shapes vs. textures, using previous tools (Geirhos et al, 2021). We found these ANNs could converge to the same result as the brain by showing a bias towards animal-related textures, even if they lacked object-centric representations. Finally, we also compared neuronal heatmaps with those derived from monkeys' free-viewing data, against three saliency maps (GBVS, Itti-Koch, and FASA). We found saliency maps and free-viewing maps correlated best with IT heatmaps (Pearson correlation; eye-viewing: 0.13, Itto-Koch: 0.31, GBVS: 0.38, FASA: 0.16). Collectively, our results provide further evidence of an organizing principle of the monkey ventral stream — to encode information diagnostic of animals.

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Poster

053. Social Vision: Face and Body Representation

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Topic: D.06. Vision

Support: the McDonnell Center for Systems Neuroscience (pre-doctoral fellowship to B.W.)
the David and Lucille Packard Fellowship #2020-71377
E. Matilda Ziegler Foundation Grant (to C.R.P.)

Title: Inferring the Geometry of Tuning Landscape of Ventral Stream Neurons via Image Optimization

Authors: *B. WANG[1,2], C. R. PONCE[2];
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Abstract: Vision scientists often characterize neuronal function using tuning curves, plots of spiking activity as a function of a continuous variable. These variables are chosen a priori and often control simple image properties (e.g., orientation). Here we generalize this notion to study neuronal responses as a continuous function over a vast naturalistic image manifold — the parametrized 4096-dimensional space of a deep generative network. In a continuous naturalistic space, a neuron's high-dimensional tuning function can be conceptualized as a landscape. To study its geometry, we searched for peaks in this landscape and measured the tuning shape around each peak. We conducted in vivo experiments on neurons from V1/V2, V4, and IT in parallel with in silico experiments using convolutional neural networks (CNNs). Specifically, for each neuron and hidden unit, we searched for an activation-maximizing stimulus using an evolutionary algorithm (CMAES). Then, we smoothly morphed the highly activating stimulus along a 2D submanifold and measured the neuronal responses. Finally, to probe the feature density of highly activating stimuli, we searched for activation maximizing stimuli in a 50d random subspace and compared the result to the full latent space. We found it was possible to increase neuronal firing rate in most experiments (72/91). From V1 to IT, neurons generally exhibited smooth bell-shaped tuning curves around their peaks, reminiscent of 1-D orientation tuning curves. Compared to the peaks measured in orientation and curvature space, the peaks on the GAN image manifold were taller and wider. When compared across the ventral hierarchy, we found tuning peaks became sharper from V1 to IT (per Kent function parameter κ, mean ± SEM, V1, 0.7±0.1; V4, 1.8±0.2; IT, 3.2±0.3). Further, we found that the gap between the max activation in the full- and random 50d spaces increased from V1 to IT (ratio of maximal activation, V1 1.01±0.02, V4 0.84±0.05, IT 0.71±0.06); and the search converged slower (V1,

12±1 generations; V4, 21±3; IT, 29±2). These three trends were consistently found in monkeys and in CNNs. We modeled the neural tuning function as a multivariate Gaussian. To explain these trends, we inferred that the neurons in higher visual cortices are likely to be tuned to more axes (i.e. be invariant to fewer axes) than neurons in lower visual cortices. For in silico CNN units, we showed that the units with narrower tuning width in the generator space, also exhibited sparser responses to natural images. These results mark an initial step in understanding the geometry of the tuning landscapes of visual neurons, and further unifying previous findings of neural tuning in the ventral stream.

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Poster

053. Social Vision: Face and Body Representation

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Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495
FWO-G0E0220N

Title: Contribution of shape and motion to body selectivity in macaque dorsal and ventral patches of anterior temporal visual cortex

Authors: *R. RAMAN[1,2], A. BOGNÁR[1,2], G. GHAMKHARI NEJAD[1,2], N. TAUBERT[3], B. DE GELDER[4,5], M. A. GIESE[3], R. VOGELS[1,2];
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Abstract: Visual information from body movements is important for non-verbal social communication and action recognition. Recent fMRI studies, from our lab, mapped dynamic body patches in the macaque temporal visual cortex that were activated more by 1 sec long videos of acting monkeys (with blurred faces) compared to videos of monkey faces and artificial objects (Bartoli et al., SfN-2021; Bognár et al., SfN-2022). However, the feature selectivity of the single cells in these patches is still unclear. In this fMRI-guided single unit study, we recorded neurons in the ventral (lower bank of and ventral to the Superior Temporal Sulcus (STS)) and dorsal (upper bank/fundus of the STS) dynamic body patches of the anterior temporal cortex of macaques. A sizable proportion of the cells responded more to the videos of bodies than videos of faces and objects that were used in the fMRI mapping study. Many neurons of both dorsal and ventral patches responded only to a few of the videos, showing a high selectivity within the body category, and responded only during a segment of the video. Most neurons had a similar response profile for the original videos and versions in which the shaded and textured

body was reduced to its silhouette. Median filtering of the body shape of each video frame showed that most neurons were sensitive to spatial smoothing of the body shape while some also responded to highly spatially smoothed dynamic displays. To assess the contribution of body dynamics, we measured the response to static frames ("snapshots") of the videos. Many neurons responded equally well to the static presentations of snapshots and the original videos and in many neurons, especially from the ventral patches, the responses to video segments were predicted by the responses to the static snapshots. Other neurons responded only to the videos requiring motion. Some neurons were sensitive to the order of the frames within the video, even when showing similar responses to the video and static snapshot presentations. The dorsal body-responsive patch showed a higher proportion of such sequence-sensitive neurons than the ventral body patches. These findings suggest that most body-selective cells in the anterior temporal cortex respond to silhouettes of bodies with various degrees of preference and tolerance for shape smoothness. Although a large fraction of the cells in the dorsal patches are sensitive to the motion and snapshot sequence, a significant fraction of the cells in ventral patches also possess similar sequence sensitivity.

Disclosures: **R. Raman:** None. **A. Bognár:** None. **G. Ghamkhari Nejad:** None. **N. Taubert:** None. **B. De Gelder:** None. **M.A. Giese:** None. **R. Vogels:** None.

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 053.21

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495

Title: Encoding models of body stimuli reveal 2D key points like representation in extrastriate body area.

Authors: *G. MARRAZZO[1], F. DE MARTINO[1,2], A. LAGE-CASTELLANOS[1,3], M. VAESSEN[1], B. DE GELDER[1,4];
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Abstract: In this fMRI study we investigated the type of representation of bodies which takes place in EBA using encoding models. The extrastriate body area (EBA) (Downing et al. 2001, Peelen and Downing, 2005) is currently considered to be a ventral cortex object category area, selective for still body stimuli but little yet understood about its computational functions. Yet understanding how whole-body postures are encoded in EBA is crucial to disentangle the role played by this area in body perception. Stimuli were generated using a variational autoencoder (VAE), via a random sampling of the latent space parameters, depicting 3D rendered body poses

(Pavlakos et al. 2019). We used 108 randomly generated unique poses from 3 different viewpoints (frontal view, 45° rotation to the right and to the left respectively), for a total of 324 stimuli. 20 participants were scanned using a 7T (T2*-weighted Multi-Band accelerated EPI 2D BOLD sequence, MB = 3, voxel size = 1.6 mm^3, TR = 1000 ms, TE = 20 ms) in a fast event-related design over 12 separate runs. Each run consisted of 54 unique stimuli (18 unique poses x 3 viewpoints) which appeared on the screen for 750 ms. Participants were asked to fixate and attention was controlled with a one-back task (6 trials per run). The fMRI response was modelled using several features extracted from the stimuli, such as VAE representation (encoding/decoding layers, latent space), 2D/3D coordinates of key joints (kp2d/kp3d), pixel space (Gabor like representation) and the 4^{th} layer (inferior temporal) of CORnet. The fMRI predicted responses from each model were generated via banded ridge regression (Nunez-Elizalde et al. 2019, Dupré La Tour et al. 2022) using crossvalidation. Results show a pattern of responses across visual cortex with Gabor and kp2d model best predicting responses to our stimuli. Specifically, the Gabor representation shows higher prediction accuracy in early visual occipital area as opposed to the kp2d representation which shows higher prediction accuracy in high-level temporal areas such as EBA. Furthermore, kp2d model (viewpoint variant) shows higher accuracy in EBA than kp3d model (viewpoint invariant). These findings suggest that EBA codes for specific features of the body, which in the case of kp2d model, are the joints position, and that this representation is viewpoint variant.

Disclosures: G. Marrazzo: None. **F. De Martino:** None. **A. Lage-Castellanos:** None. **M. Vaessen:** None. **B. de Gelder:** None.

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 053.22

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495
FWO-G0E0220N

Title: fMRI activation for body and face dynamics in the temporal visual cortex of the macaque

Authors: *A. BOGNÁR[1], R. RAMAN[1], N. TAUBERT[3], M. A. GIESE[4], B. DE GELDER[5], R. VOGELS[2];
[1]Dept. of Neurosci., [2]Neurosciences, KU Leuven, Leuven, Belgium; [3]Univ. of Tuebingen, Tuebingen, Germany; [4]Hertie Inst. For Clin. Brain Sci. / CIN, Tuebingen, Germany; [5]Maastricht Univ., Maastricht, Netherlands

Abstract: Previous fMRI studies demonstrated patches in the macaque inferior temporal cortex that are activated stronger by bodies compared to faces and objects. These studies were performed with static images. However, whole body movements contribute essential information

to non-verbal social communication and action recognition. To understand the neural substrate underlying the visual processing of dynamic monkey bodies, in addition to dynamic monkey faces, we mapped activations to dynamic and static bodies and faces with fMRI in macaques. We employed a novel set of 1 sec long videos of moving bodies, faces and artificial objects to map dynamic body and face patches. To control for low-level spatiotemporal features, we presented also mosaic and phase scrambled versions of these videos. The displays were presented on a dynamic white noise background in a block design while the monkeys performed a passive fixation task. We found 9 body patches along and ventral to the Superior Temporal Sulcus (STS) by contrasting the body videos with those of faces and objects, including both lower and upper banks of the STS. Although we observed weak body category-selective activations for both mosaic- and phase scrambled videos in posterior STS patches, body patches were still present after controlling for the activation to the scrambled displays. To examine the contribution of dynamics to the activation in the patches, we presented the original videos and 2 static images from each video for 500 ms in a random order, using the same block design during passive fixation. We extracted the percent signal changes to the dynamic and static presentations, relative to the white noise background, in regions of interest (ROI) that were centred on the local maxima of the body and face patches, localized in an independent experiment with the same dynamic displays. In most body patch ROIs, we found higher activation for dynamic than static bodies. However, the face patch ROIs showed a similar activation for dynamic and static faces, except in face patch MF which showed higher activation for the dynamic stimuli. In sum, using dynamic stimuli, we identified a set of body selective regions in macaques, which were present both in the upper and lower bank of the STS. Our data suggest that, for identical stimulus displays, dynamics of bodies and faces have a stronger effect in the body patches than in the neighbouring face patches, which aligns with behavioural results in humans on the role of dynamics in body and face perception. The current fMRI study provides the basis for single-unit studies of the feature selectivity in the identified body patches (Raman et al., SfN 2022).

Disclosures: **A. Bognár:** None. **R. Raman:** None. **N. Taubert:** None. **M.A. Giese:** None. **B. De Gelder:** None. **R. Vogels:** None.

Poster

053. Social Vision: Face and Body Representation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 053.23

Topic: D.06. Vision

Support: ERC 2019-SyG-RELEVANCE-856495

Title: Affective expression-modulated nodes in large-scale body networks in the human brain

Authors: *B. LI, M. POYO SOLANAS, G. MARRAZZO, B. DE GELDER;
Univ. Maastricht, Maastricht, Netherlands

Abstract: The body plays an important role in daily communication by conveying information about intentions, actions, and emotions. However, although multiple brain networks have been proposed for action and social cognition, questions remain on how affective body expressions can modulate network connectivity. The current study used two human body networks obtained with a fully data-driven approach to study the specific network modulations triggered by affective body expression. Ultra-high field 7T fMRI (1.6mm^3 isotropic, TR = 1s) was used to map the affective body perception with 15 participants. In the first experiment, we used a blocked design with naturalistic videos of bodies, faces and objects from both human and monkey recordings. With group independent component analysis (GICA), we identified two networks modulated by human body videos. One covered a large region of the lateral occipital cortex (LOC network) and the other one was dominated by the right STS and frontal cortex (rSTS network).In the second experiment, participants viewed a new set of affective (angry and fearful) and neutral body videos as well as neutral face and object videos included for control. The three body conditions were regressed out separately from the time courses, and the two body networks were reconstructed on the condition-regressed data. Thus, the nodes dependent on the regressed condition will exhibit lower weights in the reconstructed networks. By comparing the affective-regressed and neutral-regressed networks, we found in the LOC network significantly higher dependence for both angry and fearful bodies in the bilateral extrastriate body areas (EBA). In the rSTS network, higher dependence for the angry body was found in the left EBA and amygdala nodes, and for the fearful body, higher dependence was found in the right temporoparietal junction (TPJ), insula, premotor cortex, and along the right STS. Our study bridged the gap between voxel-level and network-level modulations during affective body perception and offers a new perspective for investigating network-wide representation of affective perception.

Disclosures: B. Li: None. **M. Poyo Solanas:** None. **G. Marrazzo:** None. **B. de Gelder:** None.

Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.01

Topic: D.06. Vision

Support: NIH Grant U19

Title: Population recordings from prefrontal and parietal cortex during a spatial categorization task

Authors: *A. ALAMRI[1], N. Y. MASSE[2], D. J. FREEDMAN[3];
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Abstract: Categorization is a remarkable cognitive function which takes sensory input and transforms it into behaviorally relevant representations to guide flexible behavior. Studies of

neural correlates of visual categorization have focused extensively on higher order cortical areas such as the posterior parietal cortex (PPC) and pre-frontal cortex (PFC), which show categorical encoding at the single neuron and population levels. Elucidating the neural mechanisms which underlie categorization is important as categorization underpins many cognitive functions such as recognition, learning, and decision making. In this study non-human primates (NHPs, n=2) performed a spatial categorization task where they were presented with a sequence of green, red, and white dot flashed targets with each target appearing for 80ms followed by an 80ms blank delay and were required to report (with a lever release) whether a colored flash appeared in a target zone which had been associated with that color during training. Analysis of the animals' behavior shows that they were able to correctly respond to the colored targets which occurred inside their respective color target zones. The two monkeys had a response rate of 83.85% ± 0.012 and 91.91% ± 0.008 for red flashes inside the red zone, 96.64% ± 0.003 and 90.06% ± 0.009 for green flashes inside the green zone, respectively. Analyses of PPC and PFC data during task performance finds that spatial receptive fields (RFs) within and around the target zones for colored targets are strongly represented, and that this over-representation persists even when the animals are performing an unrelated memory guided saccade task. These receptive fields were context dependent: during passive presentation of the stimuli where the animals were required to maintain fixation while only white targets were presented, the neural responses for targets inside the target zones appeared to be weaker than in the active version of the task. Current analyses are examining how the stimulus features (location, color, and target selectivity) are encoded in PPC and PFC in terms of both strength and latency of encoding. These results demonstrate that animals were able to learn a rapid spatial categorization task, leading to persistent over-representation of the RFs corresponding to the target zones, and that the encoding of spatial categories was strongly dependent on task context.

Disclosures: **A. Alamri:** None. **N.Y. Masse:** None. **D.J. Freedman:** None.

Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.02

Topic: D.06. Vision

Support: This project was supported by TTU start-up funds.

Title: Reconstructing Neural Representations During Category Learning in Intraparietal Sulcus

Authors: *S. JUNG[1], S. O'BRYAN[2], M. SCOLARI[1];
[1]Dept. of Psychological Sci., Texas Tech., Lubbock, TX; [2]Dept. of Cognitive, Linguistic & Psychological Sci., Brown Univ., Providence, RI

Abstract: Learning to classify objects that vary along continuous feature values requires the ability to finely discriminate between similar stimuli that nonetheless belong to discrete

categories (i.e., near-boundary stimuli). A prior fMRI study from our lab (O'Bryan et al., in progress) used an inverted encoding model (IEM) to show that this is likely supported by enhanced representations of near-boundary stimuli within the primary visual cortex. Other research has shown that attentional control regions like the intraparietal sulcus (IPS) can also contribute to stimulus enhancement, in conjunction with the visual cortex, during perceptual learning (Mukai et al., 2007). Therefore, the present study focused on topographically defined IPS (Silver & Kastner, 2009) to determine whether orientation-selective population responses within the top-down attentional control system exhibit similar patterns of sharpening during category learning. Participants were assigned to one of two groups: an experimental group learned to categorize a series of central gratings based on an orientation rule (N = 10), and a control group learned to categorize the same gratings based on a spatial frequency rule (N = 11). Identical stimuli were also presented in an initial control condition, in which all participants differentiated small, brief changes in contrast within the stimulus epoch. We expected that consistent with V1, orientation representations would be enhanced for the experimental group during the categorization task compared to the contrast task, while orientation representations should be unchanged between conditions for the control group. Instead, the IEM results showed that the shape of orientation-selective population representations in IPS were enhanced for both rule groups. Furthermore, unlike in V1, representations for stimulus values near the category boundary within orientation space were no sharper than those for category centers. These results suggest that the attentional control system is involved during active category learning, but in a more global manner than the localized effects we observed in the primary visual cortex.

Disclosures: S. Jung: None. **S. O'Bryan:** None. **M. Scolari:** None.

Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

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Program #/Poster #: 054.03

Topic: D.06. Vision

Support: NIH R01EY032878
Simons Collaboration on the Global Brain 543033

Title: Evidence that the extrinsic effects on image memorability are computed in inferotemporal cortex and inherited by the hippocampus

Authors: *C. M. HACKER, B. G. L. JANNUZI, T. MEYER, M. L. HAY, N. C. RUST; Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: The temporal context in which an image is viewed influences how likely it will be remembered. For example, an image of a dog tends to be less memorable when it is presented in a sequence of images enriched for dogs as opposed to a sequence of images with random categorical content. While the neural correlates of image memorability in random contexts have

been explored, the extrinsic effects on memorability have not, and they place important constraints on descriptions of where and how visual memories are stored in the brain. To determine the neural correlates of these extrinsic effects on image memorability, we recorded from populations of neurons in inferotemporal cortex (ITC) and the hippocampus (HC) in one rhesus macaque performing a single-exposure visual memory task. The monkey viewed one image at a time and reported whether it was novel or repeated. We manipulated temporal context by presenting images in one of two blocks: 1) random blocks with images drawn from many different categories (random) or 2) categorical blocks with 80% of images drawn from a single category (categorical) and 20% of images drawn from different categories (oddball). We found that the monkey, like humans, showed reduced memory performance for categorical images relative to random and oddball images. We compared two accounts of the relationship between ITC and HC in mediating the extrinsic effects on image memorability. In the first, transformations downstream of ITC but in or before HC are required to account for these extrinsic effects. These transformations might be linked to computations in HC such as pattern separation whereby the responses of visually similar images are separated before memory storage. In the second, HC does not perform these computations itself but instead inherits these effects from ITC. To distinguish these possibilities, we trained a linear decoder to classify the population responses to novel and repeated images in each condition for each brain region. The classifier was constrained to operate on repetition suppression - a more vigorous response to novel relative to repeated images - following on evidence from previous studies that this neural signal aligns with visual memory behavior. We found that classifiers trained on neural responses from both ITC and HC recapitulated the extrinsic effects of memorability. We also compared the amount and timing of information across regions and found nothing that conflicted with the hypothesis that HC inherits its extrinsic image memorability effects from ITC. A parsimonious account of our data is that the extrinsic effects on image memorability are computed in ITC and inherited by HC.

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Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.04

Topic: D.06. Vision

Title: Visual experience shapes identification of cardiomegaly on chest radiographs through the process of perceptual learning

Authors: *N. PANYANIRUN[1], T. CHAINIYOM[1], C. CHUNHARAS[1,2];
[1]Cognitive Clin. & Computat. Neurosci. Lab, Fac. of Med., Chulalongkorn Univ., Bangkok, Thailand; [2]Chula Neurosci. Ctr., King Chulalongkorn Mem. Hospital, Thai Red Cross Society, Bangkok, Thailand

Abstract: Multiple studies have demonstrated that visual experience shapes image recognition. Two striking examples are facial recognition and perceptual learning. Faces are recognized as a "whole" rather than the sum of their parts and could be shaped by long-term experience (e.g., Gauthier et al., 1999). For example, normal and Thatcherized versions of the same faces are easier to discriminate in the upright position as opposed to when these images were inverted (Thompson, 1980). For perceptual learning, subjects' performance in vernier discrimination task can be improved through training and is hemifield specific (Ling-Po Shiu & Harold Pashler, 1992). Here, we aimed to investigate the influence of visual experience in the real-world settings in medical context, specifically medical image recognition. Since the heart is mostly on the left side of the thorax and its general shape is highly consistent across population, it allows us to do image manipulation to check these two accounts. To study how visual experience affects the heart-image recognition, 20 participants with 1 to 2 years experience and 18 participants without experience in chest x-rays interpretation were recruited with age and sex matched. In this study, we used 20 chest x-rays images from patients with normal heart and another 20 images from patients with enlarged heart (determined by cardiothoracic ratio > 0.5 and confirmed by radiologists). The images were either oriented normally, flipped horizontally, flipped vertically, or both when shown to the participants. Each of the orientations was shown twice in a random order so that the participants experienced a total number of 320 images. We measured the recognition accuracy and reaction time when participants recognized normal versus enlarged hearts in order to compare the performance on each image orientation. The results showed that the experienced subjects performed better than the inexperienced subjects, especially when the hearts were on the left hemifield. However, inverted images did not affect image recognition in both groups. In summary, the evidence suggests that acquired experiences in chest x-rays interpretation helps image recognition through the process of perceptual learning at the level of sensory cortices rather than the process of object recognition at the ventral visual stream. In the future, we plan to recruit more medical doctors in specialized fields to investigate how the learning might change with longer experience.

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Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

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Program #/Poster #: 054.05

Topic: D.06. Vision

Support: ERA-NET NEURON, NeuroDREAM
TeleVision

Title: Visual perceptual learning in mice

Authors: ***A. CONSORTI**[1,2,3], G. SANSEVERO[1,2], I. DI MARCO[1,2], N. BERARDI[2], A. SALE[2];

[1]Florence Univ., Florence, Italy; [2]Inst. of Neurosci., CNR, Pisa, Italy; [3]F.M. Kirby Neurobio. Ctr., Boston Children's Hospital, Harvard Med. Sch., Boston, MA

Abstract: Perceptual learning refers to any change in perceptual performance as a result of practice. Perceptual learning occurs in all sensory modalities and is a fundamental phenomenon for improving response to environmental changes. In the visual system, practice improves the performance on various sensory tasks including grating, texture, hyperacuity or stereoscopic discrimination. Visual perceptual learning (vPL) is currently supposed to rely on still poorly characterized rearrangements in brain circuity occurring at early stages of sensory processing. In this study, we aimed to develop a vPL task for adult mice. A group of mice (n=14, P60) was subjected to a modified version of the Prusky water maze test. Mice were required to discriminate between two vertical sinusoidal gratings differing only for their spatial frequency (SF), until they achieved a performance level of at least 80% of accuracy in three subsequent sessions. Then, for a first group of mice (n=7), the two stimuli were made progressively more similar to each other, until the animal performance reached a steady plateau (vPL mice). In parallel, a different group of control mice were allowed to only discriminate between the two initial stimuli (first-step (FS) mice, n=7). When the performance plateau was reached, the two vertical gratings were rotated by 90° (orientational shift), and new trials were applied to assess the performance for horizontal gratings. FS mice were still able to perform the test after the orientational shift, without a significant change in their performance. On the contrary, vPL mice were totally unable to discriminate the newly oriented stimuli when the gratings were maintained at the same SFs reached before the orientational shift, with mice being able to only discriminate a difference between the SFs of the two gratings much higher than that recorded before the orientational shift. Starting from this point, additional vPL training was repeated, until the animal performance reached again a steady plateau. We found that this new threshold was not significantly different from that achieved with vertically oriented gratings. Our results provide evidence that a robust and reliable vPL can be induced in mice through an orientation-dependent process that strongly points toward a key involvement of the primary visual cortex.

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Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

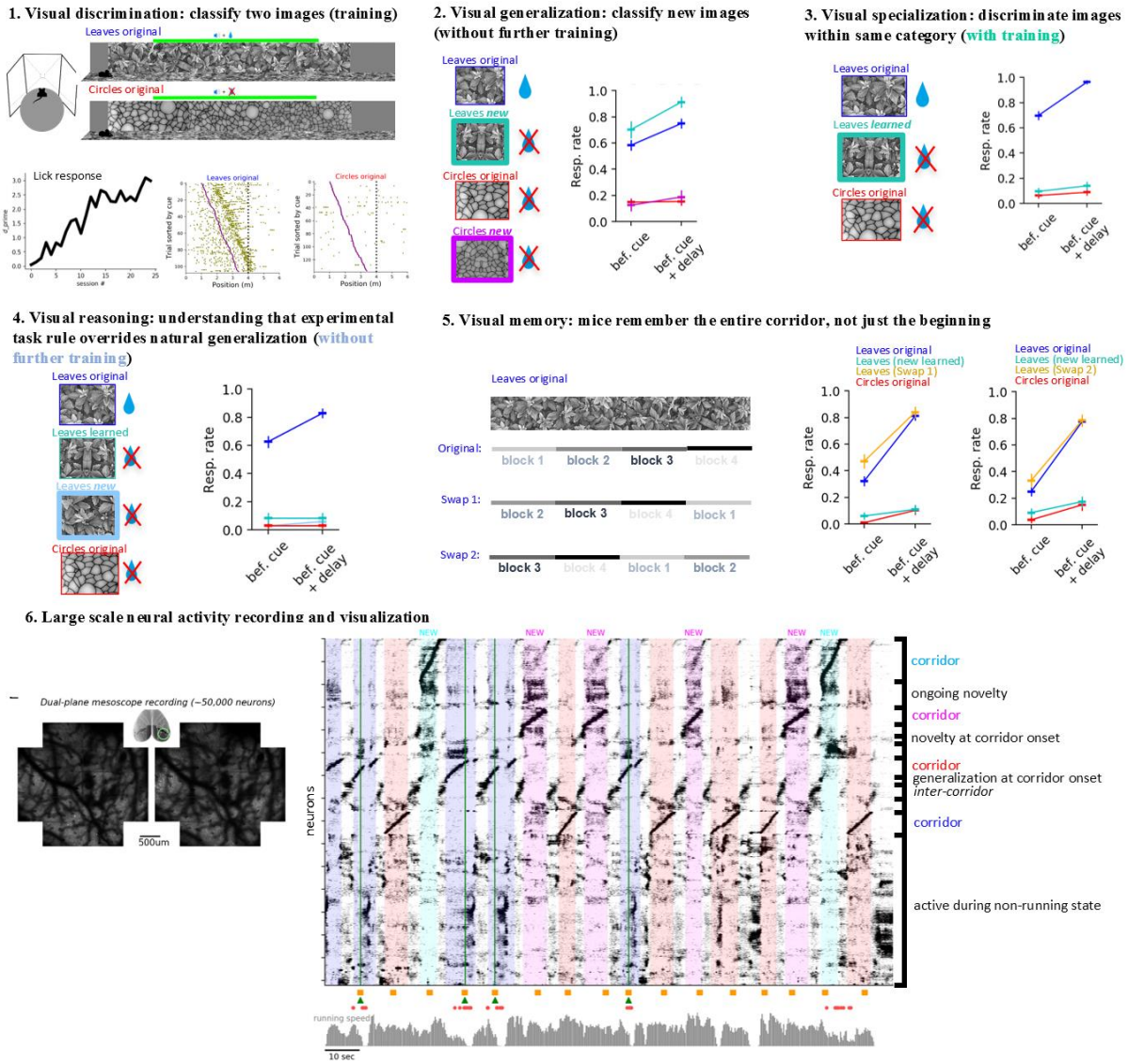
Program #/Poster #: 054.06

Topic: D.06. Vision

Title: Making sense of large-scale neural and behavioral data

Authors: *L. ZHONG, C. STRINGER, M. PACHITARIU;
Janelia research campus, Janelia Res. Campus, Ashburn, VA

Abstract: Humans and other primates possess a wide range of complex visual functions ranging from invariant object recognition to fine pattern discrimination, visual memory, spatial reasoning etc. We would ideally like to study the neural basis of such visual computations in lower animal models like the mouse, which are amenable to modern neuroscience techniques. However, it is not known if mice can perform interesting visual computations, and are notoriously known as "non-visual" animals. Here we demonstrate advanced visual functions in mice navigating and foraging in an immersive virtual reality while headfixed on an air-floating ball. The mice demonstrate: 1) visual generalization of texture class, 2) fine discrimination within a texture class, 3) visual reasoning about task rules and 4) high-capacity visual memory. While the mice were engaging in these tasks, we recorded up to 70,000 neurons simultaneously from multiple visual and non-visual cortical areas, using mesoscopic two-photon calcium imaging. We developed new analysis and visualization tools, and used them to identify several neural populations involved in these visual tasks. Our results pave the way for a new generation of high-powered visual neuroscience studies in mice.



Disclosures: **L. Zhong:** None. **C. Stringer:** None. **M. Pachitariu:** None.

Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

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Program #/Poster #: 054.07

Topic: D.06. Vision

Title: Training alters the perceptual readout of neuronal activity from cortical area V4

Authors: *P. LAAMERAD[1], D. GUITTON[2], C. C. PACK[2];
[1]Montreal Neurolog. Institute, McGill Univ., Montreal Neurolog. Institute, McGill Univ., Montreal, QC, Canada; [2]McGill Univ., McGill Univ., Montreal, QC, Canada

Abstract: The ventral visual pathway in primates is known to be responsible for the perception of shapes. Area V4 is an important part of this pathway, as it receives input from posterior visual structures and projects strongly to high-level structures like the inferotemporal (IT) cortex. Nevertheless, the role of V4 in perceptual decisions about shape is poorly understood. We therefore reversibly inactivated parts of V4, using muscimol, while non-human primates performed a challenging shape recognition task. Using response-pattern dissimilarity matrices, we found that circular and radial gratings, with ranges from low frequencies to high frequencies, are two categories that elicit the most dissimilar response patterns in V4. Previous work has suggested an over-representation of neurons that prefer circular grating in V4, and we confirmed this bias at the single-neuron level. We also found that the optimal stimuli contained low spatial frequencies. We therefore trained the animals to discriminate noisy patterns comprised of circular or radial gratings in two phases: 1) training with optimal stimuli (low-frequency gratings); 2) training with suboptimal stimuli (high-frequency gratings). Following training with optimal stimuli, inactivation of V4 severely impaired detection of the low-frequency circular, but not the low-frequency radial, gratings. In contrast, training with suboptimal stimuli,inactivation of V4 led to a large impairment in performance for both low-frequency circular and radial gratings. Neither neural response levels nor selectivity changed appreciably with training. Thus, the same neurons could contribute very differently to perceptual decisions about the same stimuli, depending on recent visual experience. The importance of biased representations in V4 was evident in the pattern of inactivation effects. When animals were trained to discriminate low-frequency circular and radial gratings, inactivation of V4 led to a strong perceptual bias for radial gratings. This was distinct from the threshold shifts that are often observed after inactivation of cortical neurons. In contrast, when the animals were trained with high-frequency gratings, inactivation led to the conventional threshold shifts in behavior. Altogether, these results suggest that subjects prefer to use a neural code based on overall population activity when possible, but that they can be trained to rely on comparisons between different neuronal subpopulations, when the task requires it. Given that many cortical areas contain biased representations, these two strategies should be considered in models of perceptual decision-making.

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Poster

054. Visual Learning, Memory, and Categorization

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Program #/Poster #: 054.08

Topic: D.06. Vision

Support: NIAAA Intramural Program ZIA-AA000421
NEI Intramural Program ZIA-EY000511
NIH Center on Compulsive Behaviors

Title: Circuits underlying striatal dopamine transmission with visual learning

Authors: *H. C. GOLDBACH[1,2,3], G. CONTRERAS[3], S. PREUSS[3], M. E. AUTHEMENT[1], K. ELLIOTT[3], V. A. ALVAREZ[1], R. J. KRAUZLIS[3];
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Abstract: The primary input area of the basal ganglia, the striatum, is thought to play a role in integrating signals from the cortex, midbrain, and thalamus to make associations between stimuli, actions, and rewards. The canonical view has been that midbrain activity drives all dopamine signals in the striatum. However, recent findings have forced the field to reconsider this viewpoint: cortical and thalamic inputs to the striatum can also produce large dopamine signals indirectly through striatal cholinergic interneurons (CINs). Here we further explore the cortical control over striatal dopamine signals and test whether sensory inputs to the striatum can also evoke dopamine release through CINs and whether training on a visual task affects the strength of this mechanism. Mice were trained on a unilateral orientation-change detection task requiring them to report a stimulus change by licking a spout. Expressing an excitatory opsin, ChR2, bilaterally in visual cortex allowed us to stimulate terminals of these inputs in the striatum in ex vivo brain slices while using fast-scan cyclic voltammetry to measure resulting dopamine signals. We found that V1 inputs could not evoke striatal dopamine release in either trained or untrained mice, which contrasted with a control experiment showing mPFC inputs could trigger striatal DA signals in this same region. Using whole-cell voltage-clamp recordings, we verified that visual inputs connect to CINs; however, the connection strength is weak and may be insufficient to synchronize the firing of CINs necessary to evoke dopamine release. We replicated these null results with auditory and somatosensory cortical inputs, demonstrating that no sensory area could evoke striatal dopamine release through CINs. Although activation of sensory inputs could not evoke striatal dopamine release, we found striking differences in electrically evoked striatal dopamine release that depended on visual task training; since visual cortical inputs could not be directly responsible for these differences, the dependence on visual learning indicates a potential role for thalamic or frontal inputs. These findings provide

surprising information regarding corticostriatal connectivity and the neural circuits involved in visual learning and sensory perception.

Disclosures: H.C. Goldbach: None. **M.E. Authement:** None. **K. Elliott:** None. **V.A. Alvarez:** None. **R.J. Krauzlis:** None.

Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.09

Topic: D.06. Vision

Support: USAMRDC/MOMRP

Title: Retinal Signaling Networks: Stress and Diet Effects Using Rodent Models

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Abstract: Eye injury and visual dysfunction resulting from military exposure affect a large number of Service Members and Veterans. Surveillance data from the Department of Defense (DOD) showed more than 275,000 eye injuries in the U.S. armed services between 2000 and 2017. Specifically, Service Members who have been diagnosed with traumatic brain injury (TBI) can have significant impairments of vision even when there is no direct injury to the eye, e.g. retina. Thus, these insults can affect the processing of the visual and nonvisual retinal signaling pathways. However, the molecular signaling mechanisms underlying this retinal damage are less studied, and hence effective therapies required to prevent the lingering and often serious symptoms, are lacking. Nutritional countermeasures could potentially be a vital therapeutic approach in diminishing stress-related debilitations. In the study, we exposed rats to simulated blast overpressure plus a weight drop head concussion to induce TBI and forced immersion under water trauma to instigate stress-like behavior. Further, to gain insights into the effects of dietary supplements on these injuries, adult male rats were fed with three different diets having different composition and ratios of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs). Long chain omega-3 fatty acids serve as the structural components of the brain and are essential for neuronal membrane synthesis. Omega-3 PUFAs also play a vital role in neuroinflammation by converting into potent anti-inflammatory metabolites while omega-6 PUFAs are known to be pro-inflammatory. Assessments were done 14 days post-insult to identify gene expression changes in the retina, using transcriptomic analysis. Our results showed that consumption of diets with higher omega-6 to omega-3 ratio in rats exposed to TBI altered differential expression

of networks mostly related to oxidative stress, vascular function and immune response. The findings in the retina demonstrated distinct transcript profiles for the traumatic stressor insult in rats fed with diets deficient in DHA (Docosahexaenoic acid) and EPA (Eicosapentaenoic acid), with particular focus on neuronal function and angiogenesis. These results suggest that having a diet with balanced omega-6/omega-3 PUFAs could potentially be beneficial in TBI victims, and the presence of omega-3 fatty acids like DHA and EPA may decrease the vulnerability to traumatic stress. This work was intramurally funded by the USAMRDC/MOMRP.

Disclosures: **M. Patel:** None. **A. Lawrence:** None. **A. Gautam:** None. **R. Yang:** None. **N. Chakraborty:** None. **J. DeMar:** None. **A. Batuure:** None. **D. Wilder:** None. **J. Long:** None. **R. Hammamieh:** None.

Poster

054. Visual Learning, Memory, and Categorization

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.10

Topic: D.06. Vision

Support: Mission Lucidity
KU Leuven C14/18/100

Title: Selective hippocampal activation and memory enhancement caused by epicranial current stimulation

Authors: ***N. PEELEMAN**[1], S. RIVA[1], H. HEYLEN[2], A. KHATOUN[1], N. VAN HELLEPUTTE[2], T. THEYS[1], M. MC LAUGHLIN[1], M. VANDENBULCKE[1], P. JANSSEN[1]; [1]KU Leuven, Leuven, Belgium; [2]Imec, Leuven, Belgium

Abstract: Many people suffer from cognitive decline caused by hippocampal dysfunction due to neurodegeneration, yet there is no effective medical intervention available. We propose a novel minimally invasive method to stimulate brain regions involved in memory by means of epicranial stimulation (ECS). In this study, three adult rhesus monkeys were implanted bilaterally with custom-built platinum stimulation electrodes on the temporal skull. We investigated the effects of ECS on behavior during a non-navigational spatial memory task, in which the animal had to remember the locations of two shapes presented on a touch screen. In one macaque monkey, we applied ECS before and during presentation of half of the patterns while the monkey performed the task, either at 0.1 mA or at 3 mA. We observed a significant memory improvement (percent correct) for the stimulated patterns with ECS at 40 Hz and 3 mA (X^2= 15.87, $p < 0.0001$). Stimulation at 10 Hz and 3 mA, however, induced a significant decrease in performance for the stimulated patterns (X^2= 11.97, $p = 0.00053$). In the same animal, we measured the changes in spike rate induced by 40 and 10 Hz ECS during single-cell recordings and passive fixation. For each neuron, we recorded a block of stimulation at 0.1 mA and at 3 mA, both at a frequency of 10 Hz and 40 Hz. We recorded from 25 neurons in the upper bank of

the lateral sulcus and 24 neurons in inferior temporal cortex (IT), directly beneath the stimulation electrode. In the lateral sulcus, the average firing rate increased significantly more after 40 Hz stimulation than after 10 Hz ECS (significant interaction between the factors frequency and pre/post stimulation, ANOVA unbalanced design, interaction effect p = 0.0091). However, we did not observe a systematic effect in the population of neurons recorded in IT. To chart the effects of ECS at the network level, we performed contrast-enhanced functional Magnetic Resonance Imaging (fMRI) during ECS at 3 mA in two other monkeys during ketamine/medetomidine sedation. In each session, stimulation blocks were alternated with no-stimulation blocks at either 40 or 10 Hz. ECS at 40 Hz resulted in significant activation of the hippocampal region in both animals (12.1% activated voxels for monkey P and 12.5% for monkey C), whereas ECS at 3 mA and 10 Hz did not produce any activations in this region (X^2= 539.20, p = 0.0001 for monkey P and X^2 = 616.56, p = for monkey C). In contrast, 40 Hz ECS did not evoke significant activations in lateral temporal cortex, consistent with our single-cell results. To conclude, 40 Hz ECS may be an effective minimally invasive approach to improve spatial memory.

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Poster

054. Visual Learning, Memory, and Categorization

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 054.11

Topic: D.06. Vision

Support: Simons Foundation (Simons Collaboration on the Global Brain award 543033)
National Eye Institute R01EY032878

Title: Disambiguating familiarity from visual modulation: a role for the hippocampus in recognition memory

Authors: *S. BOHN, C. HACKER, B. G. L. JANNUZI, T. MEYER, M. L. HAY, N. C. RUST; Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA

Abstract: Humans and other primates can accurately report whether they've seen images before, even after viewing large numbers of them, each only once. Evidence suggests that this recognition memory (or familiarity) behavior aligns with a neural signal in the brain called repetition suppression: a reduction of the overall vigor of the population response to the repeated presentation of an image, reflected in inferotemporal cortex (ITC) and the hippocampus (HC). However, at least in ITC, some visual properties of images also modulate the vigor of the population response. This leads to the question: How does the brain disambiguate whether changes in the vigor of the ITC population response follow from changes in familiarity versus

visual modulation?
To address this question, we focused on a type of visual modulation known as image memorability, a visual property whereby images that are more likely to be remembered produce more vigorous ITC responses. As a consequence of this type of visual magnitude modulation, simple repetition suppression decoding schemes cannot account for the mapping of ITC responses to memorability behavior. We hypothesized that the information that drives familiarity behavior is reflected in ITC, but that familiarity is disambiguated from this type of visual modulation downstream of ITC, in the hippocampus. To test this, we recorded neural activity in both ITC and HC as a rhesus macaque monkey performed a single-exposure familiarity task. In this task, the monkey viewed one image per trial and reported whether it was novel (never seen before) or repeated (seen exactly once). We drew images from a broad range of categories and memorabilities. Consistent with previous reports, the monkey's behavioral performance increased as a function of human-based image memorability scores.
We found several pieces of evidence consistent with our hypothesis that HC reformats information arriving from ITC to disambiguate familiarity from visual modulation. First, the vigor of the population response in both ITC and HC was modulated by familiarity. Second, modulations of population response vigor by the image property that we studied (image memorability) were reflected only in ITC, not HC. Third, the monkey's behavior was well-predicted by a simple spike count decoder that relied on repetition suppression in HC but not ITC. Finally, we found that total memory information was matched in ITC and HC. Together, these results suggest that computations performed downstream of ITC in the medial temporal lobe disambiguate familiarity from visual modulation. We speculate that this computation happens in the hippocampus.

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Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.01

Topic: E.03. Basal Ganglia

Support: The Simons Collaboration on the Global Brain

Title: Dissection of inter-area interactions of motor circuits

Authors: *E. GJONI[1], R. D. SRISTI[2], H. LIU[1], S. DROR[2], X. LIN[1], K. O'NEIL[1], O. M. ARROYO, Jr.[1], S. HONG[1], S. BLUMENSTOCK[1,4,5], B. LIM[1], G. MISHNE[2,3], T. KOMIYAMA[1,3];
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Abstract: Motor behaviors arise from dynamic interactions of interconnected neural populations across distributed brain areas. The underlying principles of information flow remain largely unknown. Here, we investigate the functional roles of motor cortex and intralaminar thalamus in driving specific subpopulations of the striatum - the input nucleus of the basal ganglia - during movements. We recorded the activity of direct and indirect pathway medium spiny neurons (dMSNs and iMSNs) in the striatum as mice performed a skilled motor task, by in vivo two-photon calcium imaging through GRIN lens. Furthermore, using monosynaptic pseudo-typed rabies virus we identified and imaged corticostriatal and thalamostriatal neurons that specifically project to dMSNs and iMSNs, through a glass window or a GRIN lens, respectively. MSNs showed a sustained population activity throughout movement duration that peaked at movement offset. Whereas, their cortical and thalamic inputs showed contrasting activity dynamics, with corticostriatal activity concentrated around movement onset and offset and thalamostriatal activity engaged during movement execution. To explore activity differences among dMSNs and iMSNs and their inputs, we developed Trial Ensemble Attention network (TEA-net) - a recurrent neural network with attention trained on ensembles of single-trial neuronal activity. This approach followed by clustering analysis identified quintessential activity patterns that were distinct between dMSNs and iMSNs and between cortical and thalamic neurons that specifically project onto them. The results provide insights on the mechanisms of integration of distinct long-range inputs carrying diverse information by MSN subpopulations.

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Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.02

Topic: E.03. Basal Ganglia

Support: NIH F31NS117093-01A1
NIH NCATS TL1TR003019
NIH F31NS124343-01
NIH R01NS094450
NSF IOS-1845355

Title: Anatomical and functional connectivity of POm projections to dorsolateral striatum

Authors: *A. YONK[1], R. S. ABDELGHAFAR[1], B. D. SANABRIA[3], D. J. MARGOLIS[2]; [1]Cell Biol. and Neurosci., [2]Cell Biol. & Neurosci., Rutgers Univ., Piscataway, NJ; [3]Cell Biol. and Neurosci., Rutgers Univ. New Brunswick, Piscataway, NJ

Abstract: The dorsolateral region of the striatum (DLS), the main input nucleus of the basal ganglia, is an essential hub that integrates sensory and motor signals from a multitude of cortical and thalamic inputs. Despite these widespread inputs, the influence of specific projections, such as posteromedial thalamic nucleus (POm), on striatal microcircuitry remains poorly understood. Therefore, it is vital to elucidate (1) where these POm-striatal inputs contact spiny projection neurons (SPNs) and interneurons and (2) how these inputs influence the striatal microcircuitry. Here, we are interrogating the anatomical and functional connectivity of thalamic input from POm onto three identified striatal cell types in anterior DLS.
Channelrhodopsin-2 (pAAV-hSyn-hChR2(H134R)-EYFP) was expressed unilaterally in POm of male and female double transgenic mice. These mouse lines permit the identification of D1-SPNs (Tg(DrD1-cre)EY262 x Ai14 (R26-LSL-tdTomato)), D2-SPNs ((Tg(Adora2a-cre)KG139Gsat x Ai14), or parvalbumin-expressing interneurons (PV-INs; Pvalbtm1(cre)Arbr/J x Ai14) via red fluorescence in the striatum. Ex vivo whole-cell current clamp recordings were performed on the three cell types within the ipsilateral anterior DLS. Identified cells were confirmed via responses to hyperpolarizing and depolarizing current injections including resting membrane potential, half-height width, and other characteristics (ramp depolarization, spike adaptation frequency, etc.).
Our initial results indicate that optogenetic activation (via 40X objective; ~1.2mW intensity) of POm-striatal terminals readily elicits moderate subthreshold postsynaptic potentials (PSPs) in D1-SPNs, D2-SPNs, and PV-INs. For the single pulse protocol (one 2.5ms pulse, 460nm), D2-SPNs exhibited the most robust PSP (7.97 ± 2.57mV) followed by PV-INs (6.30 ± 1.39mV) and D1-SPNs (2.47 ± .641mV). For the paired pulse protocol (five 2.5ms pulses, 50ms IPI), all three cell types exhibited short-term depression with D2-SPNs having mild short-term depression, while PV-INs and D1-SPNs have moderate depression. For the train protocol (twenty-five 2.5ms pulses, 97.5ms IPI, 10Hz), all three cell types exhibited mild short-term depression with little amplitude changes. Finally, preliminary 3D reconstructions of recorded PV-INs appear to show an even distribution of synaptic contacts amongst all orders of dendrites and potentially many contacts on the soma. Further data collection and analysis for both electrophysiology and reconstructions are ongoing.

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Poster

055. Basal Ganglia: Physiology and Function I

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Program #/Poster #: 055.03

Topic: E.03. Basal Ganglia

Support: NIH Grant NS125814-01

Title: Diverse responses of substantia nigra pars reticulata neurons to in vivo optogenetic stimulation of globus pallidus external segment in mice

Authors: *J. PARKER[1], A. ARISTIETA[2], A. H. GITTIS[2], J. E. RUBIN[1];
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Abstract: GABAergic signals from neurons in the globus pallidus external segment (GPe) and striatal direct pathway are assumed to exert an inhibitory effect on the basal ganglia (BG) output nucleus in the rodent substantia nigra, the substantia nigra pars reticulata (SNr). Indeed, prevalent frameworks summarizing BG contributions to action selection and tuning are based on this assumption, positing a role for the direct pathway as promoting or invigorating movement by inhibiting SNr activity and a need to reduce GPe firing in order to suppress movement. Recent experimental results, however, have revealed surprisingly diverse SNr responses to optogenetic activation of GPe and striatal direct pathway neurons. In this work, SNr neurons were recorded in vivo from both control and dopamine depleted (DD, based on 6-OHDA) awake mice during repeated GPe optogenetic stimulation. A novel classification method was developed and used to analyze the diverse set of responses recorded from the SNr neurons. Specifically, we computed a spike density function (SDF) and an interspike interval function (ISIF) both for a baseline period immediately preceding the stimulation and also during each stimulation epoch. For each neuron, the baseline results were leveraged to derive significance criteria, while averaging the SDF and ISIF across trials provided results to compare against these criteria. This analysis shows that SNr responses to GPe stimulation are strikingly diverse, including the expected sustained inhibition but also excitation, adapting and partial inhibition, and biphasic patterns. Baseline SNr neuron firing rate, CV, and burstiness do not predict the nature of this response, but responsiveness does depend on neuronal positioning within the SNr. In comparison to control, DD neurons have a lower baseline frequency distribution, produce a higher proportion of spikes within bursts, display enhanced delta-band oscillations, and exhibit fewer increases in firing to GPe stimulation; this stimulation unexpectedly can increase measures of SNr burstiness. Together, these results call for a reinterpretation of the function of the GPe projection to the SNr and suggest that this pathway's effects may vary across time, target neurons, and conditions.

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Poster

055. Basal Ganglia: Physiology and Function I

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Program #/Poster #: 055.04

Topic: E.03. Basal Ganglia

Support: R00MH109569
T32NS041234

Title: Striatonigrostriatal Circuits for Disinhibition of Dopamine Signaling

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Abstract: The basal ganglia operate largely in *closed* parallel loops, including an associative circuit for goal-directed behavior originating from the dorsomedial striatum (DMS) and a somatosensory circuit important for habit formation originating from the dorsolateral striatum (DLS). An exception to this parallel circuit organization was proposed to explain how information is transferred between striatal subregions, for example from DMS to DLS during habit formation. The "ascending spiral hypothesis" proposes that DMS disinhibits dopamine signaling in DLS through an *open* loop involving substantia nigra pars reticulata (SNr) and compacta (SNc). Specifically, this hypothesis predicts the existence of a tri-synaptic striatonigrostriatal circuit, DMS→SNr→SNc→DLS. Despite deeply influencing the habit and addiction literature, this hypothesis rests on weak anatomical evidence and lacks functional support. I tested the ascending spiral hypothesis using electrophysiology, optogenetics, and new tools available for circuit interrogation in mice. Using transsynaptic and intersectional genetic tools, I labeled SNr and SNc cells based on their inputs and outputs, respectively. Together, these tools allowed me to investigate both closed- and open-loop striatonigrostriatal circuits *ex vivo*. I found strong evidence for closed loops (e.g., DLS→SNr→SNc→DLS), which would allow striatal subregions to self-regulate their dopamine signaling. I also found evidence for functional synapses in open loops, including a descending spiral (DLS→SNr→SNc→DMS). However, the synapses in open loops were unable to modulate dopamine neuron firing, questioning their ability to mediate crosstalk between striatal subregions through disinhibition of dopamine neurons. These findings challenge key predictions from the ascending spiral hypothesis and call for alternative mechanisms of habit formation.

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Poster

055. Basal Ganglia: Physiology and Function I

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Topic: E.03. Basal Ganglia

Support: National Institute of Mental Health (R01 MH060379 to A.M.G.)
JSPS kakenhi 18H04945

Title: Striatal neurons encode rhythm parameters of mice running in complex stepping.

Authors: *K. HIROKANE[1,2], T. NAKAMURA[2], Y. KUBOTA[3], H. DAN[3], T. YAGI[2], A. M. GRAYBIEL[3], T. KITSUKAWA[1,2];
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Abstract: Many of our actions, such as playing musical instruments, playing sports, or walking and running, are continuous movements involving many body parts such as limbs and fingers at the same time. When we play the piano, multiple fingers sometimes move simultaneously, alternately, or sequentially. The movement of fingers is often repetitive and rhythmic. In addition, rhythms of each finger are coordinated temporally, as if all finger rhythms are connected to each other. It is not clear how such coordination of movements is shaped in the brain. In our previous study, to investigate the coordination of multiple body part movements, we developed a complex stepping task for mice, the 'step-wheel task'. The step-wheel is a motor-driven vertical wheel in which mice can receive water reward by controlling their speed and the step patterns as they run. The step patterns of mice can be controlled by the pattern of pegs that serve as footholds for mice. We recorded spike activity from the dorsal striatum as mice ran on the step-wheel. We found neurons responding to events, including the start or stop of drinking, licking of the spout, and touches of forelimb paws to pegs. Among them, we focused on neurons with phasic responses to touches, because their responses were not uniform for every touch; responses were high for some touches but low for others. To further analyze such responsivity of the neurons, we used two peg patterns: the Interval peg-pattern and Phase peg-pattern. In the Interval peg-pattern, the space between two consecutive pegs was gradually changed from narrow to wide. In the Phase peg-pattern, the relative positions of right and left pegs were gradually changed. We found that a considerable number of touch-responsive neurons showed the modulation of firing rate by the interval and/or by the phase. We analyzed the relationship between stepping and firing over several steps back and forth to test whether those touch-responsive neurons were associated with repetition. It became clear that the firing rate of those neurons was increased in response to repeated touches in a specific touch cycle.Finally, we investigated the relationship between the firing rate of the neurons to the coordination of licking and footfalls. We found neurons that increased their firing rates at specific phases of the licking cycle. Notably, we discovered cross-phase neurons, which increased their firing rates with a particular phase-combination of the licking and touch cycle.These results indicate that striatal neurons may serve the coordination of multiple body parts in repetitive movements using the parameters of rhythm: interval, phase, and repetition.

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Poster

055. Basal Ganglia: Physiology and Function I

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Program #/Poster #: 055.06

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NSF NeuroNex Award 1707408

Title: Striatal phase locking to the gait cycle during walking

Authors: *L. YANG, S. C. MASMANIDIS;
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Abstract: The striatum plays an important role in regulating voluntary movements, including walking. Previous work shows that striatal neurons encode the start and stop of locomotion, as well as movement speed. However, since walking speed is the result of multiple coordinated limb movements, there is still an incomplete picture of how striatal neurons encode single-limb kinematics during walking (i.e., gait). Here, using high resolution, high-speed video tracking (80 fps), we recorded mice freely walking in a large open arena (60 cm x 60 cm). Simultaneous electrophysiological recordings were performed in the dorsal striatum. The large open field enabled mice to walk at a wide range of speeds. Using open-source machine-learning based behavioral tracking techniques, we successfully extracted the 2D trajectory of each limb's position in the arena, which allowed us to examine how neural activity is related to body kinematics at the single-limb level. We then used circular analysis methods to test whether individual neurons are locked to specific phases of gait. Around half of recorded striatal neurons were significantly entrained to the phase of at least one limb. While the preferred phase varied across the population, on average the preferred phase corresponded to the start of the stance period of the contralateral rear limb. Taken together, these findings show that striatal neurons represent the phase of individual limb movements, suggesting a more nuanced coding scheme than whole-body speed. This work also opens new avenues for exploring how brain activity during walking is disrupted in movement disorder models.

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Poster

055. Basal Ganglia: Physiology and Function I

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Program #/Poster #: 055.07

Topic: E.03. Basal Ganglia

Support: NIH R01 NS105690
NIH R01 NS119520

Title: Subthalamic deep brain stimulation of an anatomically detailed model of the human hyperdirect pathway

Authors: *C. S. BINGHAM[1], C. C. MCINTYRE[2];
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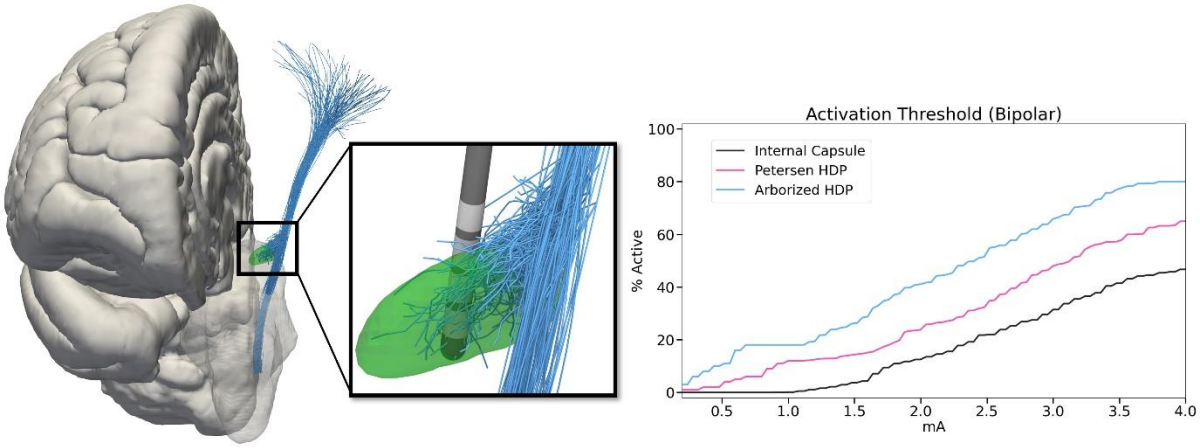
Abstract: The motor hyperdirect pathway (HDP) is considered a key target in the treatment of Parkinson's disease with subthalamic deep brain stimulation (DBS). This hypothesis is partially derived from the association of HDP activation with evoked potentials (EPs) generated in the motor cortex and subthalamic nucleus (STN) after a DBS pulse. However, the biophysical details of how and when DBS-induced action potentials (APs) in HDP neurons reach their terminations in the cortex or STN remain unclear. Therefore, we used an anatomically detailed representation of the motor HDP, as well as the internal capsule (IC), in a model of human subthalamic DBS to explore AP activation and transmission in the HDP and IC. Our results show that small diameter HDP axons exhibited AP initiation in their subthalamic terminal arbor, which resulted in relatively long transmission latencies to cortex (~3.5-8 ms). Alternatively, large diameter HDP axons were most likely to be directly activated in the capsular region, which resulted in short transmission times to the cortex (~1-3 ms). However, those large diameter HDP antidromic APs would be indistinguishable from any other IC axons that were also activated by the stimulus. Conversely, DBS-induced APs in both small and large diameter HDP axons reached their synaptic boutons in the STN with similar timings, but both spanned a wide temporal range (~0.5-5 ms). We also found that using anodic or bipolar stimulation helped to bias activation of the HDP over the IC. These computational results provide useful information for linking HDP activation with EP recordings in clinical experiments.



Disclosures: C.S. Bingham: None. **C.C. McIntyre:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.08

Topic: E.03. Basal Ganglia

Support: NIH R21 NS123763

Title: Area X marks the spot: cell type specific transcriptomic signatures of sensorimotor learning and singing in zebra finch basal ganglia

Authors: *E. COOKE[1], A. FERNANDEZ[2], S. DE FLORENCIO[1], M. FARIAS-VIRGENS[3], J. E. VAN VEEN[1], S. WHITE[1];
[1]UCLA, Los Angeles, CA; [2]Integrative Biol. & Physiol., UCLA, Rosamond, CA; [3]Univ. of California Los Angeles, Los Angeles, CA

Abstract: The basal ganglia play an important role in sensorimotor learning, in part, by providing signals about the quality of the output of varying motor patterns. Zebra finch (T.guttata) song learning occurs during a sensorimotor critical period during development and requires a subregion of the basal ganglia, Area X. Within Area X, expression of the transcription factor FoxP2 is behaviorally regulated by singing, and experimentally interfering with this behavioral regulation via knockdown or overexpression impairs song learning. Previous work in the lab used Weighted Gene Co-Expression Network Analysis (WGCNA) with RNA sequencing data to identify gene modules in Area X linked with singing and learning. However, it remains unclear which cell type(s) express genes found in these behaviorally relevant modules. Single-cell RNA sequencing (10X Genomics) allows for investigation into cell-type-specific transcriptomic changes after singing, as well as longer-term cell-type-specific shifts following critical period closure. Here we present juvenile and adult single-cell Area X transcriptomes, along with song data, to better understand the effects of singing and learning status on basal ganglia cell types. We apply differential expression analysis to specific cell types across conditions (age, singing status), and use Gene Set Enrichment Analysis to map gene modules previously identified via bulk sequencing WGCNA onto individual Area X cell types. These analyses provide insight into how acute changes in behavior, and closure of the sensorimotor critical period, shape the transcriptome of the avian basal ganglia.

Disclosures: E. Cooke: None. **A. Fernandez:** None. **S. De Florencio:** None. **M. Farias-Virgens:** None. **J.E. Van Veen:** None. **S. White:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.09

Topic: E.03. Basal Ganglia

Support: Israel Science Foundation (ISF) (297/18)

Title: Uncovering Globus Pallidus encoding of movement during normal and disinhibited states in non-human primates using temporal alignment

Authors: *E. ZINKOVSKAIA, M. BRONFELD, I. BAR-GAD;
Bar-Ilan Univ., Ramat-Gan, Israel

Abstract: Basal ganglia (BG) dysfunction is associated with multiple hyper-behavioral motor disorders such as dyskinesia, hyperactivity and stereotypy, however, the exact role of the BG in these disorders is unknown. This study aims to extend current understanding of the globus pallidus role in modulating normal and abnormal movement using previously recorded data. We invoked dyskinetic, hyperactive and stereotypic states in two male Macaca Fascicularis monkeys by disinhibiting different areas of the globus pallidus externus (GPe) using the GABA-A antagonist, bicuculline. We then analyzed the neuronal activity recorded via multi-electrode electrophysiology, which includes single units, multi-unit activity and local field potential in the GPe and globus pallidus internus (GPi). The experimental setup allows evaluating upper-limb task-related and task-unrelated movements. We extracted body pose estimation from the videos using deep neural networks (DeepLabCut) and analyzed movement kinematics. As we revisited previously evaluated data, we confirmed observations that single unit activity responds to task-related pauses in movement, i.e., pauses between movement segments. We then looked into movement motifs and pallidal modulation throughout their execution. We visually assessed the behavior for repetitive motifs and then performed automatic search for such patterns employing shift-only, linear and/or dynamic-time warping (DTW) based alignment. Applying the best-found alignment to the neuronal activity, we searched for consistent modulations and compared them during different disinhibited states. In parallel, using the affine-warp framework, we performed analysis in the opposite direction, i.e., neuronal to behavioral. The framework allows for unsupervised alignment of the neuronal activity, using analogous shift, linear or DTW alignment. This bidirectional approach to alignment provides a confirmation to the neuronal-behavioral mapping. Additionally, the framework was shown to uncover previously unseen phenomena over trials. We extended the method to non-identical repetitive movement motifs such as dyskinetic and stereotypic movements. Using bi-directional temporal alignment allows analyzing non-constrained movement and generalizing over motifs that vary in velocity, amplitude and path. This allows us to evaluate behavior directly as opposed to interpolating from experimental features. As the study includes various disinhibited states and analysis of free movement in non-human primates, it could broaden our understanding of the BG role in movement during hyper-behavioral movement disorders.

Disclosures: E. Zinkovskaia: None. **M. Bronfeld:** None. **I. Bar-Gad:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.10

Topic: E.03. Basal Ganglia

Support: F32MH125596

Title: Dissecting cortico-basal ganglia interactions controlling action selection

Authors: *A. E. GIRASOLE[1,3], G. MANDELBAUM[2], M. A. ALBANESE[4], Y. ZHANG[4], T. M. HAYNES[4], M. DIAZ BOBILLO[4], W. WANG[4], B. L. SABATINI[4];
[2]neurobiology, [1]Harvard Med. Sch., Boston, MA; [3]Dept. of Neurobio., Howard Hughes Med. Inst., Boston, MA; [4]Dept. of Neurobio., Howard Hughes Med. Inst. & Harvard Med. Sch., Boston, MA

Abstract: Selecting future actions based on previous experiences is key to an animal's survival. This process, known as action selection, depends on the proper function of cortical and subcortical basal ganglia circuits. Despite the importance of these regions for using previous experiences to inform upcoming motor choices, we do not understand the precise mechanisms by which these regions work together and the activity patterns they use to select actions. The significance of these regions in action selection is clear in disorders that arise from cortical and basal ganglia dysfunction. One hypothesis is that the cortex develops motor plans that the basal ganglia then executes and evaluates based on outcome. Based on anatomy, cortex and basal ganglia form a recurrent loop in the brain, however we do not understand how the two work together to promote and select actions. The anterior lateral motor cortex (ALM), sends strong projections to the dorsolateral striatum (DLS), a region previously implicated in action generation, specifically licking. In order to understand the functional consequences of this ALM[[Unsupported Character - Symbol Font ]]DLS circuit organization, we designed a behavioral task in which mice were required to base their next choice on their previous actions and outcomes associations (AOA). We hypothesize that striatal activity is modified based on action outcome and its recurrent feedback to ALM is necessary to update subsequent motor planning required for action selection. Optogenetic stimulation of DLS direct and indirect spiny projection neurons during the AOA formation period caused biases in action choice made several seconds later, contraversive and ipsiversive, respectively. Additionally, optogenetic inhibition of DLS direct and indirect spiny projection neurons during the AOA formation period caused biases in action choice in the opposite direction of excitation. Finally, optogenetic inhibition of ALM during the delay period after the AOA had been formed but prior to reporting the choice caused the same bias. These data suggest that the execution of actions and reinforcement are linked through the dorsolateral striatum.

Disclosures: A.E. Girasole: None. **G. Mandelbaum:** None. **M.A. Albanese:** None. **Y. Zhang:** None. **T.M. Haynes:** None. **M. Diaz Bobillo:** None. **W. Wang:** None. **B.L. Sabatini:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.11

Topic: E.03. Basal Ganglia

Support: NIH R01NS104089
NIH T32GM008444

Title: Acetylcholine release is pathologically elevated in the dorsal striatum of SAPAP3-null mice

Authors: *A. BAEZ, J. MALGADY, J. L. PLOTKIN;
Neurobio. and Behavior, Stony Brook Univ., Stony Brook, NY

Abstract: Obsessive compulsive disorder (OCD) is a neuropsychiatric disease listed as top 10 and top 15 most disabling illnesses in women and men, respectively, and has a predicted prevalence of 2.3% in the United States. SAP90/PSD95-associated protein 3 (SAPAP3) is a postsynaptic scaffold protein which is enriched at corticostriatal synapses and has been linked to OCD. The deletion of SAPAP3 in mice recapitulates aspects of OCD with biological validity (striatum hyperactivity), behavioral validity (anxiety-like behaviors and compulsive grooming), and treatment validity (reduction in compulsive behavior by chronic course of selective serotonin reuptake inhibitor). Here we show that acetylcholine (ACh) release is dysregulated in the dorsal striatum of SAPAP3-null mice. Cholinergic interneuron (CIN) soma and axonal punctae are increased in density in the dorsal striatum of SAPAP3-null mice, as determined by immunohistochemical staining for choline acetyltransferase (ChAT) and vesicular ACh transporter (VAChT). Electrophysiological recordings in *ex vivo* brain slices demonstrate that the firing rate of CINs is also increased in SAPAP3-null mice. Consistent with these findings, we found that evoked release of striatal ACh is also elevated in the dorsal striatum of Sapap3-null mice, as measured in acute brain slices using a genetically-encoded fluorescent ACh sensor. We hypothesize that dysregulated release of ACh by CINs may shape striatal circuit dysfunctions and promote compulsive motor behaviors in SAPAP3-null mice.

Disclosures: A. Baez: None. **J. Malgady:** None. **J.L. Plotkin:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.12

Topic: E.03. Basal Ganglia

Support: H2020 European Research Council (imove 755745)

Title: Complex dynamical coding of simple movements in the output of the basal ganglia

Authors: *G. ZUR, N. LARRY, M. JOSHUA;
Hebrew Univ. of Jerusalem, Hebrew Univ. of Jerusalem, Jerusalem, Israel

Abstract: The Substantia Nigra pars Reticulata (SNpr), an output structure of the basal ganglia, is known to play a role in pursuit and saccadic eye movements. Previous studies have reported

that neurons in the SNpr are active tonically and show either a pause or increase during eye movements, consistent with the gating role of the basal ganglia output. These studies have mostly focused on probing SNpr activity in a narrow behavioral regime. We recorded activity in the SNpr of two monkeys while they performed a broad regime of behavioral eye movement tasks including saccade and pursuit eye movements, with eight directions of movement and multiple reward probability conditions. SNpr neurons exhibited a highly complex reaction pattern during pursuit, including frequent increases and decreases in firing rate during movement, uncorrelated responses in different directions and reward conditions, and high variance in reaction times and reaction durations. Responses during saccades were not as complex, indicating that some of the complexity was related to the instantaneous sensorimotor control of pursuit eye movements.
Prior to movement, the color of a visual cue signaled the probability of the upcoming reward. The response of SNpr neurons during the presentation of the cue encoded the reward probability, with uncorrelated dynamic patterns between different reward conditions. Comparison of the responses of the SNpr to other brain regions in the eye movement pathway indicated that SNpr neurons had a specific high-dimensional dynamic that differentiated them from other regions in the eye movement pathway.
These complex dynamic responses contrast sharply with simple eye movement behavior, suggesting a role for the basal ganglia beyond inhibiting or permitting behavior. Instead, our results suggest a dynamic complex mapping between sensorimotor parameters and activity, akin to intermediate levels in artificial neural networks. Analogous to these networks, we suggest that the SNpr dynamically decomposes information into components with a diverse set of filters. Thus, complex activity in the basal ganglia could represent its role in extracting features of sensorimotor information into a dynamic pattern that may span a wide range of behavioral functions.

Disclosures: **G. Zur:** None. **N. Larry:** None. **M. Joshua:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.13

Topic: E.03. Basal Ganglia

Support: NIH/NINDS DP1 NS116783
NIH/NIA R01 AG064051
NIH/NIBIB R01 EB030884
NIH/NIMH RF1 MH114227
NIH/NINDS R01 NS091461
NIH S10 OD025176

Title: Investigation of basal ganglia output pathways using optogenetic fMRI

Authors: *B.-M. GU[1], G. O. CRON[1], J. LEE[1,2,3,4];
[1]Dept. Neurol. and Neurolog. Sci., [2]Dept. of Bioengineering, [3]Dept. of Neurosurg., [4]Dept. of Electrical Engin., Stanford Univ., Stanford, CA

Abstract: Basal ganglia are critical in multiple behavioral controls, including movement, decision making and reward processing. Dysfunctions of this circuit have been known to be associated with multiple neurological and psychiatric diseases. Multiple information signals converge at the basal ganglia output nucleus, substantia nigra reticulata (SNr), and then the SNr sends diverging outputs to multiple brain areas including thalamus, superior/inferior colliculus, and pontine reticular formation. Recent anatomical studies are starting to show cell-type-specific downstream targets of SNr. In particular, glutamic acid decarboxylase 2 (GAD2) and parvalbumin (PV) expressing cells in the SNr are reported to anatomically target different brain areas and are known to differentially modulate sleep. However, the downstream functions associated with these differential anatomical connections are unknown. In this study, using optogenetic functional MRI (ofMRI) and electrophysiology methods in mice, we showed the cell-type-specific effects of SNr on downstream targets. Using GAD2-cre and PV-cre mice, Cre-dependent ChR2 virus was expressed in the SNr and 10 Hz laser stimulations were applied repeatedly with 20s on and 40s off cycles. The optogenetic stimulation of specific cell types of SNr produced different whole brain activity patterns measured using BOLD signal. The findings reveal the common and distinct SNr downstream targets in a cell-type-specific manner.

Disclosures: B. Gu: None. **G.O. Cron:** None. **J. Lee:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); LVIS. F. Consulting Fees (e.g., advisory boards); LVIS.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.14

Topic: E.03. Basal Ganglia

Support: ISF Grant 297/18

Title: Striatal encoding of action sequences in normal and hyperactive rats

Authors: *O. TAHARY[1], K. LÖFFLER[2], K. BELELOVSKY[1], I. BAR-GAD[1];
[1]Gonda Multidisciplinary Brain Res. Ctr., Bar-Ilan Univ., Ramat Gan, Israel; [2]Inst. for Automation and Applied Informatics (IAI), Karlsruhe Inst. of Technol. (KIT), Eggenstein-Leopoldshafen, Germany

Abstract: The basal ganglia (BG) are a group of nuclei involved in motor, associative and limbic functions. Abnormalities in their function are associated with hyper-behavioral disorders such as Tourette's syndrome and attention deficit hyperactivity disorder (ADHD). The BG have a primary role in learning and production of motion sequences; however, the representation of

these sequences and the changes in encoding that relate to these disorders are yet unclear. This gap historically stems from the difficulty to quantify complex natural behavioral sequences. Recent advances in the fields of computer vision and machine learning mitigate some of these difficulties. This study aims to explore the relation between neuronal activity in different areas of the striatum, the main input nucleus of the BG, and the expression of behavioral sequences in normal and hyperactive rats. The rats were rendered temporarily hyperactive using local microinjections of bicuculline into the ventral striatum (Nucleus Accumbens - NAc). The rats were freely behaving in a Plexiglas arena and their behavior was recorded using high-speed cameras (120 fps). In conjunction, extracellular neurophysiological recordings were wirelessly recorded in both motor (dorsal) and limbic (ventral) areas of the striatum. [IBg1] We developed a custom landmark detection method using a stacked hourglass convolutional network to identify key landmarks on the video of the freely behaving normal and hyperactive rats. A variational autoencoder was used on the landmarks to represent the behavior in a lower dimensional space. This was followed by clustering on that latent space. Initial results suggest that there are significant differences is the expression of behavioral sequences between normal and bicuculline injected animals. Our analysis is focused on finding neuronal activity patterns unique to the hyperactive state and correlated to abnormal sequences of behavior. The findings of this study could help understand the striatal role in the organization of behavior and to better understand the causes of BG related disorders. That understanding could, in turn, translate into better diagnostic and treatment methods. In addition, software tools developed for the study could be used to improve upon existing methods and aid in projects associating behavior with neuronal activity.

Disclosures: **O. Tahary:** None. **K. Löffler:** None. **K. Belelovsky:** None. **I. Bar-Gad:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.15

Topic: E.03. Basal Ganglia

Support: BRAIN Initiative NINDS R00 Grant awarded to RCE: R00NS112417
American Parkinson's Disease Association (APDA) Grant awarded to RCE: 2021APDA00RG00000209666

Title: Evaluating the role of cholinergic inputs from the pedunculopontine nucleus on dopaminergic neurons of the substantia nigra pars compacta

Authors: *M. L. BEAVER[1], M. EZEIZA-ORTEGA[2], S. GROSSEN[2], M. KILEY[2], C. B. SCOTT[2], A. E. SWIATEK[2], M. R. CROOM[2], N. D. NECKEL[2], R. C. EVANS[2];
[1]Pharmacol. and Physiol., [2]Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: Neuronal populations that degenerate in Parkinson's Disease (PD) include the dopaminergic neurons of the substantia nigra pars compacta (SNc) and the cholinergic neurons of the pedunculopontine nucleus (PPN) that provide the main cholinergic input to the SNc. Dysfunction and degeneration of the dopaminergic neurons of the SNc cause hallmark motor symptoms of PD. The degeneration of cholinergic PPN neurons in PD may enhance degeneration of SNc DA neurons (Bensaid et al., 2015), but the cholinergic connection from the PPN to the SNc has not been fully characterized. The cholinergic input to the SNc can activate multiple types of nicotinic receptors and M5 muscarinic receptors. We use whole-cell patch clamp *ex vivo* electrophysiology in wild-type (C57B6/J) mice to evaluate the effects of muscarinic receptor activation on the most vulnerable population of SNc dopaminergic neurons. These neurons are located in the ventral tier and are characterized by a large, T-type calcium channel-mediated afterdepolarization (ADP) (Evans et al., 2017). Because both the M5 muscarinic receptor and the Ghrelin receptor are G_q-coupled receptors known to inhibit the CaV3.3 T-type calcium channel subunit in cultured cells (Mustafa et al., 2020; Hildebrand et al., 2007), we tested whether activation of these receptors in dopaminergic neurons would inhibit the T-type-mediated ADP. We find that application of either the non-selective muscarinic agonist Oxotremorine (OxoM) or Ghrelin decreases the size of this ADP. This finding is important because it suggests that muscarinic activation could alter information processing by ventral tier SNc dopaminergic neurons. Because the cholinergic PPN neurons are the most likely source for muscarinic activation of SNc neurons in healthy animals, we have developed a mouse model of PD cholinopathy by selectively lesioning the cholinergic PPN neurons by injecting a cre-dependent caspase into the PPN of ChAT-cre;Ai9 mice. Using CatWalk gait analysis, balance beam tests, and open field, we found that these model mice exhibit mild gait deficits, but do not have severe motor impairments. *Ex vivo* electrophysiological recordings of SNc dopaminergic neurons in these mice show no differences in intrinsic action potential properties or neuronal excitability. We are currently conducting experiments to determine whether the dopaminergic neurons in this PD cholinopathy model show an altered sensitivity to muscarinic receptor activation. This project will help us understand how the activity of the vulnerable SNc dopaminergic neurons is affected by the degeneration of their primary cholinergic inputs.

Disclosures: M.L. Beaver: None. **M. Ezeiza-Ortega:** None. **S. Grossen:** None. **M. Kiley:** None. **C.B. Scott:** None. **A.E. Swiatek:** None. **M.R. Croom:** None. **N.D. Neckel:** None. **R.C. Evans:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.16

Topic: E.03. Basal Ganglia

Support: BRAIN Initiative NINDS R00 R00NS112417
American Parkinson's Disease Association (APDA)
2021APDA00RG00000209666

Title: Role of pedunculopontine nucleus excitation in motor learning

Authors: *C. B. SCOTT, Z. COLON, M. R. CROOM, R. C. EVANS;
Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: The pedunculopontine nucleus (PPN) is a heterogeneous structure in the midbrain, consisting of cholinergic, glutamatergic, and GABAergic neurons (Wang and Morales, 2009). In close relation to the basal ganglia, the PPN is implicated in basal ganglia circuitry responsible for motor behaviors and gait control. Cholinergic neurons of the PPN have been implicated in modulating locomotion, particularly, increasing motion (Dautan et al., 2016, Xiao et al., 2016). Literature has shown that in Parkinson's Disease, where motor skill learning is impacted, cholinergic neurons are lost and may precede the major dopaminergic loss (Rinne et al., 2008). In rodent studies, lesioning the cholinergic neurons results in disrupted motor learning (MacLaren et al., 2014). On the other hand, literature has shown exercise greatly improves motor skill learning in both mice and humans (Li & Spitzer, 2020; Wanner et al., 2021). We have seen that a week of wheel running, in both sexes of wild-type C57BL/6J mice, significantly improves performance on an accelerating rotarod assay. Because the cholinergic PPN neurons are implicated in enhanced motor learning (Li and Spitzer, 2020), we evaluated their electrophysiological characteristics after a week of wheel running using ChAT-cre/tdTomato mice. Through *ex vivo* whole cell patch clamp electrophysiology, we found that the amplitude of spontaneous excitatory postsynaptic currents (sEPSC) were significantly increased, yet the event frequency remained comparable to controls. This suggests that the cholinergic neurons are receiving stronger excitatory input after wheel running. To explore increased excitation of the cholinergic PPN in a motor learning context, we used designer receptors exclusively activated by designer drugs (DREADDs) and optogenetic approaches to excite this neuronal population in ChAT-cre mice during motor skill learning. We hypothesize that excitation of the cholinergic PPN will enhance motor learning and that inhibition of these neurons will impair motor learning. Evaluating the electrophysiological characteristics and behavioral implications of the cholinergic neurons is an important first step in understanding the role of cholinergic PPN neurons in connecting exercise with motor skill learning.

Disclosures: C.B. Scott: None. **Z. Colon:** None. **M.R. Croom:** None. **R.C. Evans:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.17

Topic: E.03. Basal Ganglia

Support: BRAIN Initiative Grant R00NS112417 awarded to RCE
Parkinson's Foundation Summer Student Fellowship PF-SSF-941810 awarded to AES

Title: Behavioral and synaptic characterization of in vivo and ex vivo optogenetic activation of basal ganglia inhibitory inputs to the pedunculopontine nucleus

Authors: *M. FALLAH, A. E. SWIATEK, C. B. SCOTT, R. C. EVANS;
Neurosci., Georgetown Univ. Med. Ctr., Washington, DC

Abstract: The pedunculopontine nucleus (PPN) is a midbrain structure that interacts with nuclei of the basal ganglia to enable smooth movement. The PPN is thought to modulate motor output by integrating input from the basal ganglia, but direct PPN stimulation has resulted in contradictory outcomes and the circuitry involving the PPN in the motor pathway is not well characterized. We aim to use optogenetics, electrophysiology, and 2-photon calcium imaging to dissect the functional interconnectivity between the basal ganglia nuclei and specific neural PPN subpopulations underlying the motor circuit. Using *in vivo* optogenetics in wild type mice of both sexes, we selectively stimulated the axon terminals projecting from two inhibitory basal ganglia nuclei, the substantia nigra *pars reticulata* (SNr) and the globus pallidus *externus* (GPe), to the PPN. We found SNr axon stimulation evokes ipsilateral turning, while GPe axon stimulation evokes contralateral turning. Using a real-time place preference task, we found SNr axon stimulation is aversive, while GPe axon stimulation is rewarding. We hypothesize that the SNr and GPe selectively inhibit distinct subregions across the rostral-caudal axis of the PPN leading to their opposing behavioral effects on PPN innervation. Contradictory outcomes in deep brain stimulation, where caudal PPN stimulation improved gait and rostral stimulation worsened it, suggest functional heterogeneity among rostral and caudal neuronal subgroups within the PPN. We focus on cholinergic PPN neurons whose degeneration in Parkinson's disease correlates with gait impairment in both patients and rodent models. Our confocal imaging showed that axons from the SNr project throughout the PPN while axons from the GPe project to the caudal PPN in ChAT-Cre mice of both sexes. Using *ex vivo* electrophysiology and optogenetics, we stimulated axons from the SNr or GPe while recording the inputs at the cholinergic PPN neurons in whole-cell patch clamp to confirm the functional connectivity of the anatomical axonal projections and characterize the strength and synaptic pattern of the inhibitory input. We found that both the SNr and GPe send inhibitory projections that display short-term synaptic depression. The SNr strongly inhibits both the rostral and caudal PPN whereas the GPe selectively and weakly inhibits the caudal PPN. Our ongoing work to characterize the motor circuit involving the PPN and functional subpopulations among PPN cholinergic neurons will help us understand how the PPN interacts with the basal ganglia to modulate movement.

Disclosures: **M. Fallah:** None. **A.E. Swiatek:** None. **C.B. Scott:** None. **R.C. Evans:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.18

Topic: E.03. Basal Ganglia

Support: 2R01NS094754-06

Title: The striatal indirect pathway resets the neural representation of numerosity

Authors: *I. P. FALLON[1], S. O. FERNANDEZ[2], H. H. YIN[3];
[1]Duke Univ., Duke Univ. Sch. of Med., Durham, NC; [3]Duke Univ., [2]Duke Univ., Durham, NC

Abstract: The Basal Ganglia are critical for action selection. D2 receptor expressing striatal projection neurons (iSPNs) form the indirect pathway and are known to suppress actions. However, their functional contributions to action control remain controversial. Here, we selectively manipulated iSPNs using optogenetics during the performance of a novel operant counting task. Unilateral excitation of iSPNs produced directional action transitions and disrupted counting if transitions were directed towards the reward-port only. A complete reset of the count was observed following 20hz stimulation at the beginning or end of the sequence. Stimulation induced transitions depended on the count estimate and training history. In contrast, unilateral inhibition of iSPNs suppressed directional transitions to the reward-port and increased the length of the ongoing count. Across all count lengths, the learned count requirement determined the stimulation induced reset. These results suggest iSPNs signal the transition between actions and reset the neural representation of numerosity.

Disclosures: I.P. Fallon: None. **S.O. Fernandez:** None. **H.H. Yin:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.19

Topic: E.03. Basal Ganglia

Support: NIH NS094754
NIH MH112883
NIH DA040701

Title: Ventral tegmental dopamine neurons signal force vector dynamics during Pavlovian conditioning and performance

Authors: *K. BAKHURIN, R. N. HUGHES, I. P. FALLON, H. H. YIN;
Dept. of Psychology and Neurosci., Duke Univ., Durham, NC

Abstract: The dopamine (DA) neurons of the ventral tegmental area (VTA) are thought to play a critical role in motivated behavior, yet precise understanding of their involvement remains elusive. Previous work identified relationships between force exerted in specific directions and VTA dopamine activity in head-fixed animals, but many known characteristics of DA neuron firing during associative learning have not been reconciled with the natural movements produced by animal subjects in such tasks. Here we recorded and optotagged hundreds of DA neurons

during stimulus-reward learning in head-fixed mice while continuously monitoring the forces they exerted. CR force magnitude increased across training. CS-evoked phasic activity of DA neurons covaried with the integral of CR forces generated, regardless of the amount of learning. Reducing reward magnitude resulted in less CR force exerted and concurrent reductions in phasic DA neuron firing. Reward omission revealed pauses in DA activity that coincided with sudden termination of force exertion. By altering the spatial location of reward delivery around the mouth while maintaining reward predictability, we show that mice modify the direction of force generation during anticipatory CRs. This manipulation revealed systematic changes in phasic DA activity during CRs when the direction of force exertion changed. Lastly, we found that phasic activity in response to US delivery was not attenuated with learning. Instead, DA neurons became entrained reward consummatory URs, producing multiple lick-entrained bursts when their activity was aligned to these movements. Together this work suggests that VTA DA activity may be explained by the dynamic changes in performance that occur during Pavlovian conditioning rather than a reward prediction error as conventionally believed.

Disclosures: **K. Bakhurin:** None. **R.N. Hughes:** None. **I.P. Fallon:** None. **H.H. Yin:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.20

Topic: E.03. Basal Ganglia

Support: Howard Hughes Medical Institute
Helen Hay Whitney Foundation

Title: Timescale of posterior dorsomedial striatum's contribution to trial and error learning

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Abstract: To survive in a changing environment, animals learn associations between sensory cues and motor actions through a process of trial and error that unfolds over timescales from seconds through days to years. A brain area called the posterior dorsomedial tail of the striatum (pDMS) has been implicated in this process, yet whether it contributes to learning or memory is not understood. We developed an approach to control the time when pDMS is active in order to investigate precisely when and how pDMS contributes to learning or memory. We taught mice to associate a brief, optogenetic activation of pDMS-projecting neurons in visual cortex (the cue) with a forelimb reach to grab a food pellet (the action) by repeatedly presenting the cue before presenting the food pellet. Both within and across days, the mice gradually increased the number of reaches after the cue relative to spontaneous reaches before the cue. Optogenetically inhibiting pDMS for a brief, one second-long time window overlapping the cue consistently but reversibly

arrested this learning without affecting motor kinematics. Learning ability rapidly (<10 seconds) recovered with the release of inhibition and return of endogenous pDMS neural activity. However, inhibiting pDMS did not affect previously acquired improvements in performance already consolidated into short- (within a day) and long-term (across days) memories. We then measured both dopamine release and the activity patterns of various cell types in pDMS to correlate brain activity with the increments of learning. Thus we identify a mechanism involved in incremental pDMS-dependent learning over a fast timescale of seconds but that is no longer involved in memory storage and recall after minutes, hours and days, and we uncover the neural substrates of these fast, incremental updates that accumulate over time to produce trial and error learning.

Disclosures: K. Reinhold: None. **M. Iadarola:** None. **B.L. Sabatini:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 055.21

Topic: E.03. Basal Ganglia

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Simons-Emory International Consortium (Simons 717104)

Title: Striatum encodes force and action-specific signals across learning of different isometric actions

Authors: *I. RODRIGUES-VAZ[1,2], V. R. ATHALYE[1], D. S. PETERKA[1], R. M. COSTA[1]; [1]Zuckerman Mind Brain Behavior Inst., Columbia Univ., New York, NY; [2]Champalimaud Fndn., Lisbon, Portugal

Abstract: The nervous system controls muscles to produce behavior. Some muscle contractions result in motion of the muscle and joints, while other contractions denominated isometric do not involve movement. The basal ganglia, and in particular the striatum, have been proposed to be mainly involved in modulating the vigor of movements, mostly in experiments that relate neural activity with the speed of movement - which requires motion of muscles and joints. On the other hand, activity in striatum spiny projection neurons (SPNs) has also been shown to be different for different gross body movements, suggesting that activity in striatum is movement-specific and can bias which movements animals will perform. However, these actions were performed with different body parts and therefore it is unclear how granular this movement-specific activity is, and if it is mostly related to gross somatotopy. Specifically, it is unknown whether actions that require no overt movement have a representation in striatum. We investigated whether the striatum differentially encodes actions that differ mainly on the muscle pattern used in the same limb. We designed a two-action isometric force task in which the two target actions were a push or pull force exceeding a threshold for a given duration without overt limb movement. Mice

learn to perform specific isometric actions that lead to reward and shift as action-reward contingencies shift. This learning depends on plasticity at glutamatergic synapses onto SPNs. Animals lacking the NMDARs in SPNs (RGS9cre x NR1-KO) can produce both actions but are unable to learn which action leads to reward. Using 2-photon imaging through a GRIN lens, we recorded SPNs in dorsolateral striatum (DLS) throughout learning. SPNs showed action-specific activity, and a classifier can predict which isometric action was performed in individual trials. Furthermore, SPN activity predicted the amplitude of the force executed, but did so in an action-specific manner - linear regression models could predict the amplitude of a specific action but not both actions. This evidence supports the hypothesis that SPNs in the DLS encode specific fine actions executed with the same forelimb, without requiring overt movement. Interestingly, SPN activity carried action-specific signals from the onset of training, and decoders that used SPN activity predicted which action was executed and at what amplitude with stable performance across learning. Altogether, our results suggest that SPNs encode specific actions as fine as different muscle activations of the same forelimb, and that activity at the population level can be stably readout to drive action across learning.

Disclosures: I. Rodrigues-Vaz: None. **V.R. Athalye:** None. **D.S. Peterka:** None. **R.M. Costa:** None.

Poster

055. Basal Ganglia: Physiology and Function I

Location: SDCC Halls B-H

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Program #/Poster #: 055.22

Title: WITHDRAWN

Poster

056. Hand Control: Age, Pathology, Physiology

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 056.01

Topic: E.04. Voluntary Movements

Title: The effects of increased cognitive demands during complex reach and grasp tasks in older adults

Authors: *A. C. NIEMIEC, R. N. LOGUE COOK, C. TSE, S. H. BROWN;
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Abstract: Introduction: Age-associated declines in reach and grasp tasks have been well documented with factors such as task complexity, prior experience and preferred hand use

impacting the magnitude of impaired performance. The addition of concurrent cognitive demands leads to further declines in goal-directed hand tasks although most studies have examined the effects of increased cognitive loads in older adults using relatively simple tasks such as tapping or circle drawing. This study examined the effects of age and increased attentional demands during the performance of a complex, visually-guided reach and grasp sequence. Methods: Sixteen right-handed young (mean age: 24.8 ± 3.3 y) and 16 older (mean age: 75.1 ± 5.3 y) participants performed a repetitive reach, grasp, release, and return sequence that involved placing disks in a vertical board using a modified Connect 4© board game. Three tasks with varying attentional demands were examined: placing disks in the board column by column (control task), placing colored disks following a color-coded spatial pattern visible behind the board (spatial pattern task), and placing disks without a pattern while simultaneously performing a serial subtraction task (dual task). Each task was performed unilaterally by each hand and task completion time was recorded. Results: In the control task, older adults took longer than young adults regardless of hand ($p<0.05$). The dual task took the longest to complete in the older group, resulting in a 55% increase over control task scores for both hands ($p<0.001$). Prolonged but less dramatic completion times were also seen for the spatial pattern task (~20% $p<0.001$). No hand asymmetries were seen in the dual task while the nondominant hand took longer to complete the spatial pattern task ($p<0.001$). Conclusions: Despite the visual processing demands associated with the spatial pattern task and presumably involving integration across multiple cortical regions, the cognitive cost was much smaller than that seen for the dual task condition where working memory plays a critical role. Taken together, these findings underscore the impact of competing cognitive demands involving working memory, visual processing, and sensorimotor control during complex goal-directed hand manipulation tasks.

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Poster

056. Hand Control: Age, Pathology, Physiology

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 056.02

Topic: E.04. Voluntary Movements

Support: NIH T32-NS082128-06

Title: Motor plan variability, not signal-dependent noise, explains motor variability

Authors: *S. DELMAS, Y. CHOI, B. YACOUBI KEYHANI, E. A. CHRISTOU;
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Abstract: A robust observation in human motor control experiments is that the endpoint control of pre-planned (open loop) contractions is worse at low force levels relative to moderate or high force levels. Despite these observations originating in 1892, the underlying neuromuscular

control that explains such robust behavioral differences remains unknown. Here, we use the abduction of the index finger, an experimental paradigm that requires control of a single muscle contraction (first dorsal interosseous; FDI), to study the multiple motor unit discharge characteristics that underlie this phenomenon. We recruited 15 young adults who participated in two distinct experiments that used goal-directed pre-planned contractions with the index finger. For both experiments, we quantified endpoint control as the relative endpoint variability in force and time and characterized the activity of multiple motor units in the FDI. For each trial, we derived a continuous signal from interpolation of the summated active motor unit action potentials and identified the mean discharge rate (MDR), coefficient of variation of the discharge rate (DRV), and the power (PW) from 3-8, 8-13, 13-33, and 33-60 Hz. Of the identified motor unit outcome measures, we quantified their corresponding mean performance across trials ('$_{M}$') and their trial-to-trial variability ('$_{SD}$', '$_{CV}$'). In the first experiment, participants aimed to match a force target either at 10% or 40% MVC while attempting to reverse their peak force at 160 ms. The increased force endpoint variability at 10% relative to 40% ($p < 0.01$) associated only with increased DRV_{CV} ($p = 0.02$; $R^2 = 0.35$), which related to an increase in 13-33 and 33-60 Hz PW_{CV} ($p < 0.03$; $R^2 > 0.3$). In the second experiment, we examined if changing the temporal requirements of the contraction altered endpoint control and multi motor unit discharge characteristics. Thus, participants aimed to match a 40% MVC force target at either 160 or 280 ms. We found that the endpoint control and motor unit discharge characteristics were similar between the two temporal conditions. In summary, we provide novel findings showing that the worse endpoint control at low force levels relates to an inability to consistently repeat the motor unit discharge across trials.

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Poster

056. Hand Control: Age, Pathology, Physiology

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 056.03

Topic: E.04. Voluntary Movements

Support: the Norwegian Health Association (2018/A68412)
the Faculty of Health Sciences, UiT- the Arctic University of Norway (2016/5455)

Title: Kinematic assessment of manual dexterity in mild cognitive impairment

Authors: O. VASYLENKO, M. M. GORECKA, K. WATERLOO, ***C. RODRÍGUEZ-ARANDA**;
Univ. of Tromsø, Univ. of Tromsø, Tromsø, Norway

Abstract: Background. Manual dexterity is essential for daily activities and independent living. Research has indicated that hand motor control is affected in mild cognitive impairment (MCI) but detailed analyses of movements during object manipulation are necessary to better describe how the different components of dexterity are affected by MCI. **Aim.** To provide a detailed description of temporal and kinematic differences in unimanual dexterity between MCI patients and healthy older adults.**Methods.** Forty-three MCI patients and 51 healthy controls (HC), all right-handed, were assessed with two unimanual tasks of the Purdue Pegboard Test: inserting pins with right hand and with left hand. Performance was segmented into four movement types: reaching, grasping, transport, and inserting of pins; each was analyzed separately. Temporal measures were movement times (MTs) for each type of movement and total time for each task. Kinematic measures were linear and angular velocities and their coefficients of variability, and path length. Data were analyzed by 2 Group x 2 Task repeated-measures ANOVAs. Pearson correlations were employed to further explore the relationship between overall performance accuracy and kinematics for each group.**Results.** The MCI group made more errors and spent longer time on both tasks. During fine movements (grasping and inserting) the MCI group showed longer MTs, longer paths and more variable linear velocities, indicating slower and less efficient movements. However, during gross movements (reaching and transport), the MCI group had shorter MTs, higher and less variable linear velocities and shorter paths, indicating more efficient movements. Nevertheless, correlational analyses showed that this kinematic pattern was associated with higher number of errors in the MCI group; no such association was found in the HC group. For all temporal and kinematic measures, group differences were more prominent in left hand performance. **Conclusions.** Our findings demonstrate that dexterity is affected in MCI, especially when performing with the non-dominant hand. Moreover, fine and gross dexterity seem to be affected differently. Whereas fine movements showed slowing and loss of precision, gross movements appeared faster. However, because faster gross movements were associated with more errors in the MCI group, this finding indicates decline in anticipatory motor planning, such that hand velocity or position is not adjusted for object manipulation (grasping or inserting) during the preceding gross movement (reaching or transport), but rather is carried out in the form of corrective movements during the ongoing manipulation.

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Poster

056. Hand Control: Age, Pathology, Physiology

Location: SDCC Halls B-H

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Program #/Poster #: 056.04

Topic: E.04. Voluntary Movements

Support: NIH 1DP5OD029571-01

Title: Electronic Grip Gauge (EGG): Tool to Assess Sensorimotor Interplay in Regulation of Fine Hand Control

Authors: *M. K. BUCZAK, B. S. BAUM, C. D. OLSEN, J. A. GEORGE;
Univ. of Utah, Salt Lake City, UT

Abstract: Evaluating hand dexterity is a critical aspect of informing patient care for many neurodegenerative diseases. Current upper-limb dexterity assessments primarily target gross motor function and do not directly measure an individual's ability to finely regulate their grip force. An increasingly popular test of fine motor function among researchers is a fragile-object test, in which participants are instructed to lift and transfer an object while minimizing their applied grip force. Here we present another instantiation of this fragile-object test, the electronic grip gauge (EGG). This device allows us to assess force regulation and the role of sensory feedback in fine motor control. Nine healthy subjects completed a series of grasp-and-transfer tasks of the EGG with their healthy hand and with a robotic prosthesis. The robotic prosthesis was controlled in two different ways: 1) using myoelectric control to indirectly predict hand grasping, and 2) using motion capture to track hand grasping directly. Both prosthesis conditions remove cutaneous tactile feedback. The myoelectric control further limits proprioception related to joint angle (muscle spindles) and skin stretch (SA2 fibers) but keeps force feedback (Gogli tendon organs) relatively unhindered. We used the EGG to quantify grip force under three distinct conditions: 1) implicit grasping when transferring the EGG as fast as possible, 2) grasping when participants are instructed to minimize their grip force, and 3) grasping when participants are instructed to minimize their grip force and have supplemental auditory feedback proportionate to their grip force. Implicit forces exerted by myoelectric and motion-tracking control were significantly greater than the force exerted by natural hands. When instructed to minimize their force, participants were able to significantly reduce the exerted force with their natural hands and with motion-tracking control, but not with myoelectric control. With supplemental auditory feedback, the force exerted by myoelectric control was significantly reduced, while forces exerted by natural hands and motion-tracking control did not change. These results suggest that myoelectric control and motion-tracking control provide similar motor control, but that myoelectric control offered less, or less accurate, sensory feedback. Thus, improvements in sensory feedback are critical for improved dexterity with myoelectric prostheses. These results show that the EGG can disentangle sensory and motor contributions to hand dexterity. The ability to assess the role of sensory feedback in motor tasks can inform patient care and medical-device design.

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Poster

056. Hand Control: Age, Pathology, Physiology

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Topic: E.04. Voluntary Movements

Support: NIH C-STAR, NICHD Grant Number P2CHD101899

Title: Reliability of a novel test to assess precision force control in children

Authors: *V. L. ROSE[1], A. AJOY[1], G. GOGOLA[2], P. J. PARIKH[1];
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Abstract: To dexterously manipulate objects, humans utilize sensorimotor integration. Sensorimotor integration is responsible for aspects of movement such as the force magnitude and direction exerted by each digit controlling an object, and it is essential for fine motor skills such as writing, dressing, and feeding—critical milestones during development. In clinical settings, the measurement of fine motor skills is important to identify children with developmental pathology, to guide treatments, and to quantify post treatment progress. Current pediatric dexterity tests are time-based measures that are not sensitive enough to detect small (albeit clinically significant) changes in dexterity resulting from treatment. Moreover, these tests assess only a subset of functional abilities needed for manual activities. There is a need for a dexterity test designed specifically for children that is sensitive enough to measure the quality and control of movement (i.e., dynamic force control) when performing a task—a crucial aspect of performance in complex manual tasks of daily living.

To meet this challenge, we have developed an activity-based measure of hand function that objectively assesses precision force control. Our aim was to evaluate test-retest reliability of the measures obtained using this new test and build upon previous interrater reliability data. We recruited a total of 24 typically developing children aged 4-15 years. In addition to our new dexterity test, we performed a standard clinical test of dexterity (Box and Blocks) to compare measures. To assess interrater reliability, two independent researchers computed the total force and trial duration from the recorded data. To assess test-retest reliability (subset n=9), the variables were compared between 2 visits scheduled 24 hrs. to 7 days apart. A third researcher calculated intraclass correlation coefficients (ICC). For trial duration and total force measures, the interrater absolute agreement was excellent while the test-retest reliability was found to be between very good and excellent.

Our findings provide preliminary evidence of high reliability of our novel device to assess precision force control for a wide age range of children and adolescents. As this study continues, we aim to determine smallest detectable difference, the amount of change between tests needed to detect a real difference in performance. We will also explore the validity of this method in a patient population, with the goal to develop a method for evaluation of dexterity in children that integrates a measure of fine control of digit forces with time-based measures, a more sensitive assessment of pediatric dexterity.

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Poster

056. Hand Control: Age, Pathology, Physiology

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Title: Transhemispheric Cortex Remodeling Promotes Forelimb Recovery after Spinal Cord Injury

Authors: *W. WU[1], T. NGUYEN[1], J. ORDAZ[1], X.-M. XU[1], Y. ZHANG[2], N.-K. LIU[1], Y. LIU[3];
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Abstract: Understanding the reorganization of neural circuits spared after spinal cord injury in the motor cortex and spinal cord would provide insight for developing therapeutics. Using optogenetic mapping we demonstrate a transhemispheric recruitment of neural circuits in the contralateral cortical M1/M2 area to improve the impaired forelimb function after a cervical 5 right-sided hemisection in mice, a model mimicking the human Brown-Séquard syndrome. This cortical reorganization can be elicited by a selective cortical optogenetic neuromodulation paradigm. Areas of whisker, jaw, and neck, together with the rostral forelimb area, on the motor cortex ipsilateral to the lesion are engaged to control the ipsilesional forelimb in both stimulation and non-stimulation groups at 8 weeks post-injury. However, significant functional benefits are only seen in the stimulation group. Using anterograde tracer, we further reveal a robust sprouting of the intact corticospinal tract in the spinal cord of those animals receiving optogenetic stimulation. The intraspinal cortical spinal axonal sprouting corelates with the forelimb functional recovery. Thus, specific neuromodulation of the cortical neural circuits induces massive neural reorganization both in the motor cortex and spinal cord, constructing an alternative motor pathway in restoring impaired forelimb function.

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Poster

056. Hand Control: Age, Pathology, Physiology

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 056.07

Topic: E.04. Voluntary Movements

Support: NIH Grant 5R01HD075813

Title: Multi-modal treatment for stroke survivors with severe hand impairment

Authors: *D. KAMPER[1,2], A. J. BARRY[3], N. BANSAL[4], L. VIDAKOVIC[5], N. SEO[6], M. STOYKOV[7], E. ROTH[8];
[1]North Carolina State Univ., Cary, NC; [2]Univ. of North Carolina at Chapel Hill, Chapel Hill, NC; [3]Biomed. Engin., Shirley Ryan Abilitylab, Chicago, IL; [4]Marquette Univ., Milwaukee, WI; [5]Shirley Ryan AbilityLab, Chicago, IL; [6]Med. Univ. of South Carolina, Charleston, SC; [7]Physical Med. and Rehabiltiation, Feinberg Sch. of Med., Chicago, IL; [8]Northwestern Univ. Sch. of Med., Chicago, IL

Abstract: Motor control of the hand following stroke is impaired by what seems to be a paradox of involuntary motoneuronal hyperexcitability coupled with motoneuronal hypoexcitability during voluntary activation. This phenomenon is especially prevalent in stroke survivors with greater involvement of the paretic hand. In order to facilitate hand rehabilitation in this cohort, we developed a multimodal intervention to address both involuntary hyperexcitability and compromised voluntary control of neuromuscular activation patterns. Namely, we combined cyproheptadine hydrochloride to reduce involuntary activation of the long finger flexors with active therapy involving electromyographic (EMG) control of either an assistive hand exoskeleton or serious computer games. Participants received either cyproheptadine or equivalent doses of placebo and participated in therapy sessions with active control of EMG or with passive stretching provided by the hand exoskeleton. A group of 94 stroke survivors with severe chronic hand impairment were randomized into four groups with different treatment combination in this double-blinded longitudinal intervention study: cyproheptadine with active EMG control, cyproheptadine with passive stretching, placebo with active EMG control, and placebo with passive stretching. Daily dosing of both preparations was gradually increased over 3 weeks to reach a target dosage of 24 mg. This dose level was subsequently maintained over 6 weeks while the participant engaged in 18 therapy sessions. Evaluations were performed during the three weeks of the drug titration period, at the middle and end of the 6-week training period, and at a one-month follow-up session. Five of the 94 participants withdrew from the study due to unrelated medical reasons or personal reasons during the drug titration phase. Of the remaining 89 subjects, 88 (35 female, mean age = 59 years old) completed the study and attended more than 95% of all scheduled treatment sessions. Group membership impacted the change in the primary outcome measure, the average amount of time required to complete tasks for the Graded Wolf Motor Function Test, as evidenced by the significant group x evaluation session interaction ($F = 2.0$, $p = 0.026$). The group receiving placebo and passive stretching failed to show improvement while the other three groups significantly decreased the completion time for the GWMFT. Despite the severe, chronic (mean time post-stroke = 6.3 years) impairment, our cohort of stroke survivors were able to experience some improvement in hand motor control, although not strength, through administration of cyproheptadine and/or active EMG training.

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Poster

056. Hand Control: Age, Pathology, Physiology

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Program #/Poster #: 056.08

Topic: E.04. Voluntary Movements

Support: University of Michigan School of Kinesiology pilot grant

Title: Effects of age on hand asymmetries associated with tactile perception and fine force control

Authors: *R. LOGUE COOK, E. GOLDENKOFF, M. VESIA, S. H. BROWN;
Univ. of Michigan, Ann Arbor, MI

Abstract: Introduction: During object manipulation there is an increased reliance on the ability to integrate tactile information and modulate submaximal hand forces. Tactile perception and hand force steadiness have been shown to degrade with age, though less is known regarding hand asymmetries during such tasks. The purpose of this study was to further examine age-related changes in complex hand sensorimotor function using two custom-designed assessments. Methods: Thirteen healthy older adults (mean age: 72.2±5.5y) and 13 young adults (mean age: 20±1.4y) were recruited for study. Tactile pattern recognition was assessed using a device that generated various spatial patterns using a computer controlled 4x6 pin array. The pattern was applied to the finger tip for 5 s and participants then selected the pattern from 4 options displayed on a screen. Response time and accuracy were recorded. To assess control of forces typically associated with activities of daily living, participants unilaterally squeezing a hand dynamometer to match an on-screen target force level (5% or 20% maximum voluntary contraction (MVC)) for 4s. Force smoothness during the dynamic phase of the task and force maintenance during the static phase were measured. Results: Compared to young adults, older adults were less accurate ($p<0.001$) and took longer ($p<0.001$) to discriminate tactile patterns. Older adults had lower accuracy in their nondominant compared to the dominant ($p<0.01$) hand, but response time did not differ between the hands. During the submaximal force task, older adults produced force less smoothly than young adults at the 20% MVC level ($p<0.05$) and had greater variability when maintaining force at the 5% MVC level ($p<0.001$). No hand differences were observed in force smoothness, but older adults were significantly better at maintaining force in the nondominant hand compared to the dominant ($p<0.001$). In contrast, there were no to minimal group or hand differences in standard measures of grip strength and monofilament testing. Conclusions: These results demonstrate the effects of aging on hand asymmetries during tasks associated with daily activities involving integration of higher-order tactile information and the generation and maintenance of low grip forces. These findings emphasize the importance of having better assessments of hand function that are more sensitive to age-related changes in sensorimotor function needed for daily activities.

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Poster

056. Hand Control: Age, Pathology, Physiology

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Program #/Poster #: 056.09

Topic: E.04. Voluntary Movements

Support: UNG Presidential Award

Title: Interpersonal tasks reveal neuro-motor control deficits in healthy aging.

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Abstract: Aging affects peripheral and central nervous system (CNS) aspects of motor control, which can lead to cognitive and functional deficits. People frequently interact with others, and the stability of such actions is crucial (patient-caregiver interactions, etc.). However, when two persons share a task, their CNS must account for the extra-personal source of movement variability. The neuro-muscular changes of healthy aging may affect the control processes that stabilize shared motor actions. Therefore, this study explored neuro-motor control deficits in interpersonal prehension tasks performed by younger and older adults. Ten older adults (73.5±3.1yrs) and ten young counterparts (24.6±0.8yrs) performed the following clinical tests before the main experiment: self-selected 4-m gait speed, hand-held dynamometer, and sit-to-stand. Each subject sat on a chair and held an instrumented handle vertically using one hand (two-person condition) or two hands (one-person condition). In one-person condition, subjects held the handle with both hands. In two-person conditions, subjects sat side-by-side and grasped the handle with matched-aged or different age partners. Subjects cyclically transferred the handle from the left to the right hand while keeping the handle's orientation and position as steady as possible. We analyzed data from steady-states when both hands held the handle. We computed grip forces (GF) and stability indices (DV) of hand forces to maintain the handle's horizontal stability using Uncontrolled Manifold analysis (UCM). We found no differences in most clinical measures between age groups ($p>0.05$). Young adults showed shorter times ($p=0.007$) in the sit-to-stand test. In all conditions, older adults had higher GF ($p=0.01$), and their GF increased in age-matched pairs ($p=0.05$). Young adults did not modulate GF across conditions ($p=0.06$). DV values were lower in two-person compared to one-person conditions only for young adults ($p=0.03$). When older adults performed the task, the DV values did not differ between one-person and age-matched two-person conditions. DV values decreased only when older adults worked with young adults ($p=0.04$). Results show motor control changes in GF and DV values with age, while no differences emerged for clinical 4-m gait and hand-held dynamometer tests. Young adults were able to modulate stability for tasks performed with age-matched peers. The old adults modulated stability only when shared the task with younger peers. Findings suggest the potential role interpersonal tasks to assess motor control deficits in healthy aging. Future studies should clarify if targeted therapies could improve these deficits.

Disclosures: D. Piscitelli: None. **R. Walton-Mouw:** None. **K. Fitzgerald:** None. **S. Solnik:** None.

Poster

056. Hand Control: Age, Pathology, Physiology

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 056.10

Topic: E.04. Voluntary Movements

Support: SNSF NCCR robotics no: 51NF40_185543

Title: A framework for markerless wrist and finger angle extraction from a single webcam for EMG-based proportional control of robotic prosthetic hands

Authors: *V. MENDEZ[1], X. XU[2], S. MICERA[3];
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Abstract: Robotic hand prostheses (RPHs) are gaining in complexity with single finger actuation and controllable wrist joints. EMG-based proportional control is the most intuitive way to control RPHs, therefore there is a need for precise angle extraction to calibrate decoding algorithms. Motion capture systems are bulky and expensive and cannot be installed in patient homes for daily calibration required with state-of-the-art decoding algorithms. Recently, markerless pose estimation software solutions based on one or several cameras were developed to tackle this issue.This study aims to use MediaPipe, an open-source library that can infer 21 3D landmarks of a hand together with body landmarks from a single camera to calibrate a state-of-the-art EMG-based decoding algorithm that can predict wrist and finger angles for the control of RPHs. We developed a framework to synchronize video frames with EMG signals recorded from 7 channels, extract wrist and finger angles from a webcam, and create a model to predict a total of 8 degrees of freedom. A preliminary analysis was done with one healthy subject to assess the feasibility of such an approach and we show that this framework allows the creation of models that can predict accurately wrist and finger angles based on EMG signals offline (average R2 = 0.80). This framework showed promising results for proportional decoding of wrist and finger angles and could be tested on patients with amputation if they perform synchronized and mirrored movement with their healthy and phantom hands. As we extracted 21 landmarks on the hand, more degrees of freedom could be extracted if RPHs increase their complexity. However, this approach is not as precise as more complex tracking solutions but it allows for high-quality decoding and a simple calibration procedure that could be done daily at home by patients. Finally, data gathered across several days could greatly improve decoding accuracy further.

Disclosures: V. Mendez: None. **X. Xu:** None. **S. Micera:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 057.01

Topic: E.05. Brain-Machine Interface

Support: NINDS
NIH Grant R01NS111518

Title: Tissue-engineered electronic nerve interfaces (TEENI): improved fabrication and assembly

Authors: *L. G. JIRACEK-SAPIEHA[1], K. A. FLUKER[1], B. M. SMADI[2], A. S. LIM[2], C. E. SCHMIDT[2], C. M. RINALDI-RAMOS[2], K. J. OTTO[2], J. W. JUDY[1];
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Abstract: Amputees can use neural implants, which capture the activity of motor neurons and stimulate the activity of sensory neurons, to control robotic prosthetic limbs. To achieve faster and finer control of prosthetic limbs, neural interfaces for amputees need to capture and deliver neural information from more channels and at a higher spatial scale and resolution. Targeting peripheral nerves instead of the brain can minimize risk while still providing good prosthesis performance. To maximize the number of independent motor and sensory channels for each amputee (i.e., engage with spatially distributed nodes of Ranvier), peripheral-nerve interfaces need to be scalable and 3-D in nature. Our novel multidisciplinary approach uses mechanically compliant, scalable, and high-performance nerve interfaces that combine microfabricated neural-electronic interfaces with tissue engineering and nerve regeneration. Specifically, we developed a hybrid tissue-engineered electronic nerve interface (TEENI), which consists of multi-electrode polyimide-based “threads” embedded into a biodegradable hydrogel composite scaffold that is wrapped in a bioresorbable small intestinal submucosa and sutured to the ends of a transected nerve. Multiple thread sets can be stacked and incorporated in the hydrogel to enable the TEENI device to be scaled up and functionally engage with the 3-D nerve target. Inspired by pioneering work that demonstrated thinner and narrower bioelectronic brain probes cause far less foreign-body response (FBR), we recently improved the design of our TEENI by narrowing and thinning its polyimide-metal threads. However, using our prior methods it was not practical to assemble TEENI with such flexible and delicate threads. To overcome this challenge, we have added novel design features: selectively thinning only in the region implanted in the nerve and adding disposable handleability rails. In addition, we have streamlined the TEENI assembling and packaging procedure. Specifically, we replaced the use of a stiff wire bundle with the microsolder and assembly services of an external vendor with a new flexible printed circuit (i.e., the Animal Ribbon Cable (ARC)) that enables rapid and less expensive in-lab assembly.
Here we report the characterization of the narrower and thinner TEENI devices, the assessment of their reliability, their use in preclinical experiments.

Disclosures: L.G. Jiracek-Sapieha: None. **K.A. Fluker:** None. **B.M. Smadi:** None. **A.S. Lim:** None. **C.E. Schmidt:** None. **C.M. Rinaldi-Ramos:** None. **K.J. Otto:** None. **J.W. Judy:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

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Program #/Poster #: 057.02

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS111518

Title: Developing a process model to inform future designs of the magnetically aligned regenerative tissue-engineered electronic nerve interface (MARTEENI)

Authors: *A. S. LIM[1], P. P. GRACIAS[1], C. A. BOOGAART[1], M. B. JOHNSON[1], E. M. OLIVO[1], A. I. VELA[2], L. S. DEWBERRY[1], L. JIRACEK-SAPIEHA[2], K. A. FLUKER, Jr[2], V. G. RIVERA-LLABRES[3], B. M. SMADI[1], C. M. RINALDI-RAMOS[3], C. E. SCHMIDT[1], J. W. JUDY[2], K. J. OTTO[1];
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Abstract: The cumulative efforts of experts in the field of neural engineering serve to improve the quality of life for individuals living with neurodegenerative and neuromuscular conditions as well as limb amputation. Although advances in neural engineering have led to amputees being able to fully control robotic arms, neuroprostheses adoption is limited. Why? We believe this is a result of a lack of user-device embodiment, i.e., “the prosthetic does not extend my sense of self.” Inclusion of sensory feedback may improve long-term adoption and overall quality of life for the end-user.

Herein, our efforts expand on characterizing the electrode interface from where the control signals originate. Researchers recording neural signals generally implant electrodes in animal models and report the stability of the 1 kHz impedance magnitude and electrophysiology signal-to-noise ratio over the implant lifespan. With future innovation in manufacturing, electrodes will likely continue to scale down in geometric surface area, which will affect mass and charge transfer models as boundary conditions dominate the system. Thus, developing the ideal set of tools to probe the tissue-electrode interface chronically may be invaluable for the field. Here we seek to explore and improve neuroprosthetic electrochemical techniques.

We have 2 specific aims: 1) accurately model the tissue-electrode interface apart from chronic 1 kHz impedance magnitude and Nyquist plots and 2) translate in vitro measurements for in vivo configurations. The neural implant data presented here are of three different working electrode groups: platinum, platinum coated with Sputtered Iridium Oxide Film (SIROF), and voltage-cycled platinum coated with SIROF. Electrical impedance spectroscopy was performed with an AUTOLAB PGSTAT128N (Metrohm AG, Switzerland) in a 3-electrode configuration. Prior to measurements and for each channel, open circuit potential was collected for 10 min to reach steady state. Procedures for each group were parameter fixed: 10 mV sine perturbation waveforms, 3 sec integration times, and 5 integration cycles. Next, a measurement model (Orazem and Watson) is employed to generate error residuals and deduce appropriate fit to a biophysical process model. Our data suggest differences in the process model across all groups in vitro, more notably from platinum to platinum with SIROF. Future applications will focus on

how these differences scale in a chronic implant undergoing encapsulation by the innate foreign body response.

Disclosures: **A.S. Lim:** None. **M.B. Johnson:** None. **E.M. Olivo:** None. **L.S. Dewberry:** None. **L. Jiracek-Sapieha:** None. **K.A. Fluker:** None. **V.G. Rivera-Llabres:** None. **B.M. Smadi:** None. **C.M. Rinaldi-Ramos:** None. **C.E. Schmidt:** None. **J.W. Judy:** None. **K.J. Otto:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

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Program #/Poster #: 057.03

Topic: E.05. Brain-Machine Interface

Support: This work was supported by the Institute of Information & Communications Technology Planning & Evaluation (IITP) grant funded by the Korea government (No.2017-0-00451)

Title: Un-cued SSVEP BCI speller using detection method of user cognition

Authors: *H. KIM[1], M. AHN[3], S. C. JUN[4], K. WON[2];
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Abstract: Title: Un-cued SSVEP BCI speller using detection method of user cognition Authors: Heegyu Kim, Kyungho Won, Minkyu Ahn, and Sung Chan Jun***Abstract:**A steady-state visual evoked potential (SSVEP) based brain-computer interface (BCI) speller has been widely used for the advantages of high information transfer rate (ITR) and good signal-to-noise ratio(SNR). In the SSVEP-based BCI speller, each speller target flickers the fixed frequency, and each target is matched with specific command information. The SSVEP is generated when the user concentrates on a designated speller target. However, most SSVEP-based BCI spellers have been designed with a cue-guided target selection task that indicates both task-state (flickering state) and idle-state (non-flickering state); the users must keep their gaze at the flickering target for a long while. This study proposed a method to detect user cognition that provides task state information from electroencephalogram (EEG) signals in the 40-class SSVEP-based BCI speller. The EEG dataset is recorded from 40 healthy subjects; for each subject, the experiment included six blocks, each containing 40 trials corresponding to all 40 targets indicated. According to the extended inter-national 10-20 system, thirty-one electrodes over the central, parietal, and occipital areas were used to record, and nine electrodes over the parietal and occipital areas (Pz, PO7, PO3, POz, PO4, PO8, O1, Oz, and O2) were used in this research. The proposed method consists of two steps. First, the user's cognition detection method is determined by detecting a relative increase in EEG spectral power at the narrow band (±0.2Hz) and interval band (±2.0Hz)

of the fundamental stimulus frequency. Next, a classification method is used for Filter Bank-CCA that has the advantage of identifying a target with a high frequency resolution and a massive number of stimulus targets. Using the recorded EEG dataset, we investigate our proposed method's feasibility in performing the 40-class SSVEP-based BCI speller. We found that our proposed method achieved 71.7±25.2% classification accuracy without an external cue, while the conventional cue-based method achieved 73.1±22.2%. Thus, our proposed and cue-guided methods yielded no significant difference in performance (student's paired t-test, p=0.41), showing that users may operate the SSVEP-based BCI speller without an external cue with no loss of performance.

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Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

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Program #/Poster #: 057.04

Topic: E.05. Brain-Machine Interface

Support: US Federal Grant

Title: EEg-based neurotechnology on the direct to customer market: current risks and harms

Authors: *J. MOONGA[1], B. SALTER[2];
[1]Political Economy, Academic Neurosci., [2]Political Economy, King's Col., London, United Kingdom

Abstract: Brain machine interfaces as innovative neurotechnology are instrumental tools for electrical stimulation, functional monitoring and mechanistic interactions with the brain. Over the past decades, the field of neuroscience experienced major advancements through the interface of electrical engineering. Furthermore, the emergence of other innovative technologies such artificial intelligence (AI) are powerful avenues that facilitate treatments and new therapeutic approaches for brain disorders. Although, several BRAIN Initiatives set common goals to develop new tools to better understand the brain, neurotechnology pose major societal challenges. Originally, brain computer interfaces(BCIs) were designed as medical-assistive devices, neurofeedback and rehabilitation in clinical settings. Today, neurotechnologies have expanded to digital, portable and mobile devices outside clinical practice. New generations of non-invasive devices such as electroencephalograph (EEG) based-BCIs have recently gained major popularity on the direct-to-customer (DTC) market. Often advertised as 'headbands/headsets, wearable devices are compatible with other mobile gadgets for recording, scanning brain activity, in addition to cognitive enhancement, well-being, and entertainment. Yet, little research is done on the long-term implications, safety and other associated risks. Furthermore, there is no explicit law, policy or regulatory framework for their governance. These lacks create huge policy gaps as well as raising various questions on ethics and law. Previous

concerns were recently raised by authoritative bodies and sparked major debates worldwide with the UNESCO, UN and OECD. Similar concerns were echoed in the research community around safety, privacy and governance of neurotechnology. To date, no other studies have explored this area with a systematic approach. We present a systematic analysis, conducted on EEG-based BCIs neurotechnology on the DTC. Using a comprehensive electronic design, this research followed a five-stages screening using the PRISMA framework. An in-depth analysis on the science, market and regulation is presented. The findings identified five types of harms and risks with EEG-based-BCIs, including unclear efficiency and efficacy, personal and psychological harms, inadequate safeguarding and regulatory insufficiency. Additionally, the research corelates those identified risks with current social, ethical and legal challenges. The conclusion drawn from these results brings deductive arguments for immediate actions from governments, regulators and policy makers to address current problems and challenges for neurotechnology and their future development.

Disclosures: J. Moonga: None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

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Program #/Poster #: 057.05

Topic: E.05. Brain-Machine Interface

Support: NIH NINDS R44NS065545
NIH NINDS U44NS114420

Title: An integrated system for arrayed stimulation and recording of peripheral nerve and muscle

Authors: M. MCKINNON[1], D. HOCHMAN[1], A. HECKERLING[1], M. GOEBEL[1], N. MUENCHEN[1], M. JOHNSTON[1], S. WEINBERG[1], C. K. FRANZ[2], ***I. CLEMENTS**[1]; [1]BioCircuit Technologies, Atlanta, GA; [2]Biologics, Shirley Ryan AbilityLab, Chicago, IL

Abstract: Non-invasive sensing and manipulation of nerve and muscle activity is critical for both diagnosis and treatment of neuromuscular injury and disease. Typical techniques for electrodiagnosis or therapy are technically challenging and involve precise positioning of large surface electrodes by a highly trained clinician. Here we describe a multichannel transcutaneous recording and stimulation platform to facilitate and advance clinical workflows requiring targeted monitoring and manipulation of human nerves and muscles. This platform was optimized to be simple to use, and to provide precise and consistent, high-quality electrophysiological recordings in real-world environments without the need for extensive setup or training. Custom flexible, adhesive arrays of hydrogel electrodes selectively target specific peripheral nerves and muscles. Custom software simplifies acquisition and data processing of raw signals. Meaningful physiological parameters were extracted from processed data by novel algorithms. Several examples of diagnostic and therapeutic applications were tested. Diaphragm

contraction in response to phrenic nerve stimulation was recorded, and source separation methods were able to distinguish on-target versus off-target stimulation, which could improve the diagnostic specificity of phrenic neuropathy as a result of neurological disease or injury (e.g. SCI). We successfully tracked median nerve activity continuously from wrist to elbow with sufficient resolution to calculate conduction velocity. Innovative visualization methods were used to display propagating nerve activity intuitively and succinctly. This platform was also used to acquire high density surface EMG recordings from the soleus muscle in response to tibial nerve stimulation as part of a reflex operant conditioning therapy. A non-invasive, array-based system for peripheral nerve interfacing and quantitative assessment of nerve/muscle function was designed and validated within a variety of clinically-oriented applications. Ultimately this platform has the potential to advance neuromuscular research, neuromodulation, bioelectronic medicine, and neural prosthetics.

Disclosures: **M. McKinnon:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **D. Hochman:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **A. Heckerling:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **M. Goebel:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **N. Muenchen:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **M. Johnston:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **S. Weinberg:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES. **C.K. Franz:** None. **I. Clements:** A. Employment/Salary (full or part-time):; BIOCIRCUIT TECHNOLOGIES.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 057.06

Topic: E.05. Brain-Machine Interface

Title: Novel Technique for Measurement of Brain Signals with Excellent Spatiotemporal Resolution by Using Magnetically Biased Fields: Verification with Measurement of Movement Related Cortical Signals

Authors: *O. HIWAKI;
Grad. Sch. of Information Sciences, Hiroshima City Univ., Hiroshima, Japan

Abstract: Noninvasive techniques for measurement of brain signals have been utilized for brain-machine interfaces as well as for understanding and diagnosis of the brain functions. Electroencephalography (EEG), magnetoencephalography (MEG), functional magnetic resonance imaging (fMRI) and near-infrared spectroscopy (NIRS) are commonly used as noninvasive techniques for measurement of brain signals, while each of them has disadvantages. EEG and MEG signals associated with electrical neural activity are fast, but they are characterized by a poor spatial resolution. Temporal resolution of fMRI and NIRS signals are

limited by hemodynamic response time with a width of ~3s after the onset of a neural stimulus. We have developed a novel technique, called as MBF (magnetically biased field), to achieve noninvasive measurement of brain activity with excellent spatial and temporal resolution. In the MBF technique, a magnetic field emitted from a coil located on the scalp passing through the cerebral cortex is used. We have found that the fast signals can be detected as perturbation of the magnetic field according to the cortical activity with a magnetometer on the upper end of a coil. Here, we verify the effectiveness of the MBF by measurement of movement related signals. We tried to measure movement related signals by using a multichannel MBF system. Subjects, who watched a rotating clock hand on a computer monitor, were instructed to push a button with the index finger at the moment of the returning of the clock hand to the starting position after the rotation. The signals around Cz were measured simultaneously. The movement related signals were obtained by an average of 300 trials. As a result, clear movement related signals were successfully obtained by the MBF system. The signals preceding the onset by more than 1 sec were recorded in an extremely localized fashion exclusively from points in the vicinity of the precentral motor area. It is verified that the movement related signals representing the preparation of voluntary finger movements can be detected by the MBF with excellent spatiotemporal resolution.

Disclosures: O. Hiwaki: None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.07

Topic: E.05. Brain-Machine Interface

Support: NIH grant R01NS121219

Title: Mxene textile dry eeg electrodes for clinical recordings

Authors: *S. SHANKAR, F. CIMINO, D. XU, K. DAVIS, F. VITALE;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: Electroencephalography (EEG) is commonly used as a non-invasive diagnostic technique to record electrical activity from the scalp to detect and monitor underlying neurological disorders, such as epilepsy. The current clinical standard for EEG requires the use of gel-filled cup electrodes mounted on the patients' scalp. To ensure adequate contact and signal acquisition from the scalp, these types of electrodes require expert technician experience to vigorously prepare the skin using abrasive pastes and are often held in place using chemical adhesives. Such processes can be time consuming and leave patients in discomfort. Furthermore, the adhesives and their leachates can be irritating or, in some cases, toxic. Finally, current clinical electrodes might pose additional obstacles in performing further diagnostic tests, as they are not always compatible with magnetic resonance imaging or treatments such as transcranial

magnetic stimulation. To address these issues, we have developed flexible textile-based dry electrodes, using a novel material Ti_3C_2 MXene, for easy-to-use gel-free EEG. MXene electrodes are low cost in cost and are rapidly fabricated as they can be mold casted in large batches. We have also customized an elastic EEG cap, in the clinical standard 10-20 montage, for ease of electrode application and fast replacement of individual contacts. These electrodes require minimal to no skin prep, no conductive gels, and no adhesive for recording high-quality EEG. The 10 Hz impedance modulus of 5mm diameter measured on the scalp ranges from 12kΩ to 70kΩ (n = 4, 1 female, 3 males). Future work will demonstrate the use of such electrodes in patients to compare the signal quality and readability in comparison to clinical gelled EEG electrodes.

Disclosures: S. Shankar: None. **F. Cimino:** None. **D. Xu:** None. **K. Davis:** None. **F. Vitale:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Topic: E.05. Brain-Machine Interface

Support: This work was supported by the National Research Foundation of Korea (NRF) through the Korea Government [Ministry of Science and ICT (MSIT)] under Grant NRF-2022R1A2B5B01001443

Title: Distraction classification during performing motor execution using SSVEP paradigm

Authors: *H. LIM, J. KU;
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Abstract: The importance of patients' attentive participation in rehabilitation programs has been emphasized. However, during rehabilitation, patients are frequently distracted by distractors in their surroundings. These reduce attention on rehabilitation, which may reduce the effectiveness of brain plasticity. we hypothesized that the EEG responses would be modulated by one's attention while performing motor tasks if the SSVEP paradigm is adopted, and it could help classify one's state of being distracted while ensuring robust and seamless monitoring of the patient's state of being distracted from the task. We recruited 15 healthy right-handed adults (8 men and 7 women) in this study. The mean age of the participants was 25.67 years (± 3.48 years). Subjects were asked to perform a motor task in which they needed to track a semicircular target and make a circle with another semicircular cursor using a mouse. To evoke the SSVEP pattern in the brain signal, the target and cursor were flickering at 15 Hz and 12 Hz, respectively. There were two types of distractors: visual and cognitive. With visual distractors, the target comprised 20 half circles of the same radius, and their color was set as close as possible to that of the flickering target. In the cognitive distractor case, the participants were given a subtraction

problem every second. Therefore, the participants were asked to call the solution to the math problem aloud while performing the tracking task. The model trained with the data obtained from the condition including the flickering cursor and target had an average accuracy of 78%, while the non-flickering paradigm showed a lower accuracy of 61.5%. The accuracy was significantly different between the paradigm with and without flickering ($p < 0.005$). In this study, we proposed a classification paradigm for distraction during motor performance. Our method could provide a great synergy to augment the effectiveness of rehabilitation by encouraging users' attention to be directed to the flickering cursor and target, which reflect their movements when using game-based rehabilitation paradigms.

Disclosures: H. Lim: None. **J. Ku:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.09

Topic: E.05. Brain-Machine Interface

Support: KAIST-funded Global Singularity Research Program for 2022 (N11220050)

Title: Finger movement classification using ultra-high-density EEG : A pilot study

Authors: *S. JO, H. LEE, H.-S. PARK;
Korea Advanced Inst. in Sci. and Technol., Korea Advanced Inst. in Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Invasive methods such as Electrocorticography (EcoG) can be used to detect individual finger movements when high-density electrode grids are attached directly to the cortex to provide higher resolution. It supports that electrical signals originating from the brain contain enough information to detect finger movements. However, to the best of our knowledge, a classification between single finger movements using a conventional EEG system was still challenging. Non-invasive electroencephalography (EEG) is well known to have higher time resolution and lower acquisition costs compared to other electrophysiological monitoring methods such as fMRI and MEG. On the contrary, spatial resolution is lower than these alternative methods. In terms of spatial resolution, the only factor adaptable within the scope of EEG-acquisition systems is increasing the amount and density of electrodes. Our ultra-high-density EEG system has a distance between electrodes of 8.6 mm, the same as the distance between electrodes of the EcoG system. The diamond-shaped electrode grids consisting of 16 channels are combined at 16 positions each and a total of 256 channels are available. We conducted an experiment of extending fingers according to the visual cue provided through the monitor for four right-handed subjects to explore the possibility of classifying single-finger movements using an ultra-high-density electrode system. The fingers were kept in an extension position for 5 seconds, and the order of fingers was randomly provided. The experimental

protocol consisted of 10 runs executing sequentially and each finger was displayed 5 times in a single run, thus 50 extensions in total. The signals were recorded with a sampling rate of 600 Hz, low-pass filtered between 0.1 and 200 Hz. After removing artifacts in visual inspection, the common spatial pattern was applied for feature extraction. Two classification machines, linear discriminant analysis(LDA) and support vector machine(SVM), were applied to find a boundary around each cluster of a class. As a result, the classification accuracy of the LDA classifier for five fingers was 39.54% and the classification accuracy of the SVM classifier was 38.14%, higher than the chance level of 25%. The current study investigates the feasibility of classifiers using ultra-high-density EEG for individual finger movements. It is expected to expand the use of non-invasive BCI technologies in the future.

Disclosures: S. Jo: None. **H. Lee:** None. **H. Park:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.10

Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1913492

Title: Identifying Cognitive Factors Contributing to BCI Performance Variability: A multimodal Study

Authors: *S. BORGHEAI, A. H. ZISK, J. MCLINDEN, J. MCINTYRE, Y. SHAHRIARI; Univ. of Rhode Island, Kingston, RI

Abstract: Performance variability is one of major current challenges facing robust daily use of brain computer interfaces (BCI). In addition to day-to-day changes in cognitive status of healthy BCI users, the variability of disease-related conditions across the end-users particularly in people with severe motor deficits has caused inefficiency in long-term use of these systems. Additionally, motor deficits has been widely recognized as a multi-system disorder in which, not only the motor system degenerates but the non-motor system is affected as well. Identifying the major non-motor factors, including cognitive features, contributing to performance variability can potentially help develop compensatory methods to adapt BCI systems for long-term use. Here, we have explored multimodal factors causing variability in performance of electroencephalography (EEG)-based P3Speller, the most commonly used BCI for communication. 11 subjects, including five people with amyotrophic lateral sclerosis (ALS), participated in one familiarization and on average three experimental sessions in three different days. A cognitive-behavioral screen (CBS) test was performed followed by a simultaneous functional near-infrared spectroscopy (fNIRS) and EEG data recording in which subjects participated in a five-minute eyes-open resting run and P3Speller task afterwards. CBS scores, resting-state EEG spectral powers, and fNIRS correlation measures as candidate factors, and

P3Speller accuracy and precision in all and only first trial were selected as performance metrics. Statistical association between candidate factors and performance metrics were investigated using repeated measures correlations (rrm), an analysis of covariance-based regression appropriate for multiple non-independent observations. Among CBS features, fluency showed significant correlation with accuracy (rrm=0.467, p-value=0.022) and precision (rrm= 0.461, p-value=0.023), and total CBS score was significantly correlated with first trial accuracy (rrm= 0.469, p-value=0.021). Frontal- and centro-parietal resting-EEG delta power showed significant correlations with all performance features, except for accuracy in the first trial. Furthermore, right frontal-left temporal resting-fNIRS correlation and accuracy in the first trial (rrm=0.406, p-value=0.049) showed significant association. Overall our results showed prior cognitive screen and resting-state recording can help identify factors contributing to P3Speller performance variability which could potentially be extended to develop subject- and session- specific correction methods to adapt to long-term daily use.

Disclosures: S. Borgheai: None. **A.H. Zisk:** None. **J. McLinden:** None. **J. Mcintyre:** None. **Y. Shahriari:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 057.11

Topic: E.05. Brain-Machine Interface

Support: NIH Grant P20GM103430
URI Foundation's Medical Research
URI Start-Up Funding

Title: Emergence of neural activity in hand preshaping to grasp using noninvasive EEG methods

Authors: *A. CETERA[1], M. KAFI KANG[2], R. ABIRI[3];
[1]Anna Cetera, [3]Univ. of Rhode Island, [2]Univ. of Rhode Island, Kingston, RI

Abstract: Hand dexterity and grasp disability in patients with spinal cord injury has devastating impacts over their lifespan. Restoration of hand dexterity is the highest priority among this population. While some invasive brain-machine interfaces (BMI) are customized to assist such patients to perform reach-and-grasp tasks with a robotic system, they lack dexterity, generalizability, and are cost inefficient. This creates difficulties and prevents accessibility for employing these devices in larger patient populations. The purpose of this study is to develop a noninvasive BMI platform that has the ability to predict planned imaginary grip types and different preshaping actions based on electroencephalogram (EEG) recordings from a new, low-cost eight channel EEG headset (Unicorn Hybrid Black). Data collection for our pilot study included a preliminary protocol that consisted of collecting EEG recordings when the human subjects were instructed to consecutively power grip their left and right hands for a specified

duration of time. Additionally, we are in the process of enlarging our dataset to collect planned grip types for dominant hands. As the initial step, we developed a machine learning pipeline using Python language. We analyzed the EEG data from the pilot study by considering the baseline and filtering into different bands. Based on the significance of slow oscillations recorded within the premotor cortex from invasive BMI studies, we selected the power of the δ; (delta) band for feature extraction and classification. We focused on channels C3 and C4 to distinguish between left and right hand power grips. This yielded a percent accuracy of 55% (C3) and 80% (C4) using thresholding methods. Selected grip types for the new study are the transverse and lateral cylindrical grips. We hypothesize that the brain engages in preshapping of grip movement prior to the action or motor imagery of the grip movement. Classification of this period prior to the grip/imagery task will aid the process of overcoming limitations that restrict dextrous interaction of neurorehabilitation devices with objects. Our vision is to use this low cost platform to manipulate objects with a robotic arm among the disabled population.

Disclosures: **A. Cetera:** None. **M. Kafi Kang:** None. **R. Abiri:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Topic: E.05. Brain-Machine Interface

Support: NIH DP5-OD029571
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1F31NS118938

Title: Electromyographically controlled prosthetic wrist improves dexterity and reduces compensatory movements without added cognitive load

Authors: *C. D. OLSEN, T. N. TULLY, E. S. STONE, N. R. OLSEN, M. D. PASKETT, G. A. CLARK, J. A. GEORGE;
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Abstract: Though neuroprosthetic limbs are becoming more commonplace, most do not have any kind of functional wrist. Here, we highlight the development of an inexpensive prosthetic wrist that can be adapted to work with various sockets and prostheses. We validate this inexpensive prosthetic wrist alongside a commercially available prosthetic wrist while exploring the functional and cognitive impact of using an electromyographically controlled prosthetic wrist. We measured task performance, compensatory movements, and cognitive load while three transradial amputees performed a modified clothespin relocation task using the two myoelectric prostheses. Participants were asked to move a clothespin from a horizontal bar onto a vertical bar with and without use of the wrist. In aggregate, task failure rate was significantly lower in the wrist condition (39% ± 9% mean, ± standard error) than in the no-wrist condition (66% ± 12%).

Compensatory movements were also significantly less; the maximum leftward bend at the hip was less in the wrist condition (14.69° ± 1.06°) than in the no-wrist condition (22.52° ± 1.53°). We also measured cognitive load using a detection response task, where the participants were evaluated on how quickly they could respond to a vibration stimulus by clicking a button. Response time and miss percentage were used as objective measurements of cognitive load. Participants also self-reported cognitive load through the NASA Task Load Index. In all three measurements, the addition of controlling a prosthetic wrist had no significant impact on cognitive load. In fact, the addition of the prosthetic wrist trended towards reducing cognitive load. This work suggests that functional prosthetic wrists can improve dexterity and reduce compensation without substantial cognitive burden. These results, and the introduction of a new inexpensive prosthetic wrist, can aid future research and development and guide the prescription of upper-limb neuroprostheses.

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Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.13

Topic: E.05. Brain-Machine Interface

Support: NIH 1DP5OD029571-01

Title: Design and Validation of a Low-Cost Noninvasive Neural Stimulator for Functional Electrical Stimulation of Peripheral Nerves and Muscles

Authors: *M. A. TROUT, J. A. GEORGE;
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Abstract: Noninvasive functional electrical stimulation at the surface of the skin can be used to activate muscles, motor neurons, or sensory neurons for assistive and rehabilitative purposes. However, electrical stimulators with a high enough compliance voltage to reliably pass current through the skin are typically expensive and cannot readily be controlled from user-made software. Accessible stimulation hardware is necessary to promote further research, education and clinical translation of noninvasive functional electrical stimulation. We developed a low-cost (~$200), high-voltage (+/- 300 V) Arduino-based stimulator capable of delivering arbitrary stimulation waveforms with up to 16 mA on three independent channels. The stimulator design combines a waveform generator and current source into a single package. The device can be easily controlled from a computer with common software packages (e.g., Python, MATLAB). To validate the stimulator, we did a side-by-side comparison of muscle and nerve stimulation for functional reanimation of hands. Seven healthy participants had the maximum comfortable stimulation repeatedly applied to their *flexor digiti profundus* in their forearm and median and

ulnar nerves in their upper arm. Consistent with prior work, we found that nerve stimulation of the arm nerves was able to produce repeatable, isolated finger flexion and extension across all digits, as well as functional combination movements (i.e., grasps). Stimulation location was then chosen such that the participants' middle and ring fingers squeezed a hand-held force sensor against the palm. Muscle stimulation evoked 19.88 ± 5.88 N maximum grasp force which was 25 ± 8% of maximum voluntary contraction (MVC). Nerve stimulation generated a 9.12 ± 3.17 N maximum grasp force which was 10 ± 3% of the MVC. The two stimulation techniques had similar force generation times. Grip forces generated by muscle stimulation onset in 0.11 ± 0.02 s and rose to 90% of their maximum value in 0.22 ± 0.03 s. Grip forces generated by nerve stimulation onset in 0.11 ± 0.02 s and rose to 90% of their maximum value in 0.28 ± 0.03 s. Nerve stimulation demonstrated a slower fatigue rate than muscle stimulation. The force decay rate of motor stimulation was 0.12 ± 0.03 $trials^{-1}$, while nerve stimulation had a decay rate of 0.04 ± 0.02 $trials^{-1}$. The development of this low-cost stimulator and direct comparison of common motor stimulation targets constitutes an important step towards more widespread use of noninvasive stimulation for educational, research, and clinical purposes. Future work will leverage this stimulator to compare muscle and nerve stimulation for stroke rehabilitation.

Disclosures: M.A. Trout: None. **J.A. George:** None.

Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.14

Topic: E.05. Brain-Machine Interface

Support: Craig H. Neilsen Foundation Grant #725697"

Title: A broadly usable, high-performance grasp orthotic for spinal cord injury (SCI)

Authors: *S. COLACHIS, J. KECKLER, I. BAUMGART, D. GABRIELI, M. SUNDERMAN, N. ANNETTA, L. WENGERD, D. FRIEDENBERG;
Battelle, Columbus, OH

Abstract: Individuals living with spinal cord injury (SCI) have made it clear that advancing neurotechnology in research labs is important but translating advances for daily use is essential. 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. In this study, we have been evaluating two FES control modalities in an SCI population: 1) Tablet control with non-active hand and 2) electromyography (EMG) control using recorded motor

intension signals from the same FES sleeve electrodes. To date, we have tested the tablet-controlled FES system with one subject (n=1) in his home while performing activities of daily living. To evaluate effectiveness in decoding EMG activity from paralyzed limbs for future EMG controlled FES studies, we recorded muscle activity from the forearms of 3 participants (n=3) with varying levels of cervical SCI during 8 functional hand and wrist movement attempts. Using the tablet-controlled FES orthotic, the study participant was able to perform activities in his own home that he was previously unable to perform without caregiver assistance (ex. filling a cup with water from his refrigerator). Using the EMG recording capabilities of our sleeve technology, we were able to accurately decode (accuracies above chance) 4/8 movements in an individual with C5 ASIA A, 7/8 movements in an individual with C6 ASIA C, and 8/8 movements in an individual with C7 ASIA B, despite the absence of palpable movement in many cases. Our results preliminarily indicate the effectiveness of a tablet-controlled FES orthotic in the home setting for individuals with SCI and indicate that despite having palpable movement, some individuals with cervical SCI will be able to volitionally control FES driven movement using naturalistic EMG from the effected limb. With this non-invasive technology, individuals with SCI may one day soon be able to use their own hands again in their own homes and regain independence.

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Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Topic: E.05. Brain-Machine Interface

Support: NSF Grant 1724263
NSF Grant 1724345

Title: A bidirectional joint controller with admissible stiffness profiles using an FNS-based adaptive model predictive controller

Authors: *L. M. CAVALCANTI[1], W. M. THOMAS[2], D. J. WARREN[2], V. J. MATHEWS[1]; [1]Sch. of Electrical Engin. and Computer Sci., Oregon State Univ., Corvallis, OR; [2]Dept. of Biomed. Engin., Univ. of Utah, Salt Lake City, UT

Abstract: Humans modulate joint stiffness during movement through the coactivation of agonist-antagonist muscle pairs to provide stability and robustness. However, in the domain of functional neuromuscular stimulation (FNS), most control systems do not modulate stiffness and, therefore, lack an important biomimetic component. The few FNS controllers that attempt to modulate stiffness do not consider the compatibility of the desired movement profile with the desired stiffness profile. When these profiles are not compatible, FNS controllers can fail to reach either objective, leading to performance degradation. In this work, we designed and computationally evaluated a joint angle-stiffness controller which computed the closest admissible stiffness profile whenever the desired stiffness profile is not physically realizable for a given joint movement trajectory. As a result, both movement and stiffness goals can be accomplished through the assurance of an admissible stiffness modulation. To achieve this, we used an adaptive model predictive controller (aMPC) equipped with a neuromuscular model based on the summation of twitches driven by asynchronous intrafascicular stimulation. The aMPC computed the minimum perturbation δ to be applied to the original desired stiffness profile h, while guaranteeing the min-max error ε of the angle tracking goal. In every cycle, the aMPC solved the problem $\min \varepsilon^2 + \alpha\|\delta\|^2 + \beta\|\Delta x\|^2$ s.t. $0 \le x \le x_{ub}$, $\|Ax-b\|^2 \le \varepsilon$, $Gx = h + \delta$, $\varepsilon > 0$, where α, β are regularization parameters, x the stimulation intensities (x_{ub} its upper bound and Δx measures its smoothness), A is the model matrix, b is the desired angle profile, and G is the stiffness model matrix. We assessed this controller through computational simulations where the plant output was contaminated with additive white Gaussian noise (σ = 2 deg). The controller was commanded to evoke a 1-Hz sinusoidal angle profile for 3 different stiffness profiles h = 0, 10, 20 Ncm/deg and fixed α, β. In all cases, the aMPC modified the constant stiffness profiles to time-varying admissible stiffness profiles, which interestingly corresponds to the fact that stiffness is not constant in actual locomotion. The mean squared error between the desired and actual angle trajectories were 2.2 ± 0.17 deg^2, 1.3 ± 0.11 deg^2, and 3.0 ± 0.20 deg^2, respectively, n = 100. The lowest error happened at h = 10 Ncm/deg which can be considered the closest one to the admissible stiffness profile. The designed aMPC represents a significant step toward more biomimetic FNS solutions due to the incorporation of admissible stiffness modulation. We plan to test this algorithm in more realistic computational models as well in animal experiments.

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Poster

057. Brain Computer Interfaces and Neural Prosthetics: Devices and Methods

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Program #/Poster #: 057.16

Topic: E.05. Brain-Machine Interface

Support: UC Davis Academic Senate
UC Davis Early Career Faculty Award for Creativity and Innovation

Title: Bionic Prosthetic Control Leveraging Target Muscle Reinnervation for the Prevention of Neuromas and Phantom Pain

Authors: A. E. MOUKARZEL, J. FITZGERALD, M. A. BATTRAW, W. M. JOINER, *J. S. SCHOFIELD;
UC Davis, Davis, CA

Abstract: There are more than 2 million individuals with major limb loss in the United States. Initially targeted muscle reinnervation (TMR) surgery was developed as a post-amputation procedure for prosthetic control. During this carefully planned procedure, surgically intensive techniques strategically redirect severed nerves to residual muscles. Following this, the patient's intentions to move their missing limb are amplified by the residual muscles to create patterns of muscle contraction not possible with the native nerves. In turn, this activity can be used to recognize patients' movement intentions and establish a bionic link to control their prosthesis. A fortuitous side effect is improved neuroma and phantom pain outcomes. As 70-80% of individuals with amputation struggle with chronic pain, a simplified version of TMR surgery is rapidly becoming common practice at the time of initial amputation to help prevent nerve-related pain (N-TMR). This procedure typically redirects nerves to motor branches of muscles that are easily accessible during amputation surgery. The affected muscles are often deep in the residuum and thus less accessible for modern prosthesis control interfaces that measure muscle activity using sensors placed on the skin's surface (surface-electromyography, S-EMG). Additionally, N-TMR is done without the consideration of muscle orientation, signal separation, and electrical crosstalk which can lead to further limitations when using S-EMG for prosthetic control. To address these limitations, we investigated the efficiency of applying sonomyography, a prosthesis control technique that employs ultrasound imaging to detect deep muscle deformations. To classify the patterns of muscle deformation and link these to the motor intentions of the participants' missing limbs, we paired the ultrasound imaging technique with an image processing and machine learning algorithm. Results from two participants with transhumeral amputation and N-TMR surgery demonstrated that unique patterns of muscle activations were generated in the reinnervated muscles when participants thought about moving their missing hands and wrists. 4-6 hand and wrist movements could be classified with 82% to nearly 100% accuracy. Our work suggests that N-TMR can provide opportunities to establish bionic interfaces with advanced prosthesis, with similar performance to the original targeted muscle reinnervation procedure for prosthetic control.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.01

Topic: E.05. Brain-Machine Interface

Support: NIH R01 NS085167
NIH R01 NS094384

Title: Analysis of the effect of varying vagus nerve stimulation frequency on plasticity in the motor cortex

Authors: *J. J. A. ADDO[1], C. NEIFERT[1], T. DANAPHONGSE[2], S. T. ABE[2], V. EZHIL[2], M. P. KILGARD[3], S. A. HAYS[1];
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Abstract: A number of neurological insults, including stroke, spinal cord injury, and traumatic brain injury result in chronic impairments in motor function. It is widely held that approaches that facilitate synaptic plasticity in spared networks after neurological injury can promote recovery. Vagus nerve stimulation (VNS) paired with rehabilitation has emerged as one such approach. VNS elicits an increase in the release of plasticity-inducing neuromodulators which results in an increase in the cortical representation of areas activated during the paired task. As such, increasing the amount of plasticity could yield an increased degree of recovery. A number of studies indicate that the stimulation parameters impact the degree of plasticity. Here, we sought to identify the VNS frequency that results in the greatest enhancement of plasticity when delivered during a simple jaw training paradigm. Rats received 20Hz, 30Hz, and 45Hz of VNS concurrent with chewing. After 5 days of VNS pairing, all rats underwent intracortical microstimulation (ICMS) to evaluate cortical movement representations. Preliminary findings indicate that higher stimulation frequencies may yield a larger increase in jaw movement representation, consistent with a greater degree of plasticity. The results of this study will help further establish the optimal stimulation parameters for VNS and depict the importance of delivering VNS at the right frequency when used in conjunction with rehabilitation therapy.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.02

Topic: E.05. Brain-Machine Interface

Support: National Science Foundation Graduate Research Fellowship
National Institute of Health Grant R01NS106094

Title: Modulation of Caudate and DLPFC Activity During Motor BMI Control in Nonhuman Primates

Authors: E. ZIPPI[1], ***G. SHVARTSMAN**[2], N. VENDRELL-LLOPIS[3], J. WALLIS[1,4], J. CARMENA[1,2];
[1]Helen Wills Neurosci. Inst., [2]Dept. of Electrical Engin. and Computer Sci., [4]Dept. of Psychology, [3]Univ. of California, Berkeley, Berkeley, CA

Abstract: Brain-machine interfaces (BMIs) allow for the real-time transformation of neural activity into control signals for external devices. Learning to control these devices engages a wide array of learning mechanisms that rely on distributed cortical and subcortical areas. Many of these mechanisms are associated with both the caudate nucleus of the striatum (Cd) and the dorsolateral prefrontal cortex (DLPFC). Previous work in rodent BMI has demonstrated involvement of the striatum in neuroprosthetic learning, however DLPFC has been largely ignored when studying motor BMI control. To investigate the role of Cd and DLPFC in BMI learning, we performed simultaneous recordings of local-field potential in Cd, DLPFC, and motor cortex as NHPs learned to control a motor cortical BMI. We find changes in power and directed functional connectivity between these regions which are associated with BMI control. These findings provide further evidence that a distributed network of cortical and subcortical areas is involved in neuroprosthetic skill learning and control.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Title: Continuous monitoring of the subthalamic nucleus neural activity in a freely moving primate with the semi-implantable wireless system

Authors: *J.-Y. KIM[1], C. JE[1], Y. KANG[1], Y. LEE[2], K. LIM[3], C.-Y. JEON[2], J. WON[2], M. KIM[2], S.-Q. LEE[1];
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Abstract: The subthalamic nucleus (STN) is an area where deep brain stimulation (DBS) is applied to treat Parkinson's disease. In order to provide appropriate stimulation parameters according to the brain clinical state, there is an increasing need for closed loop DBS technology. However, there are few studies in long-term continuous monitoring of the clinical state of the STN region in freely behavioral states. Therefore, we developed a semi-implantable wireless system that can continuously record neural activity of the STN region in a freely moving primate and confirmed long-term stability performance. The proposed semi-implantable wireless system uses Intan Technologies chip for recording with the sampling of 8,192 Hz. The device was implanted on the head of Monkey and connected to electrodes in the STN with an impedance of about 20 kilohms (World Precision Instruments Inc.). Two channels of recorded data are transmitted through Nordic Bluetooth Low energy protocol with 720 kbps rate in more than 2.0 meters away from the primate. An adult (14-year-old) cynomolgus macaque (Macaca fascicularis) is prepared from Suzhou Xishan Zhongke Laboratory Animal Co. (Suzhou, China) and housed in individual indoor cages at the National Primate Research Center (NPRC) of the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The procedure was performed on the custom-built CT/MRI-compatible stereotaxic frame under general anesthesia. The deep brain recording electrode was inserted vertically into the right STN, and fixed to the skull using dental cement. A surgical site for the battery pocket is located on the back (interscapular space). The battery line was inserted from the skull to the back incision, using the subcutaneous tunneling technique. We wirelessly recorded local field potentials (LFP) activity in the STN region of the primate from anesthesia to a freely moving state and calculated frequency power spectral density. The LFP signal showed significant differences between anesthesia and recovery: Low frequency bands were decreased and high frequency bands were increased in the recovery state compared to the anesthesia state that correspond to the previous studies. The same spectral change was consistently observed for more than 4 weeks. Our system will be particularly useful in continuous monitoring of deep brain signals in freely moving primates. As well, the system can be used to investigate appropriate stimulation parameters according to the brain clinical state.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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DARPA N6600110C4056
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NIH NS088606

Title: Neural stability of sensorimotor activity across both hemispheres during isolated-muscle activations in a human with tetraplegia

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Abstract: Brain—machine interfaces (BMIs) to restore motor function use neural decoders to translate neural activity from movement intention into control signals for prosthetics or other devices. BMI performance is often impaired by the need to frequently recalibrate decoders throughout use sessions [Hochberg 2006, Collinger 2013], largely due to signal instabilities in underlying cortex [Downey 2018]. As decoder coverage expands outside traditional sites (e.g. M1), understanding stability across multiple brain areas and hemispheres can shed light on improved decoding approaches. In the first human with tetraplegia to be implanted with microelectrode arrays in both hemispheres (male, 49 years old at time of surgery; C5/6 incomplete, ASIA B), we characterized stability of neural representation for executed/attempted muscle contractions. We analyzed multiunit activity (MUA) recorded over six cortical microelectrode arrays (NeuroPort; Blackrock Neurotech, Salt Lake City, UT): 2 each in M1 (hand knob) and S1 of the dominant (left) hemisphere, and 1 each in M1 (hand knob) and S1 of the non-dominant. First, we verified array coverage by mapping neural activity from muscles throughout the body in consultation with physical therapists. We identified the left and right wrist extensors (extensor carpi radialis: ECR) for further stability analysis, as they were well represented and could be contracted reliably by the participant. Subsequently, we evaluated stability across 11 sessions (6.5 months) by repeatedly running a paradigm where we instructed the participant to attempt isolated ECR contractions to a metronome (period 4 s). We simultaneously recorded MUA from all arrays, and EMGs from the wrists and neighboring muscles. We analyzed stability of neural activity associated with ECR contractions at the levels of somatotopy, within-channel signaling, and population-level covariations (principal component analysis across channels). Across arrays, sensory channels registered activity over a greater number of sessions than motor. Activity patterns on the contralateral hemisphere to the contracted ECR exhibited focal regions of high stability similar to centers of gravity in fMRI, whereas ipsilateral activity was negligible. Likewise, within-channel firing stability was higher in the contralateral hemisphere, and in sensory cortex. However, these stability differences were not observed at ensemble level. Overall, our findings suggest that the stability of muscle contraction representations is heterogeneous across sensorimotor area and hemisphere, but that these differences can be compensated with population-wide (ensemble-level) approaches.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.05

Topic: E.05. Brain-Machine Interface

Support: NIDCD grant U01DC016686
NWO; Intense Project EU, work package 3
STW 12803

Title: Sleep-related fluctuations in sensorimotor cortex activity: a challenge for 24/7 BCI usage

Authors: *S. LEINDERS[1], E. J. AARNOUTSE[2], M. P. BRANCO[3], Z. V. FREUDENBURG[4], M. VANSTEENSEL[5], N. F. RAMSEY[6];
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Abstract: Background: Brain-Computer Interface (BCI) technology can be of use for people with severe paralysis who are unable to control conventional assistive devices due to limited motor control. Ideally, a BCI system should work accurately 24/7. However, research shows that widespread brain signal fluctuations occur during sleep. Specifically, sensorimotor cortex activity - commonly used for BCI control - fluctuates during sleep and is especially high during REM sleep. Here, we characterized sleep-related changes in signals from the sensorimotor cortex that are relevant for BCI control in people with severe paralysis. **Methods:** In this study, we looked at electrocorticography (ECoG) data. ECoG is placed over the cortex and offers good spatial and temporal resolution, making it suited for BCI purposes. We test feasibility of home use of a fully implanted, ECoG-based BCI system in people with severe paralysis. The two participants currently enrolled in our trial have late-stage ALS. ECoG electrodes were placed over their sensorimotor hand region and attempted hand movement was used for BCI control. We used unique ECoG datasets recorded by the participants at home over many months. Also, we included ECoG data from able-bodied epilepsy patients to ensure signal changes in the BCI participants were not related to ALS. All data was recorded from the sensorimotor hand region, as ascertained by functional mapping. Focus was on power in low (10-30Hz) and high (65-95Hz) frequency bands, which are associated with (attempted) movement and often used for ECoG-BCI control. **Results:** Analyses reveal fluctuations in low- and high frequency band power during sleep both in people with ALS and with epilepsy. Typically, we observed five fluctuations in one night, which were most pronounced in the high frequency band. This matches the number of

REM cycles people typically go through in one night. We also observed large within-and between-subject variability in fluctuations during sleep, indicating eventual BCI solutions will have to be personalized and handle a range of signals. **Conclusion:** Summarizing, sensorimotor signals used for BCI control are subject to variability during sleep. When planning 24/7 BCI usage, it is necessary to characterize the influence of sleep-related signal changes on the BCI system in question. Future BCI systems must mitigate its effects, e.g., through sleep-classification and/or by adjusting BCI parameters.

Disclosures: S. Leinders: None. **E.J. Aarnoutse:** None. **M.P. Branco:** None. **Z.V. Freudenburg:** None. **M. Vansteensel:** None. **N.F. Ramsey:** A. Employment/Salary (full or part-time):; Braincarta.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.06

Topic: E.05. Brain-Machine Interface

Support: EPSRC IRC
FET BrainCom

Title: Circumferential shape-adaptive bioelectronics enable spinal cord recording and bypass following spinal cord injury.

Authors: *L. KIANG[1], B. WOODINGTON[2], A. CARNICER-LOMBARTE[2], A. GÜEMES[2], S. HILTON[2], T. E. NAEGELE[2], S.-T. CHEN[2], R. TRIVEDI[2], G. MALLIARAS[2], D. G. BARONE[2];
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Abstract: Advances in spinal cord stimulation (SCS) have enabled the restoration of overground walking in patients with spinal cord injury (SCI) via external triggers. Closed-loop restoration of motor activity triggered by the patient's motor volition requires decoding of motor intention via neural interfaces. We designed a shape-adaptive, parylene-C based 32-electrode array with titanium/gold electronics and Poly (3,4-ethylene) dioxythiophene (PEDOT) conducting polymer doped with poly(styrene sulfonate) (PSS) capable of interfacing circumferentially around the spinal cord without causing iatrogenic SCI. In this exploratory *in vivo* study, we implanted the electrode array epidurally around the spinal cord at the T10 vertebral level in adult female rats (Sprague-Dawley, n=16) and recorded the compound action potentials evoked by stimulation of the motor cortex (motor evoked potentials (MEP)) and sciatic nerves (somatosensory evoked potentials (SSEP)). We used a thresholding method based on peak amplitude means to perform feature extraction of the signals for supervised machine learning. We achieved a classification accuracy of 93.8% using a k-nearest neighbours algorithm when categorizing the source of

evoked potentials according to the following categories: left MEP, right MEP, left SSEP and right MEP. To functionally bypass a site of SCI, we implanted recording and stimulation electrode arrays at the T10 and L1 vertebral levels respectively and transected the spinal cord at the T11 level. We then produced a low-latency communication between the 2 arrays using a threshold detection method. This enabled the recording array to detect the peak of an MEP in response to hindlimb motor cortex stimulation and trigger the stimulating array to effect hindlimb movement in paralyzed rats. In this proof-of-concept study, we have demonstrated that interfacing circumferentially around the spinal cord enables the comprehensive spatiotemporal representation of neural signals, generating control signals to trigger targeted SCS and ultimately bypass acute SCI to restore hindlimb movement.

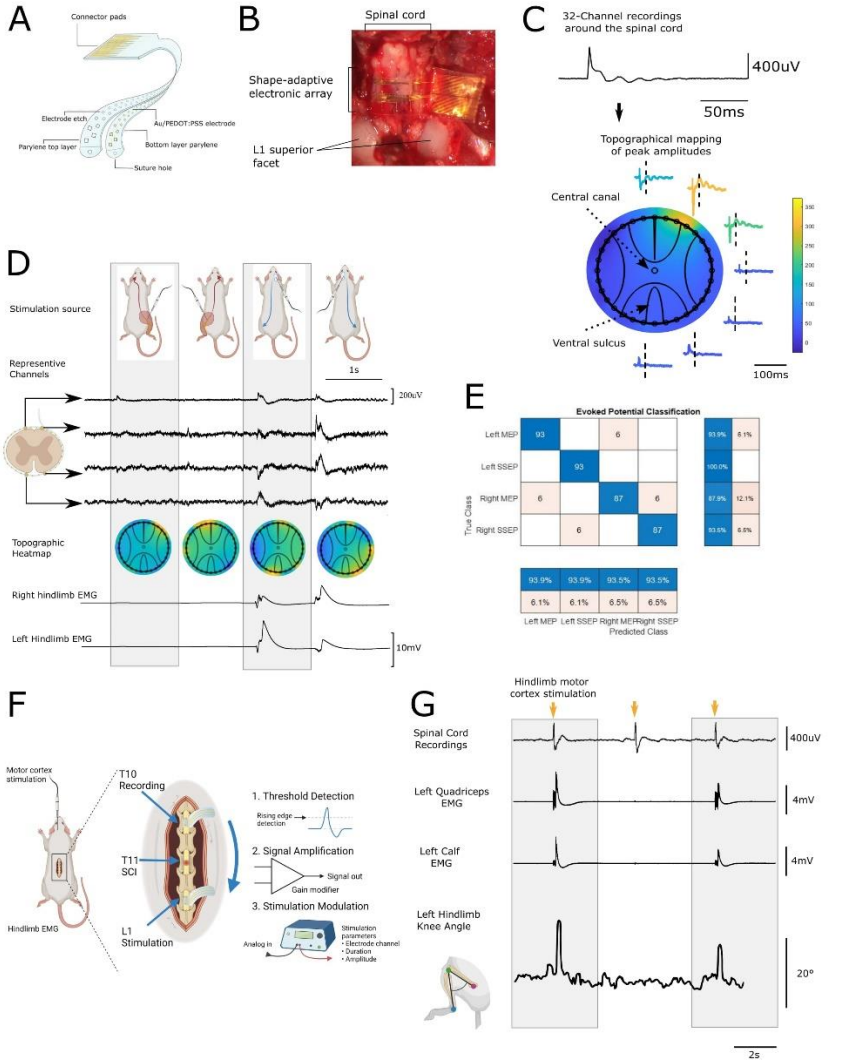

Figure 1. A) Illustration of microfabricated electrode array design. B) Intraoperative image of array placed circumferentially around spinal cord. C) Creation of spinal cord topographic map based on filtered signals from the 32-channel recordings. D) Representative recordings across 4 quadrants of the topograph during SSEP and MEP stimulation events with associated electromyography (EMG) of the hindlimbs. E) Classification matrix showing accuracy for left/right SSEP/MEP stimulation events. F) Experimental setup for spinal bypass experiments. G) Example data showing recorded signals from the ventral part of the spinal cord and quadricep/calf EMG waveforms following post-injury motor cortex stimulation. Hindlimb angles were calculated from marker-less kinematic data which corresponds to EMG and MEP waveforms.

Disclosures: **L. Kiang:** None. **B. Woodington:** None. **A. Carnicer-Lombarte:** None. **A. Güemes:** None. **S. Hilton:** None. **T.E. Naegele:** None. **S. Chen:** None. **R. Trivedi:** None. **G. Malliaras:** None. **D.G. Barone:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.07

Topic: E.05. Brain-Machine Interface

Support: UF1NS115817
R01-NS-118606-01
NSF 1707316
NIH UF1 NS107659

Title: Characterization of small surface area carbon fiber electrodes

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Abstract: Carbon fibers are an excellent material for recording neural signals. They have a small diameter, can be fabricated at the benchtop, and are biocompatible allowing for single-unit recordings. However, carbon fibers have been limited in their recording site geometry (Welle et al. 2021). The site can be small (< 10 µm in length) but unable to penetrate into tough tissues, like nerve, or self-insert further than 500 µm into brain. To access tougher tissues requires the carbon fiber to be sharpened, currently using a blowtorch. This results in a larger recording site (<140 µm in length) that makes single-unit recording difficult. Here, we focus on a small, sharpened geometry that is controllable and repeatable to increase the insertion and recording capabilities of these electrodes. Carbon fiber arrays were constructed and insulated with Parylene C (N=63 fibers). The tips of the fibers were then re-exposed using a blowtorch to melt the insulation back. Two sharpening processes were investigated: a small-flame blowtorch and chemical etch. The small-flame method exposed and sharpened the carbon fibers for a final exposed tip length of <100 µm. The chemically etched tips were blowtorched, then placed in a sulfuric acid solution and a 5 V pulse was applied in one second intervals based on the measured impedance. A subset of tips were then imaged and measured using SEM across the different etching parameters to determine repeatability for the procedure. Both electrode geometries were subjected to physical and electrical characterization including surface area, impedance, and the yield from the fabrication process itself. One perfused rat brain was also used for insertion testing at various lengths. The tip exposure length for small-flame blowtorch and chemical etch

were 43 ± 5 µm (N = 6) and 10 ± 3 µm (N=9 fibers). The success rate of each technique (sharpened vs. unsharpened) was very high. The impedances of both designs post-treatment was typically high (< 1MΩ), but adding a conductive polymer reduced the impedances to 30 ± 11 kΩ (N = 48), which is an acceptable range for a recording electrode. Comparison of sharpened fibers to historical blunt fiber data revealed that sharpened fibers penetrated the brain at longer lengths ($p<0.01$).We have shown that small tips can be achieved through two different processes that do not require specialized training or equipment. These electrodes need to be further validated *in vivo* to determine their recording capability; they have been used successfully to obtain intracellular recordings in a semi-intact preparation of *Aplysia's* feeding apparatus. Octopus axial nerve cord will be of interest due to its small signal size and tough tissue.

Disclosures: J. Richie: None. **A.K. McLane-Svoboda:** None. **J.G. Letner:** None. **Y. Huan:** None. **H.J. Chiel:** None. **G. Pelled:** None. **P.R. Patel:** None. **C.A. Chestek:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.08

Topic: E.05. Brain-Machine Interface

Support: NRF of Korea, 2021M3E5D2A0101953812

Title: Multi-site and neuroscience-based direct cortical stimulation for eliciting precise artificial somatosensation

Authors: *S. RYUN[1], J. KIM[2], C. CHUNG[3];
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Abstract: Eliciting artificial somatosensation using direct brain stimulation is one of the most essential techniques in bi-directional brain-machine interfaces. It is well-known that cortical stimulation on the primary somatosensory cortex (S1) elicits various types of artificial somatosensory responses. However, to date, fine-control of the quality of elicited somatosensation such as vibration and pressure is still challenging issue. In this study, we tackle the issue by using multi-site direct cortical stimulation and by stimulating downstream of the S1. Nine patients with drug-resistant epilepsy participated in this study. To stimulate multiple electrode sites simultaneously, we used two cortical stimulators, GRASS S12 and S12X. These two stimulators were computer controlled by customized software written in MATLAB. Duration of stimulus and dynamics of inter-pulse intervals were controlled by this software. We found that multi-site DCS on relatively distant areas of the S1 can elicit two independent sensations simultaneously. Based on the result, blindfolded patients could easily perform a real-time, DCS-guided reach-and-grasp task with high success rate (above 95%). We also found that

multi-site DCS on S1 area close to each other elicits different quality of somatosensation in the same body part. One single-site DCS on S1 induced vibrotaction on the radial side of the index finger, and the other one induced the same quality of sensation on the ulnar side of the index finger. However, DCS on both areas elicited vibration and pressure sensations on the radial side of the index finger, but no sensation was elicited on the ulnar side of index finger. Finally, we found that DCS on the ventral premotor area (vPM) can affect higher-level sensorimotor functions such as hand manipulation. During DCS on vPM, the patient consistently reported artificial vibrotaction of one hand. Simultaneously, negative motor responses were found when the patient performs grasping motions. No significant movement deficits were found during elbow flexion and reaching without grasping. In this study, we suggest that multi-site DCS on distant cortical areas can elicit simultaneous and independent tactile sensations, and its robustness is sufficient to apply the real-time somatosensory feedback system. Additionally, we demonstrate that complex multi-site DCS on the small cortical area can elicit various and controllable tactile sensations on the same body part. Finally, the results of vPM stimulation indicate that cortical stimulation on the vPM elicits function-specific sensorimotor responses such as hand manipulation.

Disclosures: **S. Ryun:** None. **J. Kim:** None. **C. Chung:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.09

Topic: E.05. Brain-Machine Interface

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NIH National Institute on Deafness and Other Communication (R01DC008358)
KIBM - Kavli Institute for Brain and Mind
The Fulbright Program
"La Caixa" Foundation

Title: Neural Population Dynamics During Vocal Behavior

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Abstract: Our increasing ability to record the activity of large numbers of individual neurons facilitates the study of brain dynamics at the population level that may appear imperceptible in individual unit responses. Previous studies have shown that covariance patterns of neural

populations in the primary motor cortex (M1) can be well-described in a low-dimensional, linear manifold during reaching movements (Gallego et al. 2017). Yet, these linear dynamics appear inexistent during grasping (Suresh et al. 2020). We investigate whether similar dynamics can be observed in the context of a complex, learned vocal behavior. To better understand the neural mechanisms that enable complex motor-vocal behavior, we use Neuropixel probes to record the simultaneous activity of tens to hundreds of single neurons in the forebrain song control nuclei HVC and the robust nucleus of the arcopallium (RA) of male zebra finches engaged in unconstrained vocal behavior. We apply latent-factor based models to gain insight into the temporal dynamics of neural populations that drive vocal production. We find that the spiking activity across populations of individual RA neurons is well described by a pattern of smooth, continuous trajectories in the latent neural space during stereotyped singing (Figure 1). Moreover, we find that neighboring neural states have a unique correspondence to song renditions that are close in the vocal space. This continuity, however, may be absent in the projection of premotor HVC activity into a linear neural manifold. Our results point to common principles that may underlie the encoding of complex natural motor actions in specific brain regions and circuits across species.

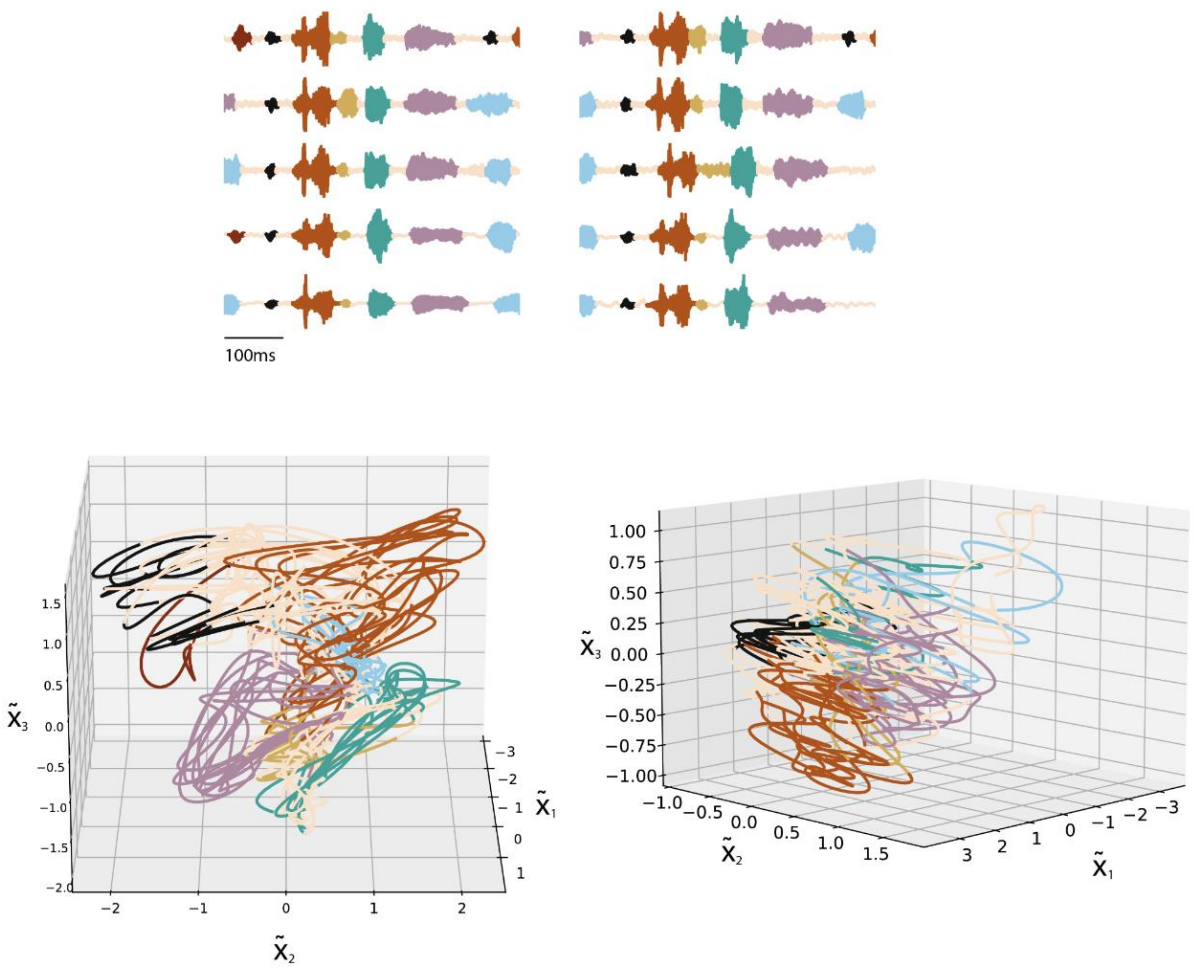

Figure 1. **Top**: Ten examples of recorded vocal renditions of zebra finch song. **Bottom left**: Neural trajectories found through the projection of the population spiking activity of RA (121 neurons) onto a latent space using GPFA during the renditions of vocal behavior shown above. **Bottom right:** Analogous to bottom left, for the HVC population (49 neurons).

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.10

Topic: E.05. Brain-Machine Interface

Support: KAKENHI 23680061
KAKENHI 18H04038
KAKENHI 18H05287
KAKENHI 20H05713
KAKENHI 16H05434
KAKENHI 19K19899

Title: Subdural spinal electrical stimulation for muscle activations of upper limb in an incomplete tetraparesis: A case report in a patient with spinal tumor

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Abstract: Spinal electrical stimulation is a promising approach for restoring the motor functions of paralyzed limbs following neurological damage to descending pathways. The present study examined the muscle responses upper limb evoked by subdural spinal electrical stimulation of the cervical cord in a patient with tetraparesis. We temporarily installed a platinum subdural electrode array over the dorsal-lateral aspect of the cervical enlargement locating caudal to the vicinity of epidural tumor. Electrical stimulation was delivered at four sites under anesthesia. The subdural electrical spinal stimulation over the cervical enlargement (C5-C7), which was located caudal to the vicinity of epidural tumor, could activate multiple muscles of the upper limb in a tetraparesis. At smaller current of 1 mA, stimulation through the rostral electrode activated muscle responses in proximal muscles, stimulation through the caudal electrode activated distal muscles. Increasing the stimulus current associated number of the activated muscles and amplitude of muscle responses. Once muscles are recruited, while the waveform of muscle response sin proximal muscles were similar among different intensity, that in distal muscles changed as current increased. Furthermore, while grasping-retracting-related muscles such as elbow flexor, wrist flexor, and thumb adductor muscles showed greater responses, their antagonists such as elbow extensor, wrist extensor, and thumb abductor muscles showed smaller responses throughout all current intensities, might suggesting stimulation activated spinal interneurons for reciprocal inhibition. These results indicate that spinal motor functions including peripheral nerves and circuits for spinal reflex of upper-limb muscles are preserved in a patient with an incomplete tetraparesis and spinal electrical stimulation is a promising neuroprosthetic technology to regain motor function in upper-limb after neural damage to the descending pathways.

Disclosures: K. Kato: None. **K. Kamada:** None. **Y. Nishimura:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.11

Topic: E.05. Brain-Machine Interface

Support: NIH RO1 NS085167
NIH RO1 NS094384

Title: Varying temporal characteristics of vagus nerve stimulation to promote motor cortex plasticity

Authors: *C. L. NEIFERT[1,2], J. J. A. ADDO[1,2], T. T. DANAPHONGSE[2], S. T. ABE[2], V. EZHIL[3,2], A. REYES[2], M. P. KILGARD[1,3,2], S. A. HAYS[1,3,2];
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Abstract: Vagus nerve stimulation (VNS) paired with rehabilitation is an emerging treatment strategy for neurological injuries. In response to injury, insufficient or maladaptive neural plasticity often prevents full recovery, leaving lasting impairments to brain function. Functional recovery of the affected areas of the brain is shown to be related to the degree of cortical reorganization in the surrounding undamaged tissue. VNS enhances this plasticity in motor networks and, when paired with rehabilitative training, increases recovery after neurological injury. Consequently, VNS strategies that generate greater plasticity may represent a means to promote greater recovery. As such, it is important to understand what elements of VNS can be changed to yield greater amounts of plasticity. Previous studies show that temporal characteristics of VNS pulses, such as stimulation train duration or bursts of stimulation, impact the degree of plasticity and recovery. In this study, we sought to systematically characterize the effect of VNS timing on motor cortex plasticity. Rats performed a simple behavioral task in which VNS was paired with jaw muscle activation during chewing. For five days, rats received 200 pairings of 16 individual pulses of VNS delivered in separate groups defined by the following parameters: a moderate frequency of 30Hz for 500 ms (Standard VNS), in bursts of 4 pulses every 500 ms (Burst VNS) and steadily over a span of 2000 ms (Long VNS). Following each animal's final behavioral session, intracortical microstimulation (ICMS) was used to assess movement representations in the motor cortex. Consistent with previous studies, our preliminary findings indicate that Standard VNS and Long VNS paradigms enhance motor cortex plasticity, whereas Burst VNS fails to enhance plasticity. Current insights from this study suggest that the timing of VNS influences the magnitude of plasticity, which has translational applications for applying these stimulation approaches to yield enhanced recovery from neurological injuries.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.12

Topic: E.05. Brain-Machine Interface

Support: NIH R01 EB027584
ANR-18-NEUC-0002-02

Title: Selective activation of nerve fiber subpopulations with intrafascicular stimulation

Authors: *A. ORTEGA SANABRIA[1], A. K. THOTA[1], L. REGNACQ[2], M. ROUHANI[3], L. M. MCPHERSON[4], J. J. ABBAS[1], Y. BORNAT[5], F. KOLBL[2], R. JUNG[1];
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Abstract: Bioelectronic therapies based on peripheral nerve stimulation (PNS) use modulation of neural activity to treat various diseases. Eliciting the PNS therapeutic effect while avoiding side effects depends on the ability to selectively target stimulation of the end-organ. Improved selectivity of the innervating nerve fibers using intrafascicular stimulation would allow enhanced on-target stimulation. Intrafascicular selectivity of nerve-fiber stimulation was investigated using multiple longitudinal intrafascicular electrodes (LIFEs) implanted in the sciatic nerve of rats. Selectivity was assessed using High Density surface EMG (HD-sEMG) that provided spatiotemporal information of the motor fiber activation of the innervated gastrocnemius lateralis (GL) muscle. Experiments were conducted in adult male Sprague-Dawley rats (n=5) under isoflurane anesthesia. 4 to 6 LIFEs were implanted in the sciatic nerve; 3-4 in the tibial and 1-2 in the peroneal fascicle. Biphasic, cathodic first, pulses were delivered at 10Hz for 5 seconds using a custom neurostimulator. Pulse width was chosen at 2 x rheobase obtained from the strength duration curves collected for each electrode. The stimulation current amplitude was increased in steps of 2µA from subthreshold to a suprathreshold maximum of 3 x rheobase. Using a 32-channel flexible electrode array (NeuroNexus), HD-sEMG of the GL was recorded. Peak to peak amplitude of the motor unit action potentials were used to assess spatiotemporal spread of muscle twitch. Results indicated that electrodes implanted within the same fascicle but in different cross-sectional and longitudinal locations activated different regions of the muscle, thereby showing targeted specificity and selectivity of nerve fiber recruitment. The recruitment of the fibers was graded with increments of stimulation amplitude. Focal recruitment can be achieved across this increment of the stimulation amplitude. These results suggest that intrafascicular selectivity can be achieved using multiple electrodes within the same fascicle. Different fibers can be selectively activated to improve on-target stimulation. Additional studies using computational models have also demonstrated the benefits of using intrafascicular electrodes and the effect of the electrode geometry, location, and stimulation waveform patterns on the nerve fiber recruitment, and could guide advanced stimulation strategies using LIFE for PNS.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.13

Topic: E.05. Brain-Machine Interface

Support: EU ERC FeelAgain Grant 759998
SNSF MOVE-IT Grant 197271

Title: Encoding natural sensory information through model-based biomimetic neural stimulation

Authors: *G. VALLE[1], N. KATIC[3,2], D. EGGEMANN[1], O. GORSKII[4], N. PAVLOVA[4], P. MUSIENKO[4], M. BUMBASIREVIC[5], S. RASPOPOVIC[2];
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Abstract: In the last decades, the fascinating opportunity to communicate artificially with the brain using peripheral nerve stimulation have been extensively explored in people with sensory-motor deficits. The use of innovative neural interfaces enables the interaction of robotic devices with the human sensory system. It has been shown that direct nerve stimulation can effectively restore somatosensation, controlling its properties by modulating the injected stimulation. However, efforts are still necessary to identify encoding strategies converting sensory information into neural stimulation patterns that would be able to elicit sensations that are effective for prosthesis use, but also perceived as natural. To this aim, we designed, implemented and tested biomimetic neurostimulation paradigms able “to write” artificial sensory information into the human peripheral nervous system. Firstly, we started designing neural modulation strategies based on the indications of realistic in-silico model able to emulate the natural touch coding. Then, we measured the effect of these model-based biomimetic approaches and compared them to traditional linear neuromodulation strategies as well as to the natural touch. We recorded and analyzed the natural patterns in the somatosensory neuroaxis (sural nerves, Dorsal Root Ganglia and Spinal Cord) that resulted from the electrically- and naturally-induced stimuli in cats. We observed multiple differences in the neural activation comparing biomimetic neurostimulations and those traditionally used in neuroprosthetics. After identifying the biomimetic patterns able to induce a neural activity more similar to the natural touch, we implemented and tested them into bionic devices in patients, achieving with very promising results. When exploited using real-time configurations of bionic legs in ecological tasks, the biomimetic neurostimulation guaranteed better performance to the device users. These findings

highlight the importance of developing neuroscience-driven technology inspired by the human natural systems (biomimicry). This will allow to finally connect humans and machines communicating directly with the brain and improving the efficacy of the neurotechnologies for people with sensory-motor deficits.

Disclosures: G. Valle: None. **N. Katic:** None. **D. Eggemann:** None. **O. Gorskii:** None. **N. Pavlova:** None. **P. Musienko:** None. **M. Bumbasirevic:** None. **S. Raspopovic:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.14

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01NS107271

Title: Effects of intracortical microstimulation on neural activity in distant cortical regions

Authors: *B. RUSZALA[1], K. A. MAZUREK[2], M. H. SCHIEBER[2];
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Abstract: The effects of electrical intracortical microstimulation (ICMS) are commonly viewed as being most influential on neurons located near the tip of the stimulating electrode. Yet, ICMS effects have been shown to be largely transsynaptic. For example, a train of ICMS delivered in the primary motor cortex (M1) evokes muscle contractions through transsynaptic activation of distant spinal motoneurons. Given the inter-areal connectivity of the cerebral cortex, ICMS may similarly have substantial effects on the activity of neurons in distant cortical areas. Here, we examined the extent to which ICMS delivered in the primary somatosensory cortex (S1) affected the activity of neurons in M1 and premotor cortex (PM), two distant cortical motor areas known to receive cortico-cortical projections from S1. Rhesus monkeys initially were trained to reach, grasp, and manipulate 4 different objects instructed by visual cues, and then implanted with multiple microelectrode arrays in S1, M1, and PM. Subsequently, the visual-cue instructions for the 4 objects were replaced by S1-ICMS instructions delivered as trains of simultaneous pulses through a set of 3-7 electrodes on a different array in S1 for each object (1-64 μA per electrode, 75 - 150 Hz). Neural data were recorded from M1 and PM as the monkey performed this task. In one monkey, we analyzed 39 M1 units recorded from 4 arrays and 43 PM units from 4 arrays (including both single- and multi-units); in another monkey, 36 M1 units from 4 arrays and 33 PM units from 2 arrays. Offline, we compiled a peristimulus time histogram (PSTH) of each unit's spike times triggered on the individual pulses from each set of stimulating electrodes. If the distribution of spike times following stimulus pulses was significantly non-uniform (KS goodness of fit test, $p < 0.01$), we considered the unit's spiking activity to have been directly modulated by those pulses. 98% of the recorded M1 units and 80% of PM units in one monkey were directly modulated by the pulses from at least one S1 array; 83% of M1 units and 94% of

PM units in the second monkey. Furthermore, many units were modulated by pulses from multiple S1 arrays. In one monkey, 54% of M1 units and 16% of PM units were modulated by pulses from all 4 S1 arrays. In the other monkey, where only 3 S1 arrays were considered, 36% of M1 units and 15% of PM units were directly modulated by pulses from all 3 arrays. S1-ICMS affected the majority of M1 and PM units, with considerable divergence of effects from each S1 array to neurons distributed widely in M1 and PM, and convergence of effects onto individual units from wide territories in S1.

Disclosures: B. Ruszala: None. **K.A. Mazurek:** None. **M.H. Schieber:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.15

Topic: E.05. Brain-Machine Interface

Support: JSPS KAKENHI 18H05287

Title: Adaptation to cortico-spinal interface to restore forearm paralysis in spinal cord injury

Authors: *K. OBARA[1,2], M. KANESHIGE[1], M. SUZUKI[1], T. TAZOE[1], Y. NISHIMURA[1,2]; [1]Tokyo Metropolitan Inst. of Med. Sci., Tokyo, Japan; [2]Grad. Sch. of Med. and Dent. Sci., Niigata Univ., Niigata, Japan

Abstract: Spinal cord injury (SCI) disrupts neural communications between the brain and the spinal circuits. Bypassing the damaged cortico-spinal pathway using brain-computer interface, which artificially-connects a preserved cortical site and spinal cord, restored volitional control of the impaired forelimb movements after SCI. However, it remains unclear how an ensemble of cortical neurons incorporates a novel artificial neural pathway into the volitional control of the paralyzed limb. Here, we investigated the effects of introducing the cortico-spinal interface on cortical neuronal activity in paralyzed monkeys that had a unilateral spinal cord lesion (SCL). Cortico-spinal interface bridged lesion at mid cervical cord and transforms firing rate of a single neuron into frequency and current of subdural spinal stimulation on cervical enlargement, to regain volitional control of a paralyzed forearm muscles. The ensemble of neurons was recorded with a multi-channel Utah array from the wrist region in the contralesional primary motor cortex. A single neuron was randomly selected to be directly linked to subdural spinal stimulation (linked neuron). Magnitude of activations in paralyzed muscles and evoked wrist torque are positively correlated with current intensity of subdural spinal stimulation. Without the cortico-spinal interface, the monkeys with SCL were unable to accomplish a wrist-torque-tracking task and most of neurons and muscles did not show task-related modulation. After introducing the cortico-spinal interface, the monkeys succeeded in performing the task by voluntarily adjusting the firing rate of the linked neuron which directly linked to subdural spinal stimulation and muscle, and achieved wrist-torque modulation depends on the magnitude of required wrist-

torque. Unlinked neurons which were not linked to the spinal stimulation also showed task-related modulation. Three types of unlinked neurons were observed: neurons that increased or decreased their activity in relation to the task, and neurons that showed unrelated activity. These findings demonstrated that the monkeys tuned the activity in the ensemble of cortical neurons in M1 to incorporate a novel cortico-spinal interface into the volitional control of paralyzed forearm.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.16

Topic: E.05. Brain-Machine Interface

Support: NSF 1954107/1734916
NSF 2124066

Title: A brain-computer interface in the prefrontal cortex that suppresses neural variability

Authors: R. WILLIAMSON[1], A. UMAKANTHA[1], ***C. KI**[1], M. A. SMITH[2], B. M. YU[3]; [1]Neurosci. Inst., [2]Biomed. Engineering, Neurosci. Inst., [3]Electrical & Computer Engineering, Biomed. Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Consider shooting free throws on a basketball court. Even if the shooter tries the same shot each time, their mind may wander. Such changes in cognitive states may manifest as neural variability, in which the brain produces variable activity when presented with the same task conditions multiple times. This variability stems from various sources, such as fluctuations in internal states (e.g., arousal and attention). Neural variability can limit the brain's ability to encode information and negatively impact one's task performance. For example, deficits in regulating neural variability have been linked to neuropsychiatric disorders. Reducing neural variability thus has implications for improving and restoring the brain’s cognitive capacity. However, the extent to which neural variability is under volitional control is unclear. We designed a prefrontal cortex (PFC) brain-computer interface (BCI) to determine if macaque monkeys can use neurofeedback to stabilize their neural activity. We challenged animals to use visual feedback (an annulus around fixation) to keep their neural activity close to a target neural state, which we defined as the population activity pattern (i.e., a multi-dimensional vector) seen at the start of an experimental session. In BCI trials, the annulus diameter reflected the distance of the current neural state from a target neural state (low distance = small annulus; high distance = large annulus). To assess how well the animals used the BCI, we interleaved blocks of BCI control trials with blocks of sham control trials (these showed feedback that did not correctly represent the animal's current neural state) in each session. We found that animals used the BCI

to stabilize their neural activity at a level above chance (defined by sham control). We identified two strategies the animals may have used to improve their performance in BCI trials. First, we found that in sham blocks, the neural activity gradually moved away from the target state. In BCI blocks, however, these slow changes were suppressed. Animals also had more stable pupil sizes in BCI blocks, which implied that animals used the BCI to self-regulate their arousal states to mitigate the distance increases seen in sham blocks. Lastly, we found that animals relied on veridical moment-to-moment visual feedback to reduce their neural distances, which suggested that within trials, they used the annulus to control their neural activity to perform better in BCI trials. Our work demonstrates that animals can suppress neural variability (i.e., "mind wandering") using our novel BCI paradigm. Moreover, these findings can inform the development of clinical BCIs that treat cognitive disorders.

Disclosures: R. Williamson: None. **A. Umakantha:** None. **C. Ki:** None. **M.A. Smith:** None. **B.M. Yu:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.17

Topic: E.05. Brain-Machine Interface

Support: UG3NS123723-01
DP2-EB029757
ECCS1542148

Title: A Thin, Flexible, and Scalable MEMS based Depth Electrode for Deep Brain Recording and Stimulation

Authors: *K. LEE[1], A. C. PAULK[4], Y. RO[5], K. J. TONSFELDT[6], Y. TCHOE[8], A. BOURHIS[2], J. LEE[7], D. R. CLEARY[1], J. S. PEZARIS[4], Y. KFIR[9], S. S. CASH[10], S. DAYEH[3];
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Abstract: Electrocorticography (ECoG) and stereoelectroencephalography (sEEG) are the gold standard for recording intracranially in the human brain to help localize epileptogenic zone and determine the onset and propagation of epileptiform discharges in patients with intractable epilepsy. Yet, the spatial resolution, or contact spacing, most commonly used is on the scale of 1 cm in existing clinical ECoG grids and strips, and 3 – 6 mm in sEEG which could limit both the diagnostic and therapeutic benefits, such as using stimulation to map epileptogenic brain

networks. To improve this spatial resolution and leveraging thin film processing with sacrificial layer deposition and etching, we developed a microelectrode-sEEG (μ-sEEG) with 128 platinum nanorod (PtNR) contacts which can achieve a minimum of 30 μm contact-to-contact spacing that can be implanted in deep brain structures. A monolithically integrated pocket on back side of the polymer electrode layers allowed us to insert a clinical grade stylet (1 – 180 mm -long-stylet) into the pocket to assist insertion of the flexible polymer electrode into deep brain structures. We could also then retract the stylet after the insertion, consistent with standard clinical procedures in implanting sEEG electrodes. The entire μ-sEEG electrode is approximately 10 μm thick which resulted in minimal tissue damage in chronic implants when compared to standard clinical sEEG electrodes. Further, we designed multiple versions of the μ-sEEG device which could be used either acutely or in a chronic preparation and could be either only spanning the cortex (3-4mm long) or extend deep into primate cortex (10 cm long).We validated the performance of our μ-sEEG electrodes in laminar recordings in acute and chronic rat cortical preparations, acutely in the pig cortex, acutely in the operating room in the human cortex, and in deep subcortical structures in an acute non-human primate (NHP) preparation. As PtNR contacts can be made to be microelectrodes while maintaining low impedances (~300 kΩ), the implantable μ-sEEG device was able to record single unit activity along with physiologically-relevant broadband dynamics including low frequency oscillations and high-gamma activity as shown in the voltage dynamics and through current-source density (CSD) analysis. When validated for safety and efficacy in animals, the μ-sEEG electrode holds the potential for recording and disrupting local brain activity on the single cell level and may help understanding and treating epilepsy and beyond.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

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Program #/Poster #: 058.18

Topic: E.05. Brain-Machine Interface

Support: NRF-2018M3C7A1022309

Title: Magnetic stimulation of peripheral nerves using an implantable miniature coil

Authors: *K. LEE, B. PARK, J.-W. JANG, S. KIM;
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Abstract: Magnetic stimulation using an implantable device is a promising technique that is free from the degradation of the device by immune responses in the body. Several studies have shown the results of applying this stimulation technique to the brain or peripheral nerves. Especially a

study on magnetic stimulation of the sciatic nerve using a coil with a diameter of 10 mm reported to cause neuronal responses by applying currents with intensities in a range typically used in transcranial magnetic stimulation (TMS). However, there are limitations that the coil was about ten times larger than the nerve diameter, and the applied current intensity was considerably high, in the order of kA. Thus, we investigated the feasibility of using a smaller coil and lower current to elicit neuronal responses in the sciatic nerve. Among many coil types tested, a small coil with a 4 mm diameter wound around a bobbin-shaped ferrite core was selected as the coil for stimulation. Due to the ferrite core, the coil had an inductance of 1 mH, hundreds of times greater than the inductance of the coils used in the previous study (ca. 5 μH). Therefore, it could induce strong electric fields with lower currents, even though it was small in size. First, the minimum stimulus intensity to elicit neuronal responses was estimated using 3D simulation software (Sim4Life). Since this simulation tool included both an electromagnetic analysis model and a computational neuronal model, it was possible to simulate the neuronal response induced by the selected coil. Sciatic nerves of Sprague-Dawley rats were targeted for magnetic stimulation. After exposing the sciatic nerve under anesthesia, the coil was placed near the sciatic nerve as close as possible. The response to stimulation was determined by the detection of compound muscle action potentials (CMAPs) from the tibialis anterior (TA) and gastrocnemius (GC) muscles. The simulation predicted that an action potential would be elicited in the sciatic nerve when a current pulse of above 3.5 A was applied and the nerve was located on the surface of the coil. In *in-vivo* experiments, CMAPs and twitching were observed simultaneously in all subjects (n=10) when the stimulus intensity was above 5.5 A. The difference between the actual threshold and the predicted value was speculated to come from the distance between the coil and the nerve axons that cannot be decreased further. The latency of CMAPs was measured to be 1.5 ms on average, and the CMAPs were observed in both TA and GC muscles. Using the small-sized coil wound on a ferrite core, we observed that the sciatic nerve could be stimulated magnetically with weak current of about 1000 times lower intensity than that used in conventional TMS.

Disclosures: **K. Lee:** None. **B. Park:** None. **J. Jang:** None. **S. Kim:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Topic: E.05. Brain-Machine Interface

Support: AHA Postdoctoral fellowship 897265
CNSM Postdoctoral fellowship D000000101
NIH 4R00NS097620
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AHA Career Development 847486

Title: Coordinated cortico-cerebellar neural dynamics underlying neuroprosthetic learning

Authors: *A. ABBASI[1], A. FEALY[1], N. DANIELSEN[1], T. GULATI[2];
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Abstract: Introduction: Brain-machine interfaces (BMIs) or neuroprosthetics allow neural control over assistive devices. They also provide an important framework for studying neural plasticity. Recent studies have suggested that optimal engagement of learning is essential for robust neuroprosthetic control. However, little is known about the neural processes in subcortical regions that support learning a neuroprosthetic skill. We performed electrophysiologic recordings in the motor cortex (M1) and the cerebellum (Cb) of rodents while they engaged M1 neurons in BMI control. By monitoring neural ensembles in both regions, we found robust indirect modulation of neurons and emergence of coordinated activity in the local field potentials (LFPs) and neural spiking across M1 and Cb. **Methods:** We recorded single units and LFPs in adult Long-Evans rats (n=8) while they performed a neuroprosthetic task by implanting microwire arrays in M1 and tetrodes/polytrodes in Cb. During the task, activity of a subset of M1 neurons was transformed into the angular velocity of a feeding tube using a linear decoder. Rats modulated the activity in these 'direct' neurons to obtain water rewards. We analyzed how the activity in these neurons, as well as all other recorded 'indirect' neurons in M1 and Cb changed while learning the neuroprosthetic task. We also analyzed band-limited oscillatory activity in both regions. Furthermore, we performed optogenetic silencing of Cb by injecting red-shifted halorhodopsin (Jaws), while rats (n=3) performed the BMI task and analyzed how Cb silencing affected the M1 activity and neuroprosthetic learning. **Results:** We found that learning BMI control was associated with the modulation of 'direct' neurons in M1, as well as robust 'indirect' neural modulation in M1 and Cb. Furthermore, we observed the emergence of task-related 3-6 Hz synchronous oscillatory activity in cortico-cerebellar LFPs which also modulated the task-related neural spiking. We also found that direct and 'task-related' indirect units were modulated by this 3-6 Hz LFP activity and canonical correlation between M1 and Cb spiking activity increased with neuroprosthetic learning. We also found that optogenetic inhibition of cerebellum prevented the acquisition of neuroprosthetic skill and in the animals that had learned the task, it led to loss of performance. **Conclusion:** Our work has identified neural mechanisms in M1 and Cb which are associated with learning of a cortically-controlled BMI skill. This underscores the importance of optimal engagement of neural learning mechanisms in an offsite motor region- the cerebellum, for successful learning of M1-controlled neuroprosthetic task.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.20

Topic: E.05. Brain-Machine Interface

Support: Dutch Technology Foundation STW NeuroCIMT project [grant 14906] (NFR)

Title: Comparison between epidural and subdural electrocorticography recordings for neural implants

Authors: *S. H. GEUKES, M. P. BRANCO, E. J. AARNOUTSE, M. J. VANSTEENSEL, N. F. RAMSEY;
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Abstract: In recent years, the development of brain-computer interfaces (BCIs) and neuromodulation devices has accelerated, with increasing translation from the laboratory to clinical practice. In this context, electrocorticography (ECoG) - the placement of electrode grids or strips on top of the cortical surface - is deemed a particularly interesting recording method, as high spatial and temporal resolution is met with relatively reduced invasiveness. In general, ECoG electrodes are placed below the dura mater (subdural), but they may be placed on top of the dura as well (epidural). It is unknown what the exact consequences of subdural or epidural electrode placement are for the signal quality and safety of the implant. As the dura is left intact, epidural ECoG may pose a lower risk for serious complications within the central nervous system, compared to subdural ECoG. However, the dura itself may hinder the signal quality of epidural recordings. To further our understanding of ECoG, we gathered all relevant epidural ECoG studies to investigate the effect of the dura on acute and chronic ECoG recordings in humans and non-human primates. We additionally compared the adverse events occurring with subdural and epidural ECoG during clinical trials. In short, the literature suggests that epidural recordings show lower signal amplitude than subdural recordings, particularly with smaller grids. However, the decodability of the epidural signal and consequently its applicability for BCI control does not seem to be considerably affected, neither in short- nor long-term recordings. Also, we find that the nature of serious complications in clinical trials is comparable between epidural and subdural ECoG. Taken together, both epidural and subdural ECoG appear well fit for high-fidelity recordings, over longer periods of time, at least for the current generation of ECoG.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.21

Topic: E.05. Brain-Machine Interface

Support: National Defense Science and Engineering Graduate (NDSEG) Fellowship (H.M.S.)

Title: Impact of Neural Variability on Brain-Machine Interface Learning

Authors: *H. M. STEALEY, Y. ZHAO, H.-Y. LU, E. CONTRERAS-HERNANDEZ, S. R. SANTACRUZ;
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Abstract: Neural variability is important when learning a new abstract skill. The specific impact neural variability has on learning remains understudied; measuring every neural pattern that is relevant to a new behavior remains difficult. To overcome this, we used a brain-machine interface (BMI) model in non-human primates (NHPs). This allowed us to create a mathematical mapping ("decoder") between the neural input and the behavioral output. Specifically, we designed a biomimetic decoder and then applied a perturbation (rotation) to the decoder to induce controlled learning. *We posit that the amount of variability in a BMI decoder population relates to the possible amount of behavioral recovery possible when learning a non-biomimetic decoder.*We trained rhesus monkeys (*Macaca mulatta*; n=2) in a center-out BMI task. In this, subjects intentionally altered their neural spiking activity to move a computer cursor. On a given trial, subjects were given 10 seconds to move the cursor from the center of the screen to a peripheral target located 10 centimeters away. Sessions were divided into two blocks of 384 trials each (48 trials x 8 targets). Block 1 served as each subject's daily control, and they used a biomimetic decoder. Block 2 instated a rotation perturbation to the decoder, driving the subject to learn an arbitrary (non-biomimetic) mapping to regain proficient (baseline) cursor control. To characterize population neural activity, we applied factor analysis (FA) to matrices of neural spiking data aligned to the cursor movements. FA extracted latent factors that partitioned neural variability into shared and unique sources. We then determined how both of these sources related to behavioral improvements on the timescale of single trials.While subjects were unable to fully recover behavior relative to baseline performance, their maximum recovery occurred rapidly – on the time scale of trials. Learning and fluctuations in performance were reliability captured by shared population variance. Additionally, we found evidence that unique baseline population variance was predictive of the amount of recovery.This work provides quantitative metrics to assess BMI learning over time. From a clinical perspective, being able to characterize the potential for recovery or assess treatment progress is critical for neurofeedback therapies for neurological disorders ranging from stroke to anxiety.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.22

Topic: E.05. Brain-Machine Interface

Support: NIH R01 NS109552-01
Craig H. Neilsen Foundation 599050

Title: Exploration of Post Stimulation Rebound Excitation Following Electrical Stimulation of the Lumbar Spinal Cord

Authors: *M. CHARDON, A. MAHROUS, M. JOHNSON, J. MILLER, C. J. HECKMAN; Northwestern Univ., Chicago, IL

Abstract: We use sub-dural electrical stimulation of the dorsal surface of the spinal cord to explain the neural mechanisms underpinning spinal cord stimulation (SCS) as a therapeutic tool. Our study has uncovered a new type of response to SCS, a potent post-stimulation rebound excitation (PSRE), directed solely to extensor muscles and controlled by stimulation parameters. Therefore, PSRE has great clinical potential to assist postural movements, such as sit-to-stand transitions. Here we report on work on the Soleus (SOL)/tibialis anterior (TA) flexor-extensor pair as a model for reciprocal inhibition between agonist/antagonist muscle pairs and their proper function is critical for movement coordination.
Stimulation of the SOL/TA circuits (10Hz-40Hz, 500µA, 5s) generated distinct force patterns comprised of 3 phases: an initial transient spike at the start of the stimulus, a profound inhibition during the duration of the stimulus and a long (< 15 s) sustained phase (rebound) after removal of the stimulus in the Sol muscle, as well as a single phase, strong activation during the stimulation period, in the TA muscle. The force responses of TA and SOL are essentially mirror opposites during stimulation most likely due to their reciprocal inhibitory relationship. The rebound force in SOL could illustrate the activation of persistent inward currents (PICs) in spinal motoneurons (MNs) or the interaction among interneurons, allowing strong synaptic activation when the stimulation ceased (rebound). We also previously showed that these patterns could be modulated by electrode location, favoring activation of TA or SOL.
In the acute thoracic transection (at T12) the responses change: transection abolished rebound activity in the SOL muscle but did not alter the interplay between SOL and TA during SCS. Rebound could not be induced even at stimulation intensities 4 times the stimulation used on the intact spinal cord suggesting dependence on a supra spinal mechanism. We explored pharmacology as a means to reintroduce supra-spinal mechanism (e.g. serotonergic modulation from raphe nuclei) which successfully regenerated the PSRE. These results suggest that the excitatory/inhibitory balance between motor pools can be effectively modulated by SCS and that pharmacology can potentially restore PSRE. These results have the potential to enhance the benefits of electrical stimulation in patients with SCI.

Disclosures: M. Chardon: None. **A. Mahrous:** None. **M. Johnson:** None. **J. Miller:** None. **C.J. Heckman:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

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Program #/Poster #: 058.23

Topic: E.05. Brain-Machine Interface

Support: Abbott Neuromodulation – contract number MSN242012

Title: Motor Thalamus Activity as a Readout of Target Engagement for Preclinical Subthalamic Nucleus Deep Brain Stimulation

Authors: *B. COVENTRY[1], J. TREVATHAN[2], K. P. CHENG[3], H.-J. PARK[7], W. LAKE[4], K. LUDWIG[5], E. ROSS[8], A. J. SUMINSKI[6];
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Abstract: Deep brain stimulation (DBS) is a therapeutic intervention where high frequency electrical stimulation is delivered to the subthalamic nucleus (STN) to disrupt pathological activity in neural circuits spanning the cortex, basal ganglia and thalamus. Computational models and experimental evidence demonstrate that the efficacy of STN DBS is due in part to modulation of downstream projections to the motor thalamus which subsequently normalizes thalamocortical information transfer, a putative measure of target engagement in DBS. Unfortunately, these predictions have yet to be fully explored in preclinical models. We describe the development of a DBS model where target engagement is examined via measurement of thalamocortical activity using simultaneous thalamus calcium imaging and cortical electrophysiology. Male Long-Evans rats (n = 14) weighing 200-250g were used in this study. Rats in the lens implant/viral expression cohort were used to develop and validate methodology for calcium imaging in the ventral anterior/ventral lateral thalamus (i.e. motor thalamus, VA/VL). Using stereotaxic technique, 800nL of AAV1.CaMKII.GCaMP6f was injected into VA/VL thalamus at rate of ~100nL/min and a gradient indexed (GRIN) lens or surrogate implanted 200µm above the injection location. Injection location, lens placement and transfection efficiency were verified using ex vivo examination of fixed, sectioned tissue. Rats in the imaging/recording cohort were chronically implanted with a bipolar DBS electrode in the STN, GRIN lens in VA/VL thalamus and microelectrocorticography (µECoG) array over the primary motor cortex (M1). Following recovery from surgery, we measured calcium events in VA/VL neurons and M1 local field potentials (LFP) while rats explored an open field with and without STN DBS. Examination of fixed tissue demonstrated our ability to successfully target injections and implant GRIN lenses in the motor thalamus. Fluorescent beads were readily visible surrounding the implanted lens. GCaMP6f expression was strong in the VA/VL thalamus when delivered with an AAV1 under a CaMKII promotor. Spontaneous VA/VL thalamus calcium events were observed during behavior and in response to STN DBS. Thalamic activity was most robust for therapeutic, high-frequency DBS (i.e. 130Hz) compared to low- frequency stimulation (i.e. 30Hz). Calcium imaging was not impacted by electrical stimulation artifacts. The ability to combine measures of target engagement with translationally relevant biomarkers (M1 LFP) and pharmacological interventions offers the possibility of faster translation of novel stimulation methodologies to clinical practice.

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Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.24

Topic: E.05. Brain-Machine Interface

Support: Corticom: RFA-NS-18-023

Title: A comparison of classification strategies for complex motor tasks using high-density electrocorticography (HD-ECoG) on the sensorimotor cortex (SMC)

Authors: *Z. FREUDENBURG[1], L. R. ENGWEGEN[2], A. SCHIPPERS[1], M. P. BRANCO[1], E. J. AARNOUTSE[1], N. F. RAMSEY[1];
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Abstract: BCIs research has made steady improvements in the complexity and number of motor tasks that can be decoded from brain activity due to the application of more advanced signal recording platforms and decoding algorithms. However, there is still much room for improvement to establish robust and reliable classifies that perform at the high level needed for practical application over long periods of time. One of the main obstacles for progress is the access to large amounts of labeled data from multiple subjects. Here we look at two HD-ECoG data sets from complex motor gestures made with either the hand (4 sign language gestures from 5 subjects, [1]) or the mouth (4 spoken phonemes from 8 subjects, [2]) and apply a range of decoding techniques to determine the strategy that performs the best across subjects and task type. Because both of these data sets have been previously reported to provide a good result with a simple a linear match filter strategy, they are well suited to evaluated the performance of more complex linear and non-linear classifiers. While we test several standard techniques (SVMs, K nearest neighbor, and a feed forward neural network), we were most interested in the performance of EEGnet (a multi-layer CNN designed specifically for EEG and ECoG signal cation [3]) and weather this performance can be improved by pre-training the network on separate rest vs. simple movement tasks before training to classify 4 complex movements. Results showed that a SVM classifier using the High-frequency band (40-150 Hz) amplitude

response performed the best for both tasks and showed a significant improvement over the simple linear match filter scheme. In addition, the EEGnet was seen to perform well despite the fact that it takes the raw time domain ECoG signal as input and needs to learn the best frequency features and the number of trials per class was very small (~30-120) compared to the data sets that multi-layer neural networks often preform well on. Furthermore, pre-training on simple rest vs active data sets was seen to improve the results for two subjects who has very little (<40 trials) and relatively noisy data due to the fact that it was recorded in the operating room during an awake craniotomy. We believe that these results motivate further exploration of multi-layer networks that can learn spectral features and transfer learned patterns from simple motor discrimination tasks to more complex tasks. [1] M. Branco et al., Neuroimage 2017. [2] N. Ramsey et al., Neuroimage 2017. [3] V. Lawhern et al., CoRR 2016.

Disclosures: Z. Freudenburg: None. **L.R. Engwegen:** None. **A. Schippers:** None. **M.P. Branco:** None. **E.J. Aarnoutse:** None. **N.F. Ramsey:** None.

Poster

058. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 058.25

Topic: C.04. Movement Disorders other than Parkinson's Disease

Support: NIH Grant R01-NS081118
NIH Grant F31-NS129241

Title: Prospective investigation of neural pathways underlying tremor suppression with deep brain stimulation in Essential Tremor

Authors: *R. D. BUTLER[1], A. K. BRINDA[1], M. BLUMENFELD[1], D. SULLIVAN[1], M. BRYANTS[2], L. SCHROCK[2], S. E. COOPER[2], J. MATSUMOTO[2], M. D. JOHNSON[1]; [1]Dept. of Biomed. Engin., [2]Dept. of Neurol., Univ. of Minnesota, Minneapolis, MN

Abstract: Background: While deep brain stimulation (DBS) is an effective treatment for medication-refractory Essential Tremor (ET), controversy exists over the optimal target for tremor suppression. Currently, therapeutic efficacy of thalamic DBS for ET has been attributed to activation of the ventral intermediate nucleus of thalamus (VIM), cerebello-thalamic tract (CTT), and the posterior subthalamic area. To determine which pathways are involved in tremor control by DBS, patient-specific computational modeling can be used to investigate neural fibers activated under various stimulation settings. In this study, we leveraged an ongoing prospective clinical study in human patients diagnosed with ET and implanted with directional DBS leads to determine how tremor control relates to activation of parcellations within the CTT and other pathways around VIM. Objective: To relate detailed, patient-specific neural pathway activation of VIM-DBS to clinical therapeutic outcomes. Methods: Patients with directional DBS leads were recruited into a prospective clinical trial investigating programming DBS systems for ET

(NCT03984643). Subject-specific finite element and pathway activation models were built from pre-operative ultra-high field (7T) MRI and post-operative CT imaging sets for 10 directional DBS leads from 6 patients. Pathways included the subdivided CTT, cortico-thalamo-cortical pathway, zona incerta, medial lemniscus, internal capsule, thalamic fasciculus, and lenticular fasciculus. During the baseline clinical trial visit, patients underwent monopolar review (10 electrode configurations), during which the Essential Tremor Rating Assessment Scale (TETRAS) was assessed at the current amplitude corresponding to the initial and maximum therapeutic effects of each electrode configuration. Mixed-effect models were utilized to determine which pathways significantly contributed to therapeutic outcomes based on TETRAS scores of the contralateral upper limb and upper extremity quantitative sensor-based measurements. Results: The lateral portion of the CTT had the highest activation at DBS settings resulting in maximum tremor suppression. Activation of the subdivided CTT pathways (i.e. deep cerebellar nuclei to the internal and external aspects of VIM) were significantly associated with decreased contralateral tremor scores (p < 0.05). Conclusion: This study provides more specific context on the pathways associated with tremor suppression in ET-DBS using high-field imaging. With directional DBS leads, these pathways can be targeted more specifically to result in improved therapeutic outcomes.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.01

Topic: E.05. Brain-Machine Interface

Title: Ability: feasibility and safety study in sheep of a fully implantable intracortical brain-computer interface

Authors: *F. M FERREIRA[1], F. BURDIN[1], M. ANDERSEN[1], J. BARROS[1], T. BERTOLOTE[1], H. FRANÇA[1], A. HERBERT[1], D. IBANEZ SORIA[1], S. PERNECKER[1], M. CORNIOLA[2,3,4,5], S. MOMJIAN[6,7], S. MONTAMAT[1], J. B. ZIMMERMANN[1];
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Abstract: Intracortical brain-computer interfaces have been used to restore communication and movement for paralyzed patients, currently using percutaneous connectors. Heat and bandwidth

limitations posed major challenges for previously reported fully implantable devices. Here, we present the first in vivo recordings with the ABILITY System, a fully implantable device and its external components, showing high-bandwidth neural data transmission at acceptable temperature levels. This study evaluated implantability and explantability of the implant, safety, performance, and usability of the system. For this, three female sheep were implanted, and functional recordings were performed three times a week for up to 13 weeks. To determine how the explantation procedure is affected by implantation duration, the implant was explanted either at week 5 (sheep 1) or 13 (remaining sheep). Overall, there were no differences between these two time points, as there was virtually no tissue adhesion to the implant, suggesting that the selected materials are adequate. Moreover, we observed complete healing after eight weeks (sheep 1). For all sheep, the study was terminated at week 13. At this time point, there were some macroscopic changes in the skin but not in the skull surrounding the implanted area. Representative areas of tissue were collected for histologically assessment of inflammation and pathological foreign body response. The duration of this study allowed us to collect data on neuronal signal stability over time. We assessed the impedance of the electrodes, neuronal data stability, and the heating of the device and of the surrounding tissue. The impedances of 90% of the electrodes remained in the 20-800kΩ. We were able to record intracortical neuronal data from all 128 channels, and in two of the sheep we recorded action potentials from at least two-thirds of the electrodes. The maximum temperature recorded by the internal temperature sensors of the implant was 41.5 °C, whereas external temperature sensors placed on areas of interest on top of the implant registered a median increase (median absolute deviation) of 1.0 (0.5) °C, across all animals and recording days. In summary, we report the first use of the ABILITY system in vivo, in sheep. From the biosafety perspective we report no peri or post operative complications, and no signs of acute or systemic infection or toxicity. The use of the system in sheep was straight forward, and the external components were deemed user-friendly. Importantly, we observed good and stable neuronal signal overtime and the heating of the system seems to fall within acceptable levels in the conditions tested.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.02

Topic: E.05. Brain-Machine Interface

Title: Ability: a fully implantable device to acquire intracortical neural signals for clinical brain-computer interface

Authors: *H. FRANÇA, M. ANDERSEN, J. BARROS, T. BERTOLOTE, F. BURDIN, F. M FERREIRA, A. HERBERT, D. IBÁÑEZ, S. PERNECKER, S. MONTAMAT, J. B ZIMMERMANN;
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Abstract: Research on intracortical brain-computer interfaces has been mainly driven by the adoption of Utah microelectrode arrays, as so far more than 30 participants have been implanted with them. In the current state of the art, a percutaneous connector gives access to these electrodes, creating risks of infection. The connector is fragile and can be easily damaged during system setup procedures. The external hardware is bulky, and it is designed for a research lab environment and not for home use. These issues are major obstacles to the further adoption of intracranial electrode technology both in clinical research as well as for independent assistive use by patients. On the other hand, wireless recording of high-bandwidth neural data from 100+ electrodes is challenging in terms of wireless power transfer, excess heat dissipation, data transfer method, hermetic encapsulation, feedthroughs, and physical volume of the device. We have designed a fully implantable device that acquires intracortical neural signals from Utah arrays with 128 channels, sampled at up to 30 kS/s with a resolution of 12 bit/sample. It is powered wirelessly, using inductive power transfer, obviating the need for an implantable battery as well as its associated risks. It uses a patented pending optical link technology for transferring the neural data at up to 50 Mbit/s, employing vertical-cavity surface-emitting lasers. We have adopted novel ceramic-metallic feedthroughs to connect the 128 electrodes and additional reference wires to the hermetically sealed electronics. The small size of the implant's housing ($25\times35\times4.5$ mm^3 for the rigid part) eliminates the need for bone flattening in most patients. The electronic components were judiciously distributed inside of the implant's housing to optimize the uniformity of the heat dissipation on its surface. The ABILITY System consists of the implant and a wearable, which has three parts: a headpiece, an earpiece, and a USB-Adapter. A headpiece is held in place over the implant by magnets. It delivers power to the implant and receives the neural data from it through the optical link. The earpiece and the USB-Adapter provide power isolation and regulation, as well as bidirectional communication between the implant and a PC. Our implant is compatible with multiple types of brain electrodes, namely high-density ECoG arrays, and manufacturing processes are being set-up for enabling their attachment to it. Additionally, design iterations are being undertaken entailing a further minimization of the power consumption, as well as the enhancement and validation of the safety and the reliability of the device, to make it suitable for long-term continuous human use.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

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Program #/Poster #: 059.03

Topic: E.05. Brain-Machine Interface

Support: NIH Grant 5UH3NS107709-03

Title: Beta burst modulation in subthalamic nucleus effects on gait impairment and freezing of gait

Authors: *J. MELBOURNE, H. BRONTE-STEWART, K. WILKINS;
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Abstract: Background: Gait impairment and freezing of gait (GI&FOG) affects 75% of people with Parkinson's disease (PD) and is a primary cause of falls and morbidity. Characterized by shortened steps and arrhythmic gait leading to cessations in stepping, GI&FOG are associated with the beta band oscillopathy in the subthalamic nucleus (STN). There is limited data published using neural and kinematic data to demonstrate the relationship between DBS intensity, behavioral kinematic variables, and beta band (13-30Hz) oscillopathy. This study explores the dose-dependent relationship between DBS intensity, beta oscillopathy, and quantitative kinematic gait variables. Methods: 7 participants (2 female, 5 male) were implanted with STN DBS using either the Medtronic Summit RC+S or the Medtronic Activa PC+S, both of which have neural sensing capabilities. Subjects completed at least 4 rounds each of a harnessed stepping in place task on force plates at randomized intensities. Subjects were blinded to DBS settings. Local field potentials were recorded using sensing-enabled implanted neurostimulators and used to calculate beta burst duration and power. Percent time freezing, gait arrhythmicity, and stride time were calculated from force plate data. Results: There was a direct relationship between DBS intensity and attenuation of beta burst duration and power. DBS improved gait metrics associated with freezing. Conclusion: This is the first evaluation of the dose dependent response of beta oscillopathy and quantitative gait metrics to DBS intensity in freely moving PD patients. The implications of this study validate the use of beta oscillopathy as a control for closed-loop DBS.

Disclosures: J. Melbourne: None. **H. Bronte-Stewart:** 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.; Medtronic. F. Consulting Fees (e.g., advisory boards); Ceregate. **K. Wilkins:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

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Topic: E.05. Brain-Machine Interface

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Michael J. Fox Foundation (9605)
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Title: Kinematic Closed Loop Deep Brain Stimulation for Freezing of Gait in Parkinson's Disease

Authors: G. C. L. ORTHLIEB[1], Y. KEHNEMOUYI[1], H. DORRIS[1], J. O'DAY[1], K. B. WILKINS[1], S. ADITHAM[1], M. N. PETRUCCI[1], E. LAMBERT[1], J. MELBOURNE[1], C. DIEP[1], S. HOFFMAN[1], J. PARKER[1], J. A. HERRON[2], *H. BRONTE-STEWART[1];
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Abstract: Gait impairment and/or Freezing of gait (GI&FOG) in Parkinson's disease (PD) often results in falls, loss of independence and significant morbidity. GI&FOG have a variable response to open loop high frequency STN deep brain stimulation (DBS) and in some instances a better response to DBS at lower frequencies. Gait arrhythmicity is a reliable marker of GI&FOG and may be a relevant kinematic controller for kinematic closed loop or adaptive DBS (KaDBS). The goal of this study is to assess the safety and tolerability of real-time adaptation of stimulation intensity or frequency in response to gait arrhythmicity or a FOG detection algorithm to minimize GI&FOG. People with PD and GI&FOG underwent bilateral STN DBS lead and the Summit™ RC+S neurostimulator implantation. During a calibration period, safe and effective neurostimulation intensity limits and ramp rates were determined, along with the arrhythmicity threshold or a FOG probability model that would be used in a participant specific single threshold control policy algorithm. Participants performed either a harnessed stepping-in-place (SIP) task on dual force plates or a free walking turning and barrier course (TBC) on closed-loop, open-loop continuous, or random intermittent open-loop DBS. Two control policy algorithms were used: one increased or decreased DBS intensity and the other switched instantaneously between 140Hz and 60 Hz DBS, based on continuous measurement of gait arrhythmicity or a FOG probability model. We present preliminary results for testing KaDBS for safety, tolerability, and preliminary outcomes. These results demonstrate the feasibility of KaDBS algorithms targeting GI&FOG in PD.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

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Robert and Ruth Halperin Foundation
John A. Blume Foundation
John E Cahill Family Foundation

Title: Calibration of closed loop deep brain stimulation in Parkinson's disease

Authors: J. A. MELBOURNE[1], ***K. B. WILKINS**[1], E. F. LAMBERT[1], M. N. PETRUCCI[1], Y. M. KEHNEMOUYI[1], G. C. L. ORTHLIEB[1], H. DORRIS[1], C. DIEP[1], S. ADITHAM[1], J. A. HERRON[2], H. M. BRONTE-STEWART[1];
[1]Stanford Univ., Stanford Univ., Palo Alto, CA; [2]Univ. of Washington, Univ. of Washington, Seattle, WA

Abstract: The growing availability of both research and commercial sensing neurostimulators has enabled the implementation of a variety of closed loop paradigms for various diseases. Although these closed loop paradigms offer promise for potential more effective therapeutic treatment of different symptoms, it also adds to the complexity required for programming and calibrating patients. Implementation of closed loop requires determination of subject-specific control policies, threshold tuning, safe and tolerable ramp rates, etc. We present our methodology for quantitatively selecting aDBS parameters for a case example of treating gait impairment and/or freezing of gait (GI&FOG) in PD, but that can be generalized to any motor symptom. Initially, the upper limits of DBS intensity are determined in order to not induce potential side effects. The range within which DBS intensity can adapt is then determined through a titration experimental protocol in which the participant performs either a stepping-in-place (SIP) task or free walking turning and barrier course (TBC) at different levels of stimulation intensity while recording synchronized neural and kinetic and/or kinematic data. A therapeutic window is then quantitatively determined from this data where I_{min} (i.e., the lower bound of stimulation intensity) represents that minimum amount of stimulation where a therapeutic level of performance is reached. Different rates of increasing/decreasing stimulation intensity (ramp rates) within this window were then established while the participants were seated. Relevant neural controllers were derived from the titration data and were then incorporated into single threshold neural control policy algorithm. The participant specific classifier and control policy algorithms were then tested during short runs of aDBS and optimized prior to the aDBS gait experiments. This quantitative and iterative approach allowed careful calibration of aDBS in a participant-specific manner and could be used to inform optimization of aDBS programming in the future.

Disclosures: **J.A. Melbourne:** None. **K.B. Wilkins:** None. **E.F. Lambert:** None. **M.N. Petrucci:** None. **Y.M. Kehnemouyi:** None. **G.C.L. Orthlieb:** None. **H. Dorris:** None. **C. Diep:** None. **S. Aditham:** None. **J.A. Herron:** None. **H.M. Bronte-Stewart:** F. Consulting Fees (e.g., advisory boards); Medtronic PLC.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.06

Topic: E.05. Brain-Machine Interface

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Michael J. Fox Foundation (9605)
Robert and Ruth Halperin Foundation
John A. Blume Foundation
John E Cahill Family Foundation

Title: Closed Loop Deep Brain Stimulation Using Beta Bursts for Freezing of Gait in Parkinson's Disease

Authors: *S. ADITHAM[1], M. N. PETRUCCI[1], K. B. WILKINS[1], E. LAMBERT[1], J. MELBOURNE[1], S. L. HOFFMAN[1], Y. M. KEHNEMOUYI[1], G. C. L. ORTHLIEB[1], J. E. PARKER[1], H. DORRIS[1], C. DIEP[1], R. W. ANDERSON[1], J. A. HERRON[2], H. M. BRONTE-STEWART[1];
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Abstract: Freezing of gait (FOG) in Parkinson's disease is a debilitating symptom characterized by brief cessation of forward progression of the feet despite the intention to walk. Prolonged bursts of beta band (13-30 Hz) synchrony in the subthalamic nucleus (STN) interfere with normal sensorimotor processing and serve as a biomarker of Parkinsonian pathology. Deep brain stimulation (DBS) can shorten abnormal bursts of beta band synchrony and improve FOG. However, neural closed-loop DBS (cl-DBS) paradigms to reduce beta burst duration in real-time have not yet been explored to reduce FOG. The objective of the current study is to assess the safety and tolerability of using beta burst durations to adapt stimulation intensity in real-time to mitigate FOG in PD. In this study, eight patient research partners with PD who had demonstrated gait impairment and/or FOG were implanted with the Summit™ RC+S neurostimulator with leads placed in the STN. Individuals initially took part in a calibration period to determine stimulation intensity limits, suitable ramp rates, and the neural parameters that would be used for the closed-loop algorithms. The individuals then performed either a harnessed stepping-in-place (SIP) task or a free walking turning and barrier course (TBC) on closed-loop, open-loop continuous, or random intermittent open-loop DBS. Stimulation frequency was set to either 140 or 60 Hz. We present preliminary results for the safety and tolerability of intensity-adapting neural closed-loop DBS at different frequencies during a harnessed stepping-in-place task and free walking in a turning and barrier course. These initial results point toward the feasibility of using beta burst duration as a control policy variable in neural closed-loop DBS algorithms targeted toward improving gait in Parkinson's disease. Initial findings also suggest the

differential effect of frequency stimulation on symptomology, with 60 Hz stimulation resulting in the brief return of tremor symptoms in three tremor-dominant individuals.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

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Program #/Poster #: 059.07

Topic: E.05. Brain-Machine Interface

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Michael J. Fox Foundation (9605)
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John A. Blume Foundation
John E Cahill Family Foundation

Title: Subthalamic Neural Correlations of an Upper Extremity Fine Motor Task in People with Parkinson's

Authors: *E. F. LAMBERT[1], K. B. WILKINS[1], K. HAN[1], S. ADITHAM[1], H. BRONTE-STEWART[2];
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Abstract: Background: The advent of sensing neurostimulators, specifically the Medtronic Activa © PC+S, allowed measurement of local field potential (LFP) recordings in the subthalamic nuclei (STN) of freely moving people with Parkinson's. In combination with a quantitative digitography task developed in the lab, this technology enabled a longitudinal study of a fine motor task with simultaneous neural recordings.
Objective: Determine the subthalamic neural signatures associated with quantitative metrics collected during a unilateral fine motor upper extremity task.
Methods: 12 participants implanted with the investigational Medtronic Activa © PC+S neurostimulator performed 30 second trials of a repetitive alternating finger tapping task on each hand. Participants were off medication and off stimulation. Neural LFP recordings from both contralateral and ipsilateral STNs were simultaneously recorded. The LFP recordings were processed and analyzed using MatLab to determine power, burst duration, and coherence within

the beta band (13-30 Hz). The finger tapping data was processed using a customized algorithm created to determine the states of finger motion and allow for calculation of kinematic metrics including the correlation of variance of the inter-strike interval (ISI-CV).
Results: There was no correlation found between beta burst duration or beta power and the arrhythmicity of finger tapping. There was a correlation found between beta coherence between STNs and arrhythmicity of finger tapping. As beta coherence increases, arrhythmicity of finger tapping (ISI-CV) gets worse.
Conclusions: Higher synchrony of STN beta was correlated with worsened performance on an upper extremity fine motor task. Given the lack of known direct connections between STN, this synchrony may arise via multi-synaptic connections via the cortex, cerebellum, or other regions in the brain.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.08

Topic: E.05. Brain-Machine Interface

Title: Evolution of BCI performance over three years of recordings with intracortical microelectrode arrays in a complete locked-in syndrome patient

Authors: *A. TONIN[1,2], D. IBAÑEZ-SORIA[1], J. RAMOS DA CRUZ[1], K. LEE[1], A. ESPINOSA[1], N. F. RAMSEY[3], J. B. ZIMMERMANN[1];
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Abstract: In March 2019, a patient in complete locked-in state due to amyotrophic lateral sclerosis was implanted with two 64-microelectrodes arrays, one in the left dominant primary and another in the supplementary motor cortex [1]. The primary goal of the study was to implement a brain-computer interface (BCI) system to restore communication. The patient is functionally blind, therefore BCI experiments have been conducted in a complete auditory mode by mapping the normalized neural modulation activity to a sound. The patient was instructed to modulate the sound to achieve a low or a high frequency target tone. These neurofeedback sessions have been structured in 20 randomized trials and were used as training for the patient and to fine tune the settings of the classification of the neural activity. If the patient was achieving an accuracy of at least 75% a similar trial structure was used in a speller paradigm that allowed the patient to communicate by modulating his neural activity.

More than three years after the implantation (1140 days) we have recorded 2628 neurofeedback sessions over 364 days and 602 speller sessions in 243 days. Most of the sessions were supervised by experimenters at bed side or remotely connected, while a significant minority (653 neurofeedback and 134 speller sessions) were run by patient's relatives. The performance did not differ with the presence or absence of experimenters. For over two years results were consistently above chance, despite a change in the acquisition software, in the selected neural feature, and in the classification method: from spike rates classified with manual thresholding to spike band power and logistic regression classifier. After 750 days the performance started to decline for reasons still not completely clear: in the same period a decrease in signal quality was observed, but a concurrent degradation in cognitive capabilities of the patient cannot be excluded.
Consistency was observed in modulation of activity on individual electrodes, both in terms of which electrodes showed differential activity between conditions as well as which condition was the preferred one per electrode. These results suggest that the patient has been using the same strategy to modulate neural activity, and that little plasticity occurred.
[1] Chaudhary U, Vlachos I, Zimmermann JB et al. Spelling interface using intracortical signals in a completely locked-in patient enabled via auditory neurofeedback training. Nat Commun 13, 1236 (2022)

Disclosures: **A. Tonin:** None. **D. Ibañez-Soria:** None. **J. Ramos da Cruz:** None. **K. Lee:** None. **A. Espinosa:** None. **N.F. Ramsey:** None. **J.B. Zimmermann:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.09

Topic: E.05. Brain-Machine Interface

Title: Intracortical SSVEPs and Auditory Oddball for BCI control in a Completely Locked In-Patient

Authors: *J. RAMOS DA CRUZ[1], K. LEE[1], A. TONIN[1], D. IBÁÑEZ-SORIA[1], A. ESPINOSA[1], N. F. RAMSEY[2], J. B. ZIMMERMANN[1];
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Abstract: Amyotrophic lateral sclerosis (ALS) patients may progress into completely locked-in state (CLIS), eliminating neuromotor ability to communicate. Still, we have shown that brain-computer interface (BCI) communication is possible in an CLIS patient implanted with microelectrode arrays. Through neurofeedback, he learned to modulate neuronal activity to control BCI applications. However, the patient's ability to modulate these signals has strongly deteriorated. Here, we explored steady-state visual evoked potentials (SSVEP) and auditory

oddball paradigms as alternative BCI paradigms. The patient was implanted with two 64 microelectrode arrays in the dominant left motor cortex. Here, we also recorded data with electroencephalogram (EEG). First, we conducted feasibility experiments to evaluate if SSVEP and auditory oddball evoked-related potentials (ERPs) could be recorded from EEG and microelectrodes. For the SSVEP, we used two flickering LEDs mounted on goggles, one on each side with different frequencies, to present visual stimuli to the patient's closed eyes. For the auditory oddball, we used headphones to present sequences of repetitive sounds infrequently interrupted by deviant stimuli to the patient. We asked him to count the number of deviant stimuli. In a second set of experiments, we evaluated whether the patient could use the two paradigms for BCI control. For the SSVEP, each of the two LEDs was flickering at a different frequency. On each trial, we asked the patient to focus on one of the light sources. For the auditory oddball, the left (right) headphone was playing a stream of repeating words containing the word "No" ("Yes") interrupted by a deviant "Nein" ("Ja"). On each trial, we asked the patient to focus on one of the streams. The feasibility experiments confirmed that SSVEP and auditory oddball ERPs can be recorded by EEG and microelectrodes. Offline classification of each session of the BCI control experiments suggested that in both paradigms the attended stimuli could be decoded from the neuronal data, both motor cortex microelectrodes and EEG (accuracy>80%). However, the best hyperparameters used for classification highly varied between sessions and classifiers trained on one session could not correctly classify trials in a subsequent session. Our results suggest that either the patient cannot modulate the signals with selective attention or that due to excess between-session variability, the paradigms could not be used for BCI control in our case study.

Disclosures: J. Ramos da Cruz: None. **K. Lee:** None. **A. Tonin:** None. **D. Ibáñez-Soria:** None. **A. Espinosa:** None. **N.F. Ramsey:** None. **J.B. Zimmermann:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.10

Topic: E.05. Brain-Machine Interface

Title: Evolution of performance and signal quality during the long-term use of an intracortical BCI

Authors: *D. IBANEZ[1], A. TONIN[1,2], J. RAMOS DA CRUZ[1], K. LEE[1], A. ESPINOSA[1], N. F. RAMSEY[3], J. B. ZIMMERMANN[1];
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Abstract: We recently demonstrated the use of an intracortical brain-computer interface (BCI) by a person in completely locked-in state (CLIS) for communication [1]. The patient was

implanted in March 2019 with two 64-microelectrode arrays (MEAs) in the dominant left motor cortex. The first array was inserted into the hand area of the primary motor cortex (M1), and the second array into the supplementary motor area (SMA). BCIs using intracortical MEAs have shown promising potential for assisting communication and movement, but data on their chronic performance and eventual failure is published only for few study participants. To enhance the common knowledge we have profiled the system for over three years with more than 300 days of experimental sessions studying: 1) the MEA impedances, 2) the signal quality, and 3) the performance in a binary BCI task.
The impedances decreased over time in both arrays, following an exponential decay. This decline was more prominent on average in the M1 array, with all electrodes reporting values below the manufacturer's recommended range (100-800KΩ) around 800 days after implantation. On the contrary, as of today in the SMA array, more than 75% of the channels are still within the range. The signal quality measured in terms of power in the spike band and presence of artifacts deteriorated over time with a high presence of power line noise interferences starting around 700 days after implantation. As the power line noise interference increased, the correlation across electrodes consistently incremented. The spike rate, the neural feature used for communication, also decreased gradually, from 65% of the SMA channels showing spiking activity 250 days after implantation to 50% after 500 days, and none or very few channels after 1,000 days. Finally, the BCI performance also declined over time and was highly correlated with the progressive loss in signal quality. This work contributes to a better understanding of long-term MEA use and chronic implantable BCI devices in humans.
Our results demonstrate that signal quality and environmental control are fundamental to ensure a lasting performance in BCI systems. Their exhaustive and periodic profiling has proved to be essential in order to carry out their translation to home use.
[1] Chaudhary, U., Vlachos, I., Zimmermann, J.B. *et al.* Spelling interface using intracortical signals in a completely locked-in patient enabled via auditory neurofeedback training. *Nat Commun* **13,** 1236 (2022). https://doi.org/10.1038/s41467-022-28859-8

Disclosures: **D. Ibanez:** None. **A. Tonin:** None. **J. Ramos da Cruz:** None. **K. Lee:** None. **A. Espinosa:** None. **N.F. Ramsey:** None. **J.B. Zimmermann:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.11

Topic: E.05. Brain-Machine Interface

Support: Graphene Flagship Core 3 WP5 881603

Title: Mapping the continuum of spreading depolarisation induced haemodynamic responses in the post-stroke brain using graphene micro-transistor arrays and cerebral blood flow imaging

Authors: *S. M. FLAHERTY[1], A. ELADLY[1], E. MASVIDAL-CODINA[2], E. FERNANDEZ[3], X. ILLA[4], E. PRATS-ALFONSO[4], K. KOSTARELOS[1], S. M. ALLAN[1], J. A. GARRIDO[2], A. GUIMERÀ-BRUNET[4], R. C. WYKES[5];
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Abstract: Soon after ischaemic stroke, spontaneous waves of depolarisation originating from metabolically compromised, but not yet irreversibly damaged ischemic penumbra, termed spreading depolarisations (SD) are observed. SD is the largest disruption of brain homeostasis possible in living neural tissue. SDs play a critical role in reducing the viability of penumbra tissue in ischemic stroke, and SD frequency has been shown to correlate with infarct size. In normoxic brain the haemodynamic response to an SD is a profound vasodilation, a mechanism to increase supply of oxidative substrates required for energy-dependent activation of membrane pumps to repolarise neurones and restore ionic balance. However, in the penumbral region surrounding the lesion core an inverse haemodynamic response can occur; a prolonged hypoperfusion due to severe arteriolar constriction coupled to the SD. This spreading ischemia, not only delays energy-dependent recovery from SD, but results in a vicious cycle where the perfusion deficit maintains the depolarisation, and the continued depolarisation results in sustained release of vasoconstrictors, promoting tissue damage. We are applying novel arrays of graphene micro-transistors gSGFETs, capable of high-fidelity DC-coupled recordings to map SD propagation and characterise SD waveform with distance from the ischemic core. Due to their transparency and high channel count (16-32 channels), it is possible to correlate SD waveform with the regional haemodynamic response using laser speckle contrast imaging (LSCI). We can map the continuum of response from spreading ischemia to hyperaemia to spreading oligemia and relate this to SD waveform. To induce ischaemia, the photothrombotic and the distal middle cerebral artery ferric chloride occlusion models were employed in mice under isoflurane anaesthesia. Simultaneous DC/ LFP in vivo electrophysiology using gSGFETs and LSCI was used to map the post-stroke brain. We noted three regional cerebral blood flow (CBF) events, vasoconstriction, vasoconstriction and hyperaemia, and hyperaemia. Principle component analysis identified 3 SD waveforms that could be separated by their duration and amplitude. Each waveform was predominately recorded within one of the three identified haemodynamic responses; i.e the largest SDs were detected in areas with a vasoconstrictive response, whereas the smallest SDs were recorded from areas of vasodilation. Therefore, the waveform characteristics of the SD could predict the haemodynamic response. Application of gSGFET arrays to preclinical stroke research will aid investigations into the impact of SDs to the post-stroke brain.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.12

Topic: E.05. Brain-Machine Interface

Support: NIH 5R01NS116464
NIH T32 EB029365

Title: The eDura: a monolithic functional surface array for neural recording from non-human primates

Authors: *S. MONTALVO VARGO[1], T. BELLOIR[2], I. KIMUKIN[1], D. J. GRIGGS[2], Z. AHMED[1], A. YAZDAN-SHAHMORAD[2], M. CHAMANZAR[1];
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Abstract: Chronic monitoring of the brain's activity for studying neurological function and designing effective therapeutics is highly desired. However, current micro-electrocorticographic (μECoG) arrays for large animal models like non-human primates (NHP) do not match the lifetime and stability of passive devices like the artificial dura, an elastomer-based device used as a chronic optical port to the cortex. The eDura is a novel and functional version of the artificial dura, with a transparent substrate and integrated electrophysiological electrodes that can provide a chronic interface for high density neural recording of brain activity in NHPs and rodents. The monolithic design of the eDura obviates the need to replace the artificial dura when recording the brain, thus reducing the chance of tissue growth and infection.
The eDura is fabricated using thin-film microfabrication techniques to monolithically integrate a high density electrode array with high spatial resolution into a flexible and transparent substrate i.e. Polydimethylsiloxane (PDMS). PDMS conforms to the neural tissue contours and can maintain the cranial pressure. The eDura covers a large area of the NHP cortex ($300mm^2$) to allow monitoring of multiple regions of the brain, simultaneously. Our initial design includes 32 and 64-electrode devices but our lithographic technique enables increasing the number of channels to 512 in each single thin-film layer. The eDura is transparent, which allows for a hybrid modality with external optical stimulation or imaging to augment the electrical recording and stimulation capability of the eDura. With 20-40 μm diameter electrodes, electrochemical impedance spectroscopy (EIS) measurements confirmed an average impedance of < 1MΩ at 1 kHz for Platinum electrodes, consistent with the previously reported results of the same size electrodes. Additionally, emulated neural signals were recorded to characterize the performance of the eDura system to confirm an acceptable signal to noise ratio needed for electrophysiological recording of multi-units and local field potentials from macaque brains. The demonstrated transparent eDura can be used for chronic high resolution recording in NHPs and can be combined with optical modalities.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.13

Topic: E.05. Brain-Machine Interface

Title: The Brain Interchange system - chronic neural recording and stimulation in preclinical research

Authors: *C. A. GKOGKIDIS[1], M. MOSER[1], Y. TONG[2], J. WESSOLLECK[2], L. MIGUEL TELEGA[2], M. SCHUETTLER[1], M. DÖBRÖSSY[2], V. A. COENEN[2];
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Abstract: The increasing employment of bi-directional neural implants in preclinical and translational medical research is growing more impactful in today's neuroscientific landscape. Fully implantable devices ideally provide high technical flexibility, long-term implantation suitability and meet the complex requirements for potential medical applications. The system employed here meets these requirements: recording configuration choice, open- and closed-loop stimulation functionality, flexible electrode type choice, inherent safety features and biocompatible materials.
The utilized Brain Interchange system was employed in several preclinical chronic implantation studies in sheep. ECoG and DBS electrode implantations were utilized, and target brain areas comprised somatosensory, auditory, and prefrontal cortices as well as deep brain structures. Different sensory stimulus types (tactile, electric, acoustic) were used to elicit neural activity modulations in the respective cortices, to prove the recording capabilities of the chronically implanted system. In addition, several electrical brain stimulation paradigms were applied to underpin the flexibility of the system's stimulation functionality. All conducted implantations and experiments were approved by the Animal Ethics committee of the Regierungspraesidium Freiburg and followed the EU-directive 2010/63/EU.
The outcomes demonstrate the system's capability to record somatosensory-related and auditory-related neural activity from the respective sheep cortices on a long-term scale in both the time and frequency domain. Peripheral electrical stimulation under anesthesia and tactile stimulation during wakefulness modulated somatosensory activity, acoustic stimulation elicited clear auditory evoked potentials and recording from prefrontal and deep brain structures showed good consistency over time. The different stimulation paradigms (i.e., single pulse, high-frequency, and DBS) show the system's potential for highly flexible electrical brain stimulation sequences. The data presented demonstrate that the fully implantable Brain Interchange system is suitable to record neural activity over an extended period using different electrode types and targeting different brain areas. These outcomes show that the system meets the requirements for highly flexible recording and stimulation functionality for neural interfaces from both a technical and neurobiological perspective. The chronic implantations in these preclinical studies in sheep show

are the cornerstone for the next step: the transition to human patients in investigational device exemption studies.

Disclosures: **C.A. Gkogkidis:** A. Employment/Salary (full or part-time):; CorTec GmbH. **M. Moser:** A. Employment/Salary (full or part-time):; CorTec GmbH. **Y. Tong:** None. **J. Wessolleck:** None. **L. Miguel Telega:** None. **M. Schuettler:** A. Employment/Salary (full or part-time):; CorTec GmbH. **M. Döbrössy:** None. **V.A. Coenen:** F. Consulting Fees (e.g., advisory boards); CorTec GmbH.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

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Program #/Poster #: 059.14

Topic: E.05. Brain-Machine Interface

Support: NSF award 1533589
NEI award R01EY015545
NIH/BRAIN UG3NS107688
DARPA Contract # HR001120C0120

Title: Scanning electron microscopy data of ten intracortical microelectrode arrays, previously implanted in three tetraplegic humans for recording and stimulation of cortical networks

Authors: L. RIETH[1], D. A. BJANES[2], S. KELLIS[5], B. BAKER[6], T. AFLALO[3], L. BASHFORD[4], S. CHIVUKULA[2], M. S. FIFER[7], L. OSBORN[8], B. CHRISTIE[9], B. A. WESTER[10], P. A. CELNIK[12], N. E. CRONE[13], W. ANDERSON[14], K. PEJSA[2], N. POURATIAN[15], B. LEE[16], C. LU[2], F. TENORE[11], R. A. ANDERSEN[17];
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Abstract: Long-term stability of the electrode interface with cortical tissue is a fundamental requirement for viable brain-machine interfaces (BMIs) as therapeutic devices. These BMI devices hold significant promise to accomplish a variety of clinical outcomes, by capturing neural activity and using signal processing to decode an extraordinary amount of detailed information: motor planning and intent, high-level cognitive goals, speech and language, and

dysregulated neural activity. Furthermore, they can inject information into cortical networks via electrical stimulation, creating novel sensory percepts, visual stimuli and stabilizing dysregulated neural networks. The purpose of this work is to understand the relationship between the physical state of the electrode and its ability to record and stimulate cortical tissue. Using scanning electron microscopy (SEM), we were able observe physical changes in the electrode metallization and insulation after long-term implantation in the human cortex. We examined ten arrays (NeuroPort, Blackrock Microsystems, Salt Lake City, UT), seven with platinum (Pt) electrode tips and three with sputtered-iridium oxide (SIROF) tips. These arrays were implanted across three human participants with tetraplegia. Two participants were implanted in anterior intraparietal area (AIP) and Brodmann's area 5 (BA5) for a duration of 5 yrs, 5 months, 10 days and 5 yrs, 9 months, 30 days, respectively. One participant was implanted in primary motor (M1) and sensory (S1 area 1) cortices, bilaterally, for 2 years, 7 months, 13 days. Three different clinical sites were used to perform the implant and explant surgeries (Caltech - UCLA/USC and Johns Hopkins).An SEM was used to image 880 electrodes from ten microelectrode arrays. We scored each electrode from 0 (high quality) to 5 (poor quality) for the condition of the Pt or SIROF tip metal and the Parylene insulation on the electrode shaft. We found metallization quality often predicted electrode impedance (as measured in vivo prior to explant). Longitudinal impedance and signal to noise-ratio data were collected for each shank. The charge delivered through each stimulation electrode was also calculated. Tip metal damage was very frequently associated with erosion of the silicon shank beneath it, suggesting a mechanism for the process.These findings begin to quantify the relationship between the physical condition of the microelectrodes and their capacity to record and stimulate. These data are especially important as multi-year clinical trials of BMIs are becoming more common and could lead to improved manufacturing or novel electrode designs to improve long-term performance of BMIs.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.15

Topic: E.05. Brain-Machine Interface

Support: GrapheneCore3 WP5 Grant agreement ID: 881603

Title: Mechanically-flexible, graphene-based, microelectrodes for simultaneous recording and electrical stimulation of deep brain microstructures: an acute in vivo study

Authors: *A. ELADLY[1], R. WYKES[1], N. RIA[2], E. MASVIDAL-CODINA[2], K. KOSTARELOS[1], X. IIIA[3], A. GUIMERA-BRUNET[3], J. GARRIDO[2];
[1]Univ. of Manchester, Manchester, United Kingdom; [2]Catalan Inst. of Nanoscience and Nanotechnology, Barcelona, Spain; [3]Inst. de Microelectrònica de Barcelona, IMB-CNM, Madrid, Spain

Abstract: Parkinson's disease (PD) is a neurological motor disorder that negatively impacts the quality of life of its patients. Deep brain stimulation (DBS) is a well-established therapy used to alleviate the symptoms of PD. However, standard DBS systems consist of stimulating electrodes also known in the field as 'leads' that are mechanically rigid since metal is used in their construction. The rigid nature of these electrodes can result in excessive glial scarring, lead displacement, or fracture, limiting the longevity of the DBS system. In addition, standard stimulating leads are bulky (⌀ > 1 mm, larger than the brain structures they stimulate) and are rarely equipped with the ability to record. The ability to record is a useful feature, since these recordings can help surgeons quickly localize deep brain structures and achieve accurate electrode placement. Thus, to overcome the above limitations of current DBS systems, we have developed a mechanically-flexible, 8-channel, graphene-based microelectrode (⌀ < 1 mm) where each channel can either be used for stimulating or recording. Sprague Dawley rats were rendered hemi-parkinsonian with an intracranial injection of 6-hydroxydopamine (25 ug/4ul) to their right Medial Forebrain Bundle. Four weeks post-lesioning, the rats were anesthestized with an i.p. injection of urethane (1.2 g/kg) and subsequently underwent a burr hole procedure over the subthalamic nucleus (STN). The microelectrode was lowered into STN using a microdriver at an insertion speed of 3μm/s. Our microelectrode was able to electrographically map the STN i.e. channels within the STN recorded fast spiking activity (10-30 spikes/s) while those outside showed a few number of spikes. Once the STN was reached, a DBS protocol consisting of 75 μA biphasic pulses with duration of 100 μs/phase was applied at 130 Hz for 1 min. This was preceded and ensued by a 2 min period of recording to capture STN activity pre and post-stimulation respectively. The delivered DBS was able to suppress the excessive firing of the STN neurons which is thought to underlie the motor symptoms of PD. In conclusion, we demonstrate that the recordings from this novel flexible microelectrode allowed for the successful localization and neuromodulation of STN neurons.

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Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

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Program #/Poster #: 059.16

Topic: E.05. Brain-Machine Interface

Support: DARPA Next Generation Non-surgical Neurotechnology
NIWC Pacific Contract No. N66001-19-C-4019

Title: High temporal resolution magnetoelectric nanoparticle mediated wireless stimulation of neurons in vitro and in vivo

Authors: *E. ZHANG[1], M. ABDEL-MOTTALEB[2], M. CAMPOS[1], B. NAVARRETE[3], Y. AKIN[1], V. ANDRE[2], M. SHOTBOLT[2], I. T. SMITH[2], B. YILDIRIM[1], P. LIANG[4], B. R. NOGA[5], S. KHIZROEV[1];
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Abstract: Direct, electrical stimulation of neurons is a vital treatment pathway for Parkinson's, essential tremor, epilepsy, depression, and other neurological diseases. As used in methods such as Deep Brain Stimulation (DBS), it provides a highly responsive way to modulate neuronal firing rates with a large degree of control. Despite the efficacy of DBS, it suffers from several known drawbacks, which stem from the requirement that stimulation electrodes be implanted in the brain. In addition to carrying the surgical risks of infection, stroke, and brain hemorrhage, the inflammatory response to the wires can degrade their performance over time. In this work, we demonstrate the ability to wirelessly modify the inherent firing frequency of neurons in vitro and in vivo in a controllable, rapid, and reversible manner using magnetoelectric nanoparticles (MENPs). These nanotransducers convert externally applied magnetic fields into locally applied electric fields, modulating the firing rate of neurons in their immediate vicinity. MENP nanoparticles make possible a wireless approach to controllably stimulating neurons deep within the brain, potentially replacing the need for surgical methods.

Disclosures: E. Zhang: None. **M. Abdel-Mottaleb:** None. **M. Campos:** None. **B. Navarrete:** None. **Y. Akin:** None. **V. Andre:** None. **M. Shotbolt:** None. **I.T. Smith:** None. **B. Yildirim:** None. **P. Liang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cellular Nanomed Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cellular Nanomed Inc.. **B.R. Noga:** None. **S. Khizroev:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cellular Nanomed Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cellular Nanomed Inc..

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.17

Topic: E.05. Brain-Machine Interface

Support: DARPA next generation non surgical neurotechnology
NIWC Pacific Contract No. N66001-19-C-4019

Title: Barrel Cortex Stimulation Using Magnetoelectric Nanoparticles

Authors: *V. ANDRE[1], M. ABDEL-MOTTALEB[1], E. ZHANG[2], M. CAMPOS[2], B. NOGA[3], Y. YILDIRIM[1], B. YILDIRIM[1], M. SHOTBOLT[1], S. KHIZROEV[2], P. LIANG[4];
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Abstract: The development of wireless brain stimulation techniques is important to more easily study brain activity and to advance the onset of noninvasive brain machine interfaces. Magnetoelectric nanoparticles (MENPs) have been shown to transform energy transmitted through externally applied magnetic fields into local electric fields. In this study, we have demonstrated how MENPs can modulate motor cortical neurons to elicit whisker movement in rats. Using intracortical microstimulation (ICMS) the location which generates whisker responses was determined in both cerebral cortices and MENPs were injected into these regions. Thereafter, a mounted electromagnetic transducer setup was aligned to the cortical regions and a magnetic field was applied to activate the MENPs. This was able to evoke whisker activity on both the left and right side of the face according to the orientation of the magnet. This study shows that we can non-invasively target different brain regions using MENPs and external magnetic stimulation, which paves the way for a wireless brain machine interface with many channels. This would advance the development of noninvasive brain machine interface technology for a range of applications.

Disclosures: V. Andre: None. **M. Abdel-Mottaleb:** None. **E. Zhang:** None. **M. Campos:** None. **B. Noga:** None. **Y. Yildirim:** None. **B. Yildirim:** None. **M. Shotbolt:** None. **S. Khizroev:** None. **P. Liang:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.18

Topic: E.05. Brain-Machine Interface

Support: DARPA Next-Generation Nonsurgical Neurotechnology
NIWC Pacific Contract No. N66001-19-C-4019

Title: Wireless localized brain stimulation using magnetoelectric nanoparticles

Authors: *M. ABDEL-MOTTALEB[1], E. ZHANG[2], M. CAMPOS[2], V. ANDRE[1], M. SHOTBOLT[1], I. SMITH[1], Y. YILDIRIM[2], B. YILDIRIM[2], P. LIANG[4], B. NOGA[3], S.

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Abstract: Wireless localized brain stimulation is important to study brain circuits and augment brain function. Magnetoelectric nanoparticles (MENPs) provide a minutely invasive brain stimulation approach, by transforming the energy transmitted through remotely applied magnetic fields into local electric fields. In our applied studies, we demonstrated how magnetoelectric nanoparticles can modulate motor cortical neurons to elicit motor responses in rodents. We confirmed the location of the primary motor cortex M1 using intracortical microstimulation (ICMS), before injecting MENPs into it and stimulating them by applying an external AC magnetic field. We found that stimulation of MENPs injected brains could evoke contractions, with either no or low response in the control (no MENPs) condition. Both the magnitude of response (relative amplitude) and the rate of response were greater in MENPs injected rats We have also found that we can produce different motor responses by modifying the applied magnetic field gradient and orientation, which can allow for a brain-machine interface with a very large number of channels. Thus, MENPs could enable non-invasive and localized deep brain stimulation.

Disclosures: M. Abdel-Mottaleb: None. **E. Zhang:** None. **M. Campos:** None. **V. Andre:** None. **M. Shotbolt:** None. **I. Smith:** None. **Y. Yildirim:** None. **B. Yildirim:** None. **P. Liang:** C. Other Research Support (receipt of drugs, supplies, equipment or other in-kind support); Cellular Nanomed Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cellular Nanomed Inc.. **B. Noga:** None. **S. Khizroev:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cellular Nanomed Inc..

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.19

Topic: E.05. Brain-Machine Interface

Title: A thin-film multi-channel electrode based on neurotrophic principles

Authors: *P. R. KENNEDY[1], A. J. CERVANTES[2];
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Abstract: The Neurotrophic electrode has several advantages over metal electrodes: stable single units with no need to reclassify them over time (1), long term survival (13 years) with no scarring and no loss of signal (2) similar to data rat and monkey histological analysis (3).

However, it has one major disadvantage, namely, an inadequate number of single units - about 20 per electrode wire pair, producing 40 single units per pair of wires. To correct this deficiency, NeuroNexus Technologies, Inc., has developed an electrode with 16 contacts inside the glass tip instead of four. This has been achieved by using a flexible polymer substrate with 16 contacts that is rolled up before being placed inside the glass cone that is 2 x 0.5 mm (upper end) and 0.05 mm (deep end). The monolithic cable is serpentine allowing 3D movement that reduces the strain on the implant and assists with longevity. Trophic factors are placed inside the glass cone prior to Implantation in rat vibrissa cortex. Recording studies are underway. The expectation is for approximately 160 clearly identified single units per electrode. 1. Kennedy P.R., Andreasen D.S., Bartels J., Ehirim P., E. Joe Wright, E.J., Seibert, S., Cervantes, A.J. 2018. Validation of Neurotrophic Electrode long term recordings in Human Cortex. Handbook of Biomedical Engineering. 2. Gearin M and Kennedy PR. Histological confirmation of myelinated neural filaments within the tip of the Neurotrophic Electrode after a decade of neural recordings. Frontiers in Human Neuroscience 21 April 2020 3. Kennedy PR, Mirra S, and Bakay RAE. The Cone Electrode: Ultrastructural Studies Following Long-Term Recording. Neuroscience Letters, 1992 142: 89-94. Normal 0 false false false EN-US X-NONE X-NONE /* Style Definitions */ table.MsoNormalTable {mso-style-name:"Table Normal"; mso-tstyle-rowband-size:0; mso-tstyle-colband-size:0; mso-style-noshow:yes; mso-style-priority:99; mso-style-parent:""; mso-padding-alt:0in 5.4pt 0in 5.4pt; mso-para-margin-top:0in; mso-para-margin-right:0in; mso-para-margin-bottom:8.0pt; mso-para-margin-left:0in; line-height:107%; mso-pagination:widow-orphan; font-size:11.0pt; font-family:"Calibri",sans-serif; mso-ascii-font-family:Calibri; mso-ascii-theme-font:minor-latin; mso-hansi-font-family:Calibri; mso-hansi-theme-font:minor-latin; mso-bidi-font-family:"Times New Roman"; mso-bidi-theme-font:minor-bidi;} <![endif]-->

Disclosures: P.R. Kennedy: E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Kennedy owns 98% of Nerual Signal Inc.. **A.J. Cervantes:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.20

Topic: E.05. Brain-Machine Interface

Support: NIH Grant R01DC008358

Title: Unsupervised Channel Compression Methods in Neural Prostheses Design

Authors: *A. ALOTHMAN[1], C.-L. WEI[1], P. M. TOSTADO[2], E. ARNEODO[3], T. GENTNER[4], V. GILJA[5];
[1]Electrical and Computer Engin., [2]Dept. of Bioengineering, [3]Biocircuit institute, Univ. of California San Diego, San Diego, CA; [4]Psychololgy, Univ. Of California San Diego

Neurosciences Grad. Program, La Jolla, CA; [5]Electrical and Computer Engin., UCSD, La Jolla, CA

Abstract: High-performance brain machine interfaces (BMIs) require scaling recording channel count to enable simultaneous recording from large populations of neurons. Unfortunately, proposed implantable neural interfaces have power requirements that scale linearly with channel count, introducing a critical constraint on the number of channels that can be recorded simultaneously. To facilitate the design of interfaces with reduced power requirements, we developed an unsupervised-learning-based compressed sensing strategy that suggests novel neural interface architectures which compress neural data by methodically combining channels of single-unit spiking activity. Specifically, we model the neural population activity as being generated from a lower dimensional set (low-D) of latent variables and aim to minimize the loss of information in the latent variables due to compression. This strategy assumes that the low-D latents can describe the activity of a high-dimensional neuronal population and enable better performance in online motor BMIs (Kao et al. 2015). Furthermore, multi-units can be emulated as a random projection of single units which can be used to infer low-D latents without measuring the activity of every single unit (Trautmann et al. 2019). This provides motivation to study whether informed mixtures of single-unit features, based on designed compression methods, can yield high accuracy estimates of low-D latents with lower numbers of channels. We introduce two evaluation metrics to assess the latents inferred from compressed features to quantify the loss of information, how well the low-D latents can predict the activity of neurons and how well they can relate to the behavior. By combining channels, we reduce count while maintaining good scoring on multiple evaluation metrics that estimate information in the compressed channels. We apply these methods to data from different species during various behavioral experiments, such as recording from Pmd/M1 in macaque monkey during reaching tasks (182 neurons, Utah Arrays) and from RA in zebra finch in a free vocal behavioral experiment (102 neurons, Neuropixel) (Tostado, SfN 2022). For reaching tasks, we assess behavioral evaluation by measuring the Euclidean distance (in mm) between kinematics, reconstructed using compressed latents, and true kinematics. Our initial results suggest that we observe 3%, 10% and 20% in mm of error when we reduce channel count by 50%, 66% and 79% respectively. These results suggest that the proposed novel neural interface design can yield reduced power requirements, scaling input recording channel count sublinearly with minimal impact on functional BMI performance.

Disclosures: A. Alothman: None. **C. Wei:** None. **P.M. Tostado:** None. **E. Arneodo:** None. **T. Gentner:** None. **V. Gilja:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); V.G. holds shares in Neuralink, Corp., and Paradromics, Inc. These organizations had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract.. F. Consulting Fees (e.g., advisory boards); V.G. currently consults for Paradromics, Inc. They had no role in study design, data collection and analysis, decision to publish, or preparation of the abstract..

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.21

Topic: E.05. Brain-Machine Interface

Support: NINDS (UF1NS107659)
NSF (1707316)

Title: Post-explant profiling of carbon fiber electrodes and surrounding neurons enables modeling of recorded signals

Authors: *J. G. LETNER[1], P. R. PATEL[1], J.-C. HSIEH[2], I. M. SMITH FLORES[1], L. A. WALKER[3], E. DELLA VALLE[1], J. D. WEILAND[1], D. CAI[4], C. A. CHESTEK[1];
[1]Biomed. Engin., [2]Neurosci. Grad. Program, [3]Biophysics, [4]Cell and Developmental Biol., Univ. of Michigan, Ann Arbor, MI

Abstract: Characterizing the relationship between signals recorded on intracortical electrodes and the contributing source neurons is integral to understanding neural circuits and can inform clinical brain machine interface design. Highly biocompatible electrodes may sample naturalistic neural populations and improve signal quality by inducing minimal tissue damage. For instance, carbon fiber electrodes with 6.8 µm diameter cause minimal immune responses (Patel 2016) and yield high signal recording (Welle 2020) after long-term implantation. However, the precise positions of surrounding neurons must be measured to relate recorded signals to function (Yang 2019). Here, for the first time in motor cortex, we attempt to precisely localize the recording site tips of carbon fiber electrodes and positions of surrounding neurons. This enabled modeling spike sortability and measuring the biocompatibility of individual fibers within the 50 µm single neuron recording zone (Henze 2000). We implanted High Density Carbon Fiber Electrode arrays (Huan 2021) for 6 (N=2) or 12 weeks (N=2) into Long-Evans rats targeting layer V motor cortex and measured electrophysiology regularly. Recording site tips were functionalized with PEDOT or PtIr. We explanted the arrays, stained the brain sections by immunohistochemistry, and imaged the recording sites with subcellular-resolution confocal microscopy. Following putative electrode tracts throughout the image volumes enabled 3 reviewers to localize 61 tips of 63 implanted fibers with a median absolute difference of 5.2 µm (6w rats) and 14.7 µm (12w rats). We then 3D segmented neuron somata within a 50 µm radius of the tips (12w rats). Although neurons surrounding implanted fibers were elongated with a cell shape strain index (Du 2017) that was 112±89% ($\bar{X}$±S) higher ($p<0.001$, Kolmogorov-Smirnov test), the nearest 6 neurons positions were similar to those surrounding hypothetical fibers in healthy contralateral cortex, differing by 2.6±1.1 µm. This naturalistic neuron distribution encouraged us to examine the relationship between the nearest neurons and spikes recorded with a point source model fit with recorded electrophysiology. Predicting average spike amplitudes of the nearest 10 neurons relative to implanted fibers suggests spike clusters may become indistinguishable beyond the fourth closest neuron (30.5±4.5 µm), as neurons clustered in space with similar amplitudes are difficult to spike sort (Pedreira 2012). Our data and simulations show the first direct evidence that neuron number and distribution in the immediate vicinity of the recording site determines how many spike clusters can be identified with spike sorting.

Disclosures: **J.G. Letner:** None. **P.R. Patel:** None. **J. Hsieh:** None. **I.M. Smith Flores:** None. **L.A. Walker:** None. **E. della Valle:** None. **J.D. Weiland:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); JW has a financial interest in PtIr material (Epic Medical, Inc.). **D. Cai:** None. **C.A. Chestek:** None.

Poster

059. Neurophysiology: Implanted Electrodes and Other Direct Interactions With Neurons II

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 059.22

Topic: E.05. Brain-Machine Interface

Title: Motor outcomes with phase-dependent stimulation of motor cortex in Parkinson's disease

Authors: *K. MILLS[1], Y. SALIMPOUR[2], M. J. KIM[1], W. S. ANDERSON[2];
[1]Neurol., [2]Neurosurg., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Neuromodulation of the cortico-basal ganglia-thalamo-cortical network for Parkinson's disease currently consists of applying high frequency stimulation to subcortical structures, regardless of pathological oscillation amplitude, phase, or temporal dynamics. Cortical neuromodulation of this network is limited by side effects and inefficacy of high frequency stimulation. The association between abnormal phase-amplitude coupling (PAC) in the motor cortex and Parkinson's disease has recently been explored in PD patients as a marker of motor symptom severity where excessive coupling between the phase of beta rhythms and the amplitude of the gamma activities is correlated with the parkinsonian motor state compared to other diseases yet these associations are at the cortex, not typical subcortical structures targeted by deep brain stimulation (DBS). Effective cortical neuromodulation, using PAC as biomarker of parkinsonism, needs to reduce beta oscillation amplitude or decouple it from gamma power without impacting other cortical functions that would cause side effects. We applied stimulation pulses triggered by specific phases of the beta oscillations (phase-dependent stimulation; PDS) of the motor cortex of five PD patients during DBS lead placement surgery while they were performing a motor task. To capture hand position information, we used a sensitive computerized system of two LEAP motion sensors while patients were involved in a series of clinically relevant hand motion tasks, which included finger tapping, hand movements, and pronation/supination. Each trial of the motor task has three temporal sequences including the rest phase and ready and go phases. We applied PDS in the entire session and measured the effect on PAC modulation index in the motor cortex relative to the motor task temporal phases. Our results demonstrate that stimulation locked to the phase of the peak of beta increased beta-gamma coupling during the preparation phase of the task and increase the severity of the motor symptoms during the execution phase. However, the opposite phase (trough) stimulation trended toward reducing the magnitude of coupling in the preparation phase and reduction of the motor symptoms in the execution phase of the task. These results demonstrate the capacity of the motor

cortex phase-dependent stimulation to modulate oscillopathy signatures and alter the severity of the motor symptoms, allowing targeting of cortical network nodes in the treatment of network-based brain disorders such as PD.

Disclosures: **K. Mills:** None. **Y. Salimpour:** None. **M.J. Kim:** None. **W.S. Anderson:** F. Consulting Fees (e.g., advisory boards); Longevity.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.01

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF Grant DMS-1715808

Title: Long-term Absence of Neuromodulators Alters Network Robustness to Temperature Perturbation

Authors: *S. MORE-POTDAR, J. GOLOWASCH;
New Jersey Inst. of Technol., Newark, NJ

Abstract: Robust neuronal activity is important in networks that generate rhythmic behaviors, like walking, breathing, or heart beating. Robustness in such behaviors is particularly essential for the animal's survival as it receives various perturbations like injuries, diseases, or environmental changes. In crabs, it has been shown that the pyloric network generates a robust rhythm over a wide range of temperatures, provided that neuromodulatory inputs to the network are intact (Haddad & Marder, 2018). Removing neuromodulators (decentralization) drastically slows down the pyloric rhythm, upsets the relative timing of bursting (phases) between component neurons, and reduces the robustness of the rhythm. After several hours the pyloric rhythm's phasing and frequency largely recover, but it is not known if robustness is also restored. We define a *robust rhythm* as the one whose variability across perturbation states (e.g., temperature) is constant within a physiological range. Additionally, a *stable rhythm* is one whose properties remain constant over time. Here we explored whether the pyloric rhythm could regain robustness and stability in the prolonged absence of neuromodulators. We recorded pyloric activity extracellularly and studied its robustness in response to temperature perturbations (9ºC - 30ºC, 3ºC intervals) under different modulatory conditions: intact, 6, and 24 hrs after decentralization (n=46). When not changing the temperature, we maintained the preparations at 12ºC. We examined four activity features: frequency of the pyloric rhythm and three phases of neuronal bursting. PD and LP are core bursting neurons in the pyloric network. The PDoff, LPon, and LPoff bursting phases describe their activity onset and termination. Our results (ANOVA analyses) show that the means and variances of all four activity features do not change across time and temperatures in the intact modulatory condition, consistent with a robust and stable system. On the other hand, a significant change was observed in both means and variance

of the same four activity features soon after decentralization ($P < 0.05$), consistent with a loss in robustness and stability. Although, at ~24 hours, both means and variances of phases and frequency return to levels indistinguishable from the control condition. In conclusion, the pyloric network loses activity robustness to temperature perturbations as well as stability shortly after decentralization but then regains both in parallel with a recovery of frequency and phase relationships. The mechanism of this recovery is not known, but it must include a reorganization of cellular and network properties since neuromodulation is not restored.

Disclosures: S. More-Potdar: None. **J. Golowasch:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.02

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF Grant 420161

Title: The impact of plastic contaminants on central pattern generator circuits controlling vertebrate locomotion

Authors: V. RIZZIERI, *M. E. DIAZ-RIOS;
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Abstract: Recent research has drawn attention to a highly toxic industrial chemical: plasticizers. Plasticizer-derived chemicals are used in regularly used products such as medical devices, cosmetics, and children's toys enhancing their flexibility and durability. However, recent studies have shown how toxic plasticizers can be for living organisms. More specifically, pollutant plasticizers tributyl phthalate (TBP) and dibutyl phthalate (DBP) pass through the blood brain barrier and contaminate the central nervous system inducing such events as oxidative stress and inflammation which can lead to neuronal death. Thus, there is a need to assess the cellular and molecular effects that these plasticizers products have on central nervous system viability and function. Our study examined the effects of TBP on vertebrate neural circuits for locomotion using a neonatal mouse model. Locomotor-like activity was induced in a neonatal mouse lumbar spinal cord preparation with the use of serotonin (5-HT) and NMDA. Ventral root recordings were performed using suction electrodes and bursting parameters (amplitude, duration, and cycle period) were measured and analyzed over time in the presence of different TBP concentrations. The L2 (flexor-related) ventral root activity was significantly increased in all parameters measured when concentrations of 10µM and 50µM TBP were applied to the perfusate. The application of 100µM TBP reversibly abolished both L2 flexor and L5 extensor ventral root activity. Pre-incubating spinal cords with Rosmarinic acid (an active ingredient of the Rosemary plant) reduced the effects of TBP effects in all concentrations used supporting its role as a

neuroprotective agent. Future experimentation will aim to identify specific cellular mechanisms of action including TBP-induced damage via oxidative stress and/or inflammation.

Disclosures: V. Rizzieri: None. **M.E. Diaz-Rios:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.03

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS029436

Title: Feeding-related microcircuit responses to some neuropeptides are selectively strengthened by a naturally occurring hormonal milieu

Authors: L. J. FICKLING, A. P. COOK, *M. P. NUSBAUM;
Perelman Sch. of Medicine, Univ. of Pennsylvania, Philadelphia, PA

Abstract: We aim to establish that a naturally occurring hormonal milieu has a distinct impact on the influence of different modulators on the same microcircuits. We are addressing this issue by studying the response of the gastric mill (chewing) and pyloric (filtering of chewed food) rhythms in the isolated crab (*Cancer borealis*) stomatogastric ganglion [STG] to neuromodulators co-applied with hemolymph obtained from a different, unfed crab [unfed hemo]. The applied modulators (10^{-6} M) include the peptides Gly^{1}-SIFamide [G-SIF] and Proctolin [Proct] plus the muscarinic agonist oxotremorine [OXO]. In the isolated STG, unfed hemo alone either did not alter the pyloric cycle period (saline, 2.0 ± 0.2 s; hemo, 3.0 ± 0.7 s, n=5, $p>0.05$) or it terminated the rhythm (n=6), and it did not activate the gastric mill rhythm (n=11). In contrast, G-SIF in unfed hemo elicited a gastric mill-like rhythm which was stronger and longer-lasting than G-SIF in saline. For example, G-SIF in hemo elicited more coordinated IC, LG and DG neuron bursts than in saline (saline: 2.3 ± 1.6/15 min; hemo: 47.0 ± 13.5/15 min, n=4, $p<0.05$). Notably, in G-SIF with saline most IC bursts had <10 spikes but there were often >20 spikes/burst in G-SIF plus hemo (%bursts with >20 IC spikes: saline, 0.6 ± 0.3; hemo: 10.5 ± 1.2, n=4, $p<0.01$). There were not only more prolonged IC bursts in G-SIF plus hemo (#/15 min: saline, 6.0 ± 2.0; hemo, 67.3 ± 9.5; n=4, $p<0.01$) but the pyloric cycle period slowed during the longer bursts (G-SIF + hemo: IC brief/silent, 1.4 ± 0.1 s; IC prolonged, 3.1 ± 0.3 s, n=4, $p<0.05$). These hemo effects on G-SIFamide actions were similar to increasing the G-SIF concentration in saline from 10^{-6} M to $5X10^{-6}$ M (Blitz et al., 2019 J Neurophysiol). In contrast, unfed hemo did not appear to alter Proct (n=2) and OXO (n=2) actions; no gastric mill activity occurred and there was little impact on the pyloric rhythm (Proct-saline: pyloric cycle period- 1.2 ± 0.2 s, LP spikes/burst- 8.8 ± 3.0; Proct-hemo: cycle period- 1.2 ± 0.1 s, LP spikes- 10.0 ± 3.5; OXO-saline: cycle period- 1.4 ± 0.5 s, LP spikes- 6.6 ± 1; Proct-hemo: cycle period- 1.0 ± 0.1 s, LP spikes- 6.4 ± 0.1). It is unlikely that the hemo-enhancing effects on G-SIF are boosted by G-

SIF in the hemolymph, as hormone levels tend to be low ($<10^{-7}$ M). Many modulators, including Proct and OXO, converge to activate the same current (I_{MI}) in STG neurons (Swensen & Marder, 2000 J Neurosci), but this is not yet known for G-SIF. Thus we will test the hypotheses that one or more hemolymph constituents selectively amplify a distinct, G-SIF activated ionic current, and/or act as an extracellular peptidase inhibitor to prevent G-SIF degradation and prolong its presence and influence in the STG neuropil.

Disclosures: L.J. Fickling: None. **A.P. Cook:** None. **M.P. Nusbaum:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.04

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant NS094176
University of Minnesota Foundation Grant

Title: Dopaminergic signaling in the spinal cord exhibits inhibitory control of locomotion during key developmental timepoints in larval zebrafish

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Abstract: In vertebrates, dopamine (DA) modulates locomotion by altering neural circuits in the brain and spinal cord. The dopaminergic diencephalospinal tract (DDT) is highly conserved, and the exclusive source of DA in the spinal cord in most vertebrates. Although there is limited understanding of the role of the DDT in directly modulating spinal locomotor circuits *in vivo*, a recent study demonstrated an increase in spinal motor neuron recruitment through activation of D1-like receptors leading to increased swim speed and amplitude (Jha and Thirumalai 2020). Further, our lab previously demonstrated a DDT mediated developmental switch from an immature-like to a mature-like motor activity in larval zebrafish that was dependent on D4 receptor signaling in the spinal cord (Lambert et al 2021). This switch was PREVENTED with chronic DAR antagonism and REVERSED by targeted diencephalic dopaminergic neuron (DDN) ablation. We hypothesized that the mechanism for the switch was due to developmental differences in either DDN activity levels or DAR expression in the spinal cord, or both. To test the hypothesis that DARs were present in immature (3 days post fertilization (dpf)) larvae and could ADVANCE the switch in motor activity, exogenous DA was applied during optogenetically-induced fictive swimming in spinalized preparations (Montgomery et al 2021). Locomotor properties (e.g., episode durations and burst numbers) were measured and compared to baseline (before drug) and drug free controls. Application of DA decreased the number of bursts (baseline: mean = 163.3 SD 16.1 bursts; treatment: mean = 117.2 SD 41.6 bursts; n = 6,

paired t-test, t = 3.82, p = 0.01). First episode duration also decreased (baseline: mean = 6.0 SD 1.7s; treatment: mean = 4.3 SD 2.7s), which was not significant (n = 6; paired t-test, t = 1.44, p = 0.21). This application of DA to immature larvae, produced activity that mimicked mature (4 dpf), post-switch larvae. We next demonstrated that selective activation of the D2-like DARs with quinpirole was sufficient to ADVANCE the developmental switch in motor activity, decreasing number of bursts (baseline: mean = 134.7 SD 27.2 bursts; treatment: mean = 83.0 SD 39.5 bursts; n = 6, paired t-test, t = 6.77, p = 0.001) and first episode durations (baseline: mean = 4.7 SD 1.7s; treatment: mean = 4.7 SD 1.7s; n = 6, paired t-test, t = 4.27, p = 0.008). These results demonstrated that DARs were present and functional in the spinal cord of immature larvae, suggesting the differences in motor activity between 3 and 4 dpf is inconsistent with differential DAR expression. Thus, differences in DDN activity during development are hypothesized to be the mechanism for motor activity changes.

Disclosures: B. Mercier: None. **E. Amon:** None. **M.A. Masino:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.05

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF Grant 1454904
NIH Grant R25GM064120

Title: Expression of voltage-gated ion channels and biogenic amine receptors in segmental ganglia of the leech after nerve cord transection and recovery of crawling

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Abstract: It has been demonstrated previously that after brain removal (or transection between the brain and segmental ganglia) leeches are refractory to crawling. However, after 10-14 days they remarkably recover their ability to crawl, in part, through the emergence of a new “lead” ganglion directly below the transection site. Subsequently, it has been shown that peripheral remodeled proprioceptors likely play a key role in this crawl recovery. However, not much is known about other changes that might occur in neurons within the ventral ganglia to promote recovered crawling. We have hypothesized that neurons of the lead ganglion undergo changes in excitability and receptiveness to external modulation that may underlie the recovery of crawling. To begin to test this hypothesis, we used real time quantitative RT-PCR (RT-qPCR) analysis on the anterior and posterior ganglia of transected and sham control animals to measure ion channel and amine receptor mRNAs after the recovery of transected animals. The nerve cords of animals were transected between ganglion M2 and ganglion M3 and all ganglia were then harvested for

analysis. RT-qPCR results indicated a significant 2 to 3-fold increase in mRNAs encoding voltage-gated potassium and sodium channels in ganglia M3 (specifically Shaw1, Shab1, ShaL1, and NAV1). In contrast, results from the adjacent posterior ganglia M4 and M5 revealed modest differences in channel expression; more posterior ganglia showed no differences in gene expression. We also found substantial changes in the biogenic amine receptors, including those for serotonin and dopamine. Specifically, D3r and HTr4 were significantly elevated in the lead ganglia and lowered in the ganglia immediately anterior to the nerve cord transection. Lastly, we detected substantial changes in Actin expression in these same ganglia, which may indicate morphological reorganization as well as excitability/receptivity. Our results suggest that neuroplasticity associated with crawl-related compensation likely include changes in ion channel and receptor expression that influence the firing properties of neurons in the lead ganglion. Future experiments will take a more cell-specific approach to determine in which cell types these changes may be most prominent to influence locomotor recovery.

Disclosures: **A. Rosete:** None. **V. Garcia:** None. **K. Mesce:** None. **D.J. Schulz:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.06

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF-IOS 1755283

Title: Neuropeptide modulation activates multiple intrinsic bursters and enhances synapses to integrate a switching neuron into a second network

Authors: *S.-R. H. FAHOUM, D. M. BLITZ;
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Abstract: Coordination of network neurons and network flexibility are important for appropriate and adaptable behaviors. Network flexibility includes neuronal switching, where neuronal participation changes from one network to another via neuromodulation of synaptic or intrinsic properties (Meyrand et al 1994 J Neurosci; Hooper & Moulins 1990 J Neurophys; Fahoum &Blitz 2021 J Neurosci). When recruited via synaptic modulation, a switching neuron is typically passive in the second network. Here we ask if a switching neuron recruited via intrinsic property modulation contributes to pattern generation in a second network. Small, well-characterized feeding-related networks (pyloric [fast], ~1 Hz; gastric mill [slow], ~0.1 Hz) and identified modulatory inputs of the *Cancer borealis* stomatogastric nervous system make it a useful model to study neuronal switching. The neuropeptide Gly^1-SIFamide (SIF) causes the LPG neuron (2 copies) to switch from fast-only to dual fast-slow activity. SIF modulation of intrinsic properties enables LPG to generate slow-timed bursting (Fahoum & Blitz 2021) which is regulated by slow network neurons LG, IC, and DG (Fahoum & Blitz SfN Abstr 2019). Here,

we examined whether LPG contributes to slow network pattern generation by comparing LG, IC, and DG activity in LPG intact vs. LPG photoinactivation (LPG kill) during SIF (5 µM) application. We found that LG (n = 10) and DG (n = 9) intraburst firing frequency was decreased ($p < 0.01$) in SIF:LPG kill. Categorical analysis of the variable SIF slow network pattern determined that the amount of LG:IC:DG (1:1:1) coordination was not altered by LPG kill (LPG intact vs. LPG kill: 35% vs. 35%, n = 7). Thus, LPG regulates firing frequency, but is not necessary to coordinate slow network neurons, pointing to synapses among LG, IC, and DG for coordination. However, blocking LG, IC, and DG chemical synapses with picrotoxin (PTX) during SIF application (SIF:PTX) elicited co-active LG, IC, DG bursts that alternated with LPG (n = 4/4), indicating a partial role for LPG in slow network coordination. When LG, IC, DG, plus LPG synapses were eliminated (SIF:PTX + LPG kill), LG, IC, DG were uncoordinated (n = 6/7), but generated bursts (LG, DG, IC, n = 5/7, 6/7, 2/7) suggesting that in addition to LPG, LG and DG are intrinsic bursters. Although synapses among LPG, LG, IC, DG are not functional in control conditions, thus far we found some to be modulated by SIF (Fahoum & Blitz 2019). Our findings suggest that modulation of intrinsic properties enables switching neuron recruitment (LPG) and intrinsic bursting of network neurons (LG, DG), while modulation of intra-network and switching neuron synapses coordinate the intrinsic bursters.

Disclosures: S.H. Fahoum: None. **D.M. Blitz:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.07

Title: WITHDRAWN

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.08

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF IOS:1755283

Title: Differential regulation of CPG network frequency across multiple phases of a related CPG network

Authors: *B. GNANABHARATHI, S.-R. H. FAHOUM, D. M. BLITZ;
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Abstract: Internetwork coordination is necessary for proper function of related behaviours, e.g., breathing and vocalization (Schmidt et al., Exp Physiol 2012). The cellular mechanisms controlling such coordination remain unidentified in most systems. We use the stomatogastric nervous system of the crab *Cancer borealis* to investigate mechanisms of internetwork coordination, due to its small, well characterized gastric mill ("slow": ~0.1 Hz, chewing) and pyloric ("fast": ~1 Hz, filtering food) networks. Modulatory or environmental conditions determine coordination of these networks (Stein and Harzsch, Zoology 2021; Bartos et al., J Neurosci 1999). Activation of the modulatory neuron MCN5 or bath application of its neuropeptide Gly^1-SIFamide (SIF, 5µM) elicits a unique slow rhythm (Blitz et al., J Neurophys 2019; Fahoum & Blitz, J Neurosci 2021). In saline, the 2 LPG neurons are coactive with the fast network pacemaker neurons. In the SIF rhythm, LPG switches into dual fast/slow network participation and the fast rhythm frequency varies during the slow rhythm (Blitz et al, 2019; Fahoum & Blitz 2021; Fahoum & Blitz, SfN abstract 2021). Dual-network activity identifies LPG as a likely contributor to internetwork coordination. We first compared the fast rhythm frequency during each phase of the SIF slow rhythm to the fast rhythm frequency during cycles that did not overlap with any slow network neurons (BaseFreq: 0.77 Hz, n=17). Fast rhythm frequency was lower during co-activity of LG and IC neurons (0.61 Hz, n=17, $p<0.001$), higher during LPG activity (0.94 Hz, n=17, $p<0.001$), and not different from BaseFreq during DG neuron activity (0.81 Hz; n=17, p=0.267). When LPG (2 copies) was photoinactivated (LPG kill), the fast rhythm frequency during the LG/IC phase was still lower than BaseFreq (n=8, p=0.01). However, in the LPG kill condition, the difference in fast rhythm frequency between the LG/IC phase and BaseFreq was smaller (LPG Intact, -27%, LPG kill, -13%, p=0.02, n=8). These data suggest that LPG alters LG/IC activity, indirectly contributing to internetwork coordination even when it is not active. In fact, LG firing frequency is lower in LPG kill (Fahoum and Blitz, SfN abstract 2022). Thus, we show that fast network frequency is differentially regulated during two of the three slow rhythm phases (lower during LG/IC, higher during LPG). This dual regulation is likely due to distinct mechanisms, as LPG vs LG/IC act on the fast network via electrical vs chemical synapses. Differential, multi-phase regulation and the use of multiple synapse types can provide further complexity as well as additional pathways for altering internetwork coordination.

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Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 060.09

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R01GM140415-02

Title: Neurogenetic Mechanisms Underlying Sexually Dimorphic Behavioral States in C. elegans

Authors: *G. REILLY[1], D. PORTMAN[2];
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Abstract: Being able to flexibly adapt and move around an environment is imperative for an organism's survival. This plasticity in locomotion requires an animal to integrate both environmental stimuli as well as the internal state of the organism itself. In *C. elegans*, previous work has shown that under specific conditions worms will switch between stereotypical forms of locomotion known as locomotor states depending on both the environment around them and their internal states. Generally, on a patch of bacteria, hermaphrodites will stochastically switch between two locomotor states: roaming and dwelling. Furthermore, the amount of time spent in each state can be altered by various perturbations to both the environment and internal condition of the organism. However, little is known about how the internal state of genetic sex affects these locomotor states. Studies from our lab have shown that genetic sex influences general locomotion in *C. elegans* by modulating both musculature and the nervous system. Moreover, work from other labs has suggested that male locomotor states differ from that of their hermaphrodite counterparts. To investigate sex differences in locomotor states, video recordings of worms (n= 60-80) were taken to analyze locomotor states. Using a combination of the open-source program WrmTrck, custom R code, and a custom Hidden Markov Model analysis, we have found evidence suggesting males have two locomotor states like hermaphrodites, but the kinetics of male locomotor states are sex-specific. Using tissue-specific sex reversals, we aim to determine where genetic sex may be acting to achieve these sex-specific locomotor states. Preliminary results indicate that genetic sex appears to modulate the nervous system and not musculature to achieve these differences. Furthermore, the known neurochemical modulators of locomotor states in hermaphrodites are well defined (PDF signaling and serotonin) but poorly described in males. Genetic sex could potentially use these neurochemicals to achieve the sex differences seen in locomotor states. Using a combination of mutants and tissue-specific knockouts, we aim to determine which neurochemicals may have a sex-specific function and where they might be acting within males. Preliminary data suggest that PDF neuropeptide signaling may be where genetic sex achieves this modulation. My results will give further insight into how genetic sex can tune neural circuitry to achieve sex-specific behaviors and more broadly give insight into the complex interplay between genetics, neural circuitry, and behavior.

Disclosures: G. Reilly: None. **D. Portman:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.10

Topic: E.07. Rhythmic Motor Pattern Generation

Support: 1 R21 NS111355
NSERC and Frank LeBlanc Chair in Spinal Cord Injury Research

Title: An electrically-coupled glutamatergic spinal network for the generation of episodic rhythmicity

Authors: *J. J. MILLA CRUZ[1], S. A. SHARPLES[3], S. M. KOROGOD[4], J. PARKER[5], A. LOGNON[2], N. CHENG[2], A. SHONAK[2], G. S. CYMBALYUK[6], P. J. WHELAN[1];
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Abstract: Spinal circuits produce a diverse array of motor outputs that coordinate the fundamental rhythm and pattern of locomotor movements. However, locomotor behaviours are often episodic. Despite the ubiquitous nature, we know very little about the underlying neural mechanisms that encode locomotor episodes; however, mounting evidence supports a spinal origin for episodic rhythm generation. Here we explore spinal mechanisms that underlie the generation of episodic bursting. To address this aim, we studied episodic bursting elicited by dopamine in isolated spinal circuits of the newborn mouse and the underlying mechanisms that were dissected pharmacologically. Dopamine-evoked episodic activity was abolished by high (10 µM), but not low (1 µM) concentrations of riluzole. Moreover, selective blockers against persistent sodium (NaV1.6: 4,9, AH-TTX) or persistent calcium currents (ICaL: Nimodipine) had no effects on episodic activity. Given that high doses of riluzole can decrease NMDA conductances, we next examined the role for glutamatergic transmission in the generation of episodic bursting. Blockade of AMPA (DNQX) or NMDA (APV) receptors eliminated fast intraepisode bursting but did not alter the underlying slow episode. Interestingly, slow episodes were completely abolished following blockade of both AMPA and NMDA receptors. Further, consistent with the presence of electrical coupling within rhythmogenic glutamatergic interneurons of the developing mammalian spinal cord, we also found that episodic rhythmicity could be abolished following blockade of gap junctions (carbenoxolone). We therefore hypothesize that episodic bursting in the mammalian spinal cord is generated by a recurrently-connected population of electrically-coupled glutamatergic interneurons. Considering these experimental results, we examined with a computational model how AMPA, NMDA, and electrical coupling can lead to the generation of episodic rhythmicity within spinal circuits. Our results suggest that NMDA receptors play a key role in the generation bursting activity within episodes.

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Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

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Program #/Poster #: 060.11

Topic: E.07. Rhythmic Motor Pattern Generation

Support: BBSRC Grant BB/T015705/1

Title: Effects of hydroxylamine on spinal locomotor output in Xenopus tadpoles: evidence for hydrogen sulphide modulation

Authors: L. HACHOUMI, R. RENSNER, *K. T. SILLAR;
Univ. St Andrews, Univ. St Andrews, St Andrews, United Kingdom

Abstract: Hydrogen sulphide (H_2S) is a gaseous neuromodulator produced in the brain enzymatically from L-cysteine that is involved in regulating neuronal excitability and synaptic transmission. Evidence from hippocampus, for example, suggests cystathionine β-synthase (CBS) derived H_2S enhances NMDA receptor-mediated currents and facilitates long-term potentiation (Abe and Kimura, 1996). Pharmacological inhibition of CBS also modulates rhythm generation and excitability in the pre-Bötzinger complex respiratory network (da Silva *et al.,* 2017). In this investigation we applied hydroxylamine (HA), an inhibitor of CBS, to test whether endogenous H_2S modulates locomotor activity in *Xenopus laevis* frog tadpoles. We performed ventral root recordings of rhythmic motor bursts in combination with whole-cell patch clamp recordings of spinal neurons in immobilised stage 42 *Xenopus* tadpoles. Experiments utilised HA (0.1 - 2 mM) to block CBS and decrease H_2S biosynthesis, which lead to profound changes in the locomotor network output. Fictive spontaneous swim episodes were briefer and more frequent under HA (n=9). Swimming activity was also more intense, especially near the starts of episodes, with increased burst durations and decreased inter-burst intervals, but there was no significant change in swim cycle frequency. This increase in swimming intensity induced by HA occurred without significant changes in resting membrane potential, but correlated with other changes in neuronal properties (n=8). Spinal central pattern generator (CPG) neurons fired more spikes per cycle at swim onset, displayed decreased spike amplitudes and reduced sag potentials. The synaptic drive underlying fictive swimming increased; the tonic depolarization was larger near the onset of swimming episodes. HA also increased both the frequency and amplitude of spontaneous synaptic potentials (n=6), consistent with strengthened excitatory connections. Finally, HA increased the amplitude and duration of an ultraslow hyperpolarization mediated by activity-dependent sodium pumps (n=3; Zhang and Sillar, 2012).In summary, these findings suggest that rhythmic locomotor output and spinal CPG neurons are regulated by endogenous H2S signaling and that blocking H2S production with HA removes an inhibitory tone in the spinal cord of Xenopus tadpoles, causing the spinal swim network to become more excitable.
References: da Silva et al. 2017, Front Physiol. 8, 425; Abe and Kimura 1996, *J Neurosci*. 16, 1066; Zhang and Sillar 2012, *Current Biol.* 22, 526.

Disclosures: L. Hachoumi: None. **R. Rensner:** None. **K.T. Sillar:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Title: The neuropeptide myosuppressin modulates cardiac contractions in the lobster, Homarus americanus, in part by exerting effects on the cardiac muscle itself

Authors: I. S. PETROPOULOS, A. JORDAN, D. J. POWELL, *P. S. DICKINSON; Bowdoin Coll, Brunswick, ME

Abstract: The cardiac neuromuscular system in the American lobster, *Homarus americanus*, includes a central pattern generator (the cardiac ganglion, CG), neuromuscular junctions (NMJ), and the peripheral cardiac muscle itself. This system is heavily modulated by neuropeptides and is consequently capable of flexibly producing varied outputs that allow these organisms to adapt to changes in their environment or sensory input. Neuromodulators such as peptides have the capacity to alter muscle dynamics peripherally both via the neuromuscular junction and by acting on the muscle itself. Myosuppressin (pQDLDHVFLRFamide) is an endogenous and highly conserved neuropeptide that decreases contraction frequency and increases contraction amplitude in the lobster heart by modulating both the CG and the periphery. In the isolated CG, myosuppressin increases the duration of action potential bursts and decreases cycle frequency. Peripherally, it increases contraction amplitude through a previously unknown mechanism. Here, we investigated the remaining question, whether myosuppressin exerts its peripheral effects on the cardiac muscle, the NMJ, or both. Because it is spontaneously active and drives the heart contractions via the NMJ, the CG was removed for all experiments. To determine whether myosuppressin alters the NMJ, excitatory junction potentials (EJPs) were evoked by direct stimulation of the motor nerve. EJPs were recorded with a microelectrode inserted into a single muscle fiber both in control saline and in the presence of myosuppressin. Myosuppressin did not modulate the amplitude of EJPs. To determine whether myosuppressin directly alters contraction of the cardiac muscle, the muscle fibers were stimulated by puffing L-glutamate (the neurotransmitter used by CG motor neurons) onto individual fibers while recording contractions, both in control saline and while superfusing myosuppressin. Myosuppressin increased glutamate-evoked contraction amplitude in the isolated muscle, suggesting that myosuppressin exerts its peripheral effects directly on the cardiac muscle.

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Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Program #/Poster #: 060.13

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant R34NS127106

Title: Oscillators driving spontaneous exploratory behavior in Ciona larva

Authors: *Y. MIAO[1,2], J. CHUNG[1,2], C. BORBA[1,2], E. NEWMAN-SMITH[1,2], W. C. SMITH[1,2];
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Abstract: The primitive chordate *Ciona robusta* is an emerging model for neural circuit analysis, and is the only chordate with a characterized connectome. Most studies focus on *Ciona* larvae, which exhibit a number of behaviors, including negative phototaxis. To initiate negative phototaxis, *Ciona* larvae first exhibit spontaneous exploratory swims in an attempt to discern the direction of light. Our group is investigating the mechanism driving spontaneous swims. The spontaneous swim behavior of the *Ciona* forebrain mutant *frimousse* (*frm*) proves important insight. In *frm* larvae the forebrain is entirely absent, while the midbrain, hindbrain, and the caudal nerve remain intact. While the spontaneous swim periodicity of wild type larvae appears stochastic, the *frm* larvae exhibited more stereotyped swim intervals with the two most frequent swim-to-swim intervals in *frm* mutant being ~2s and ~9s.
Imaging of calcium transients using VGAT- and VAChT-promoter driven GCaMP7f revealed two previously uncharacterized oscillating neurons: an inhibitory *forebrain oscillator* (FO) and an excitatory *midbrain oscillator* (MO). Significantly, *frm* larvae lack the FO, suggesting that the inhibitory FO may interact with the excitatory MO downstream, contributing to the stochastic swims seen in wild type larvae. Moreover, the absence of the inhibitory FO appears to make the excitatory MO the sole driver of spontaneous swim period.
Fourier transformation of the GCaMP recording shows the FO has an oscillation period of ~2.5s, while the MO has a period of ~14.2s. The MO frequency closely matches the predominant swim-to-swim interval (~15s) for the spontaneous swims of wild type larvae. The GCaMP recording of the *frm* MO shows a different frequency compared to the wild type. Fourier transformation of the *frm* GCaMP recording shows two periods of interest at ~9.1s and ~1.8s. Those frequencies also match the two most frequent swim-to-swim intervals of *frm*. We hypothesize that the *frm* MO frequency is the intrinsic frequency, and the interaction between FO and MO produces the lower frequency in wild type.
Our lab has been able to isolate the FO and confirm it is an autonomous oscillator. We have tentatively identified FO as the brain vesicle intrinsic interneuron (BVIN) 13 of the *Ciona* connectome. Consistent with an interaction between the FO and the MO, BVIN-13 projects to midbrain excitatory relay neurons. With this information, we are modulating the activity of the FO to better understand its potential role in spontaneous swimming. We also propose a model for the interactions of the two oscillators in order to explain the change in MO frequency in the wild type.

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Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Program #/Poster #: 060.14

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NSF IOS-1856433

Title: Dietary diversity correlates with modulatory capacity in three species of majoid crabs

Authors: *D. J. POWELL[1], J. T. SEDDON[2], E. M. MARTIN[2], K. GARCIA[2], A. I. MILLER[2], J. S. KAZMI[2], G. BUKOWSKI-THALL[2], P. S. DICKINSON[1];
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Abstract: A large number of amines, amino acids, and peptides modulate the central pattern generating (CPG) circuits found within the stomatogastric nervous system/stomatogastric ganglion (STNS/STG) and cardiac ganglion (CG) of decapod crustaceans; over 30 modulators have been identified in the Jonah crab (*C. borealis*). This is surprising given that these CPGs are comprised of only a small number of neurons; the STG contains 25 neurons, while the CG contains only 9 neurons. CPGs in the STG are responsible for internally chewing and subsequently filtering food into the midgut; the rhythmic output of the CG drives the heartbeat. Modulation consequently allows these circuits to flexibly generate a variety of outputs. However, the modulatory capacity, a measure of the extent to which a neuronal circuit can be modulated, exceeds the breadth of observed CPG output, leading us to ask why so many modulators are present. Although modulatory capacity may be solely an inherited trait, we hypothesize a functional correlation, with decapods that require greater behavioral flexibility utilizing a broader range of modulators than those with more limited behaviors. We thus predicted that decapods with broad diets would have a larger modulatory capacity in their STNS, but not their CG, than those with limited diets. Therefore, we compared responses of the STNS and CG CPGs in three species of majoid crabs (*Pugettia producta*, *Libinia emarginata*, and *Chionoecetes opilio*) to the same neuromodulators. *L. emarginata* and *C. opilio* both have broad diets, while *P. producta* primarily eats kelp. However, *L. emarginata* and *P. producta* are more closely related to one another evolutionarily than either species is to *C. opilio*. We show that CG responses across species did not correlate with the animal's diet; however, STNS responses did. Crabs with broader diets responded to a majority of applied modulators; the kelp crab's STNS responded to the fewest modulators. Modulators used include: *Cancer borealis* Tachykinin Related Peptide (CabTRP), Crustacean Cardioactive Peptide (CCAP), Red Pigment Concentrating Hormone (RPCH), two FLRFamides: Myosuppressin and NRNFLRFamide, the mAChR agonist Oxotremorine, Dopamine, Proctolin, Adipokinitic Hormone/Corazonin Related Peptide (ACP), Corazonin, Gly[1]-SIFamide, Calcitonin-Like Diuretic Hormone (CLDH/DH31), and HIGSLYRamide.

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Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Title: Dopamine stabilizes postsynaptic response by direct modulation and presynaptic spiking activity by generating ectopic axonal action potentials

Authors: D. M. BUCHER[1], *N. DAUR[1], M. WALKOW[2], F. NADIM[1];
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Abstract: Neuromodulators can shape neuronal activity in response to context and behavioral demands. However, modulators such as dopamine are often present at tonic levels in target neural circuits and recent studies have proposed that neuromodulator actions at tonic levels may enhance the stability of neural circuits. We explored this hypothesis in a lobster motor circuit. An important aspect of EAP initiation is the interaction with proximally generated activity. In the pyloric dilator (PD) motor neuron of the lobster stomatogastric nervous system, low tonic concentrations of dopamine elicit axonal EAPs in the peripheral nerves. Proximally generated bursting activity weakens or suppresses EAP initiation through activity-dependent hyperpolarization of the axon, so that EAP firing occurs preferentially during absence or weaker forms of proximal activity but will itself be weak or absent during stronger proximal spiking (Daur et al., Front Cell Neurosci, 2019). We show that this interaction results in a stabilization of overall mean spike rate across different modes of bursting activity, including changes in mean cycle frequencies and burst strengths, temporary suppression of centrally generated rhythmic activity by inhibitory neuromodulators, and slow modulation of rhythmic activity through intercircuit interactions.
The target muscles of the PD neurons show substantial short-term synaptic dynamics, dominated by facilitation, at different time scales , and their postsynaptic potentials are highly sensitive to the fine temporal structure of bursting and spiking input (Daur et al., eNeuro, 2021). We show that facilitation results in a priming effect by low-frequency spiking on the subsequent postsynaptic response to a burst input. Therefore, EAPs during the interburst intervals substantially increase the postsynaptic responses to bursts. Remarkably, this effect is exquisitely sensitive to the number and timing of EAPs during the interburst period. Because EAPs preferentially occur during weaker bursting activity, such priming stabilizes the magnitude of postsynaptic responses across inputs at different cycle- and spike frequencies.
Finally, low tonic concentrations of dopamine do not just cause EAPs in the PD axon, but also directly modulate postsynaptic responses of the muscles. While dopamine reduces the priming effect of EAPs, it also decreases the sensitivity of postsynaptic responses to the fine temporal

structure of bursting input. Therefore, dopamine can have both an indirect and a direct stabilizing effect on synaptic readout of varying rhythmic motor neuron input.

Disclosures: D.M. Bucher: None. **N. Daur:** None. **M. Walkow:** None. **F. Nadim:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 060.16

Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH060605

Title: Convergent co-modulation promotes consistent output of a central pattern generator circuit

Authors: E. M. CRONIN, F. NADIM, ***D. BUCHER**;
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Abstract: All neurons and circuits are under control of multiple neuromodulators that can adjust membrane excitability and synaptic interactions to modify circuit activity. Experimentally, cellular and synaptic actions are typically tested one neuromodulator at a time, even though most circuits are always exposed to several modulators. The consequences of such co-modulation depend on the overlapping targets and pathways of different modulators (convergence) and distinct receptor expressions of neurons for the different modulators (divergence). These patterns of convergent and divergent actions are crucial for understanding circuit function.We tested the effects that different combinations of neuropeptides have, across animals, on the triphasic rhythmic motor output in the pyloric circuit of the crab, *Cancer borealis*. We used the neuropeptides proctolin, crustacean cardioactive peptide, and red pigment concentrating hormone and quantified circuit output during co-application of all combinations of two out of these three. In this circuit, neuropeptides converge on a limited number of subcellular targets. However, their actions diverge at the circuit level, as each neuropeptide targets a different subset of circuit neurons. Consequently, at saturating concentrations, different neuropeptides can elicit different circuit outputs on their own. In addition, there are quantitative differences between the cellular effects of these neuropeptides on the same ion channel, and combined effects can be sublinear instead of simply additive. Despite this, the co-modulated circuit outputs were virtually indistinguishable in cycle frequency, relative timing between neurons, and spike frequencies within bursts.These findings indicate a dominance of additive and convergent effects. Generally, the presence of multiple modulators that can act on a circuit is interpreted as a means to expand the flexibility of circuit output through different combinations of co-modulation. However, if co-modulators have convergent actions and combined effects are simply additive, the effects of different modulators on a given neuron may be indistinguishable and an increasing number of co-modulators will eventually target all neurons and synapses in a circuit. Therefore, circuit

outputs should also converge with increasing numbers of modulators present, so output patterns become more consistent and independent of the actual identity of the modulators.

Disclosures: **E.M. Cronin:** None. **F. Nadim:** None. **D. Bucher:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH Grant MH060605
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Title: Modeling shows how convergent co-modulation could reduce inter-animal variability of network output

Authors: *O. ITANI, A. C. SCHNEIDER, E. CRONIN, H. G. ROTSTEIN, D. BUCHER, F. NADIM;
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Abstract: Neuronal networks can produce consistent output across animals despite several fold differences in ionic current levels and synaptic strengths in constituent neurons. Furthermore, networks are targeted by multiple neuromodulators, whose effects also vary in magnitude across animals. How networks can generate consistent output despite variability at the levels of intrinsic, synaptic, and modulatory properties is an open question. We address this question using the pyloric circuit of the crab stomatogastric ganglion (STG). Excitatory peptide neuromodulators in the STG converge to activate a fast voltage-gated inward current (IMI). We previously showed that raising excitability by peptide modulation of IMI maximal conductance (gMI) decreases inter-animal variability of the activity of a synaptically-isolated lateral pyloric (LP) neuron (Schnieder et al, eNeuro, 2022). Here, we build on recent experimental results in the pyloric circuit, which demonstrate that increasing the number of peptide modulators with overlapping targets (while keeping a fixed total non-saturating concentration) reduces variability of network output across animals, while producing no reduction of variability of the activity of individual neurons in isolation.
To address why LP output variability is not reduced by increasing the number of modulators targeting IMI, we used computational modeling. We found three factors can contribute to this experimental observation. First, as suggested previously (Li et al, J Neurosci, 2018), convergent modulator pathways may have mutually inhibitory effects. Second, there may be variability in receptor expression of different modulators. Third, increasing the modulator numbers, while maintaining a constant total concentration, may lead to a decreased total gMI (vs. one modulator), depending on differences in the dose response curves.
We then asked how co-modulation could lead to the reduction of variability at the network level.

Using families of reduced and biophysical network models we show that raising excitability through adding sub-saturating levels of gMI to all neurons is not sufficient to decrease network variability, but a balanced increase of gMI according to neuron type could lead to this result. Further, we show how modulator actions on certain attributes of activity (e.g., cycle frequency) can lead to outsized effects on variability of other attributes (e.g., spike frequency). These results highlight the importance of the relationship among modulator dose-response curves across cell-types of network in order to produce population-wide consistent network output.

Disclosures: O. Itani: None. **A.C. Schneider:** None. **E. Cronin:** None. **H.G. Rotstein:** None. **D. Bucher:** None. **F. Nadim:** None.

Poster

060. Rhythmic Motor Pattern Generation: Neuromodulation

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Topic: E.07. Rhythmic Motor Pattern Generation

Support: NIH grant MH060605
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Title: Co-modulation reduces inter-individual variability to produce similar neural circuit output

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Abstract: The ionic and synaptic current levels of identified neurons vary substantially across individual animals. Yet, under similar conditions, the output of neural circuits, particularly motor circuits, often remains remarkably similar. Neuromodulators provide flexibility to a neural circuit, but, at any given time, a neural circuit is subject to modulation by multiple neuromodulators. Different neuromodulators often overlap in their targets by modulating the same subcellular component (ion channel type or synapse). However, such converging modulators have different actions on different neurons due to the variability of their receptor expression. In the presence of multiple neuromodulators, however, their common target is potentially more uniformly activated across neurons. We therefore propose that a baseline tonic (non-saturating) level of co-modulation by convergent neuromodulators can improve circuit output similarity across individual animals.
We test this hypothesis in the pyloric circuit of the crab stomatogastric ganglion (STG). This circuit produces regular triphasic bursting oscillations and, across animals, the cycle frequency is variable (0.5-2.5 Hz) but, within each cycle, individual identified neurons retain remarkably similar activity phases. However, when neuromodulatory inputs to the STG are removed (decentralization), the rhythm deteriorates and activity phases become variable.
We quantified the inter-individual variability of the decentralized pyloric circuit output by measuring the activity phases, cycle frequency and within-burst spike number and frequency. We

then examined the variability levels in the presence of different combinations and concentrations of the neuropeptides proctolin, CCAP, and RPCH. These and other excitatory neuropeptides converge to activate the same voltage-gated inward current, but different subsets of pyloric neurons have receptors for each peptide. We find that at mid-level (0.3 μM combined), but not at low near-threshold (1nM), concentrations co-modulation by multiple neuropeptides reduced the variability of the circuit output compared to fewer modulators.
We have previously shown that inter-individual output variability of the isolated lateral pyloric (LP) neuron is reduced at saturating neuropeptide concentrations (Schneider et al, eNeuro, 2022). However, at the mid-level concentration, variability of the (isolated) LP output was not reduced by 3 compared with 1 or 2 modulators. Therefore, the reduction of circuit output variability is not due to a simple reduction of the variability of individual neurons but emerges as a network effect.

Disclosures: **A.C. Schneider:** None. **E. Cronin:** None. **D. Bucher:** None. **F. Nadim:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.01

Topic: E.07. Rhythmic Motor Pattern Generation

Support: Santa Clara University

Title: Characterization of a stretch receptor located in the pyloric region of the crab stomatogastric nervous system

Authors: P. K. D. HOVLAND, *J. T. BIRMINGHAM;
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Abstract: The activity of a number of different stretch receptor neurons has been shown to affect the rhythmic outputs of the stomatogastric nervous system (STNS) in the crab *Cancer borealis*. We found and characterized a previously unidentified stretch receptor in *C. borealis* that appears to be homologous to the hepatopancreas duct (HD) sensory neuron that has been described in *Panulirus* (spiny lobsters). Methylene blue staining revealed a cell body in a nerve branching off of the end of the pyloric nerve (*pyn*) in only half of the 10 preparations studied, but this appears to be a function of the difficulty in locating the neuron using this method. Manual stimulation of the pyloric 8 (p8) muscle significantly affected the pyloric rhythm produced by the STNS in preparations in which the inferior esophageal nerves (*ions*) were not present, reducing the period of the rhythm from 1.23 ± 0.19 s to 1.05 ± 0.14 s and the number of spikes produced by the lateral pyloric (LP) neuron from 6.9 ± 2.3 to 4.8 ± 2.1 (n=8 for both). These effects persisted when the stomatogastric nerve (*stn*) was severed, suggesting that synaptic connections within the stomatogastric ganglion (STG) are responsible for the modifications of the pyloric rhythm.

However, spiking by the stretch receptor was also observed on the *stn* (n=4), suggesting that synaptic connections may also be made within anterior ganglia of the nervous system.

Disclosures: P.K.D. Hovland: None. **J.T. Birmingham:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.02

Title: WITHDRAWN

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.03

Topic: E.07. Rhythmic Motor Pattern Generation

Support: The Physiological Society UK

Title: Midbrain control of the initiation and direction of swimming in the hatchling Xenopus laevis tadpole.

Authors: *M. LARBI[1], G. MESSA[1,2], H. JALAL[1], S. KOUTSIKOU[1];
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Abstract: In challenging environments, the communication between the brain and motor circuits leads to the selection and execution of movement, an essential component of most behaviors[1]. The midbrain is an integral part in this communication. The mesencephalic locomotor region (MLR), a functionally defined portion of the midbrain, is important in the start and control of movement[1,2]. Although midbrain descending projections influence premotor and motor circuits, the detailed nature of this influence and the underlying midbrain neuronal circuitry remain unclear. Here, we used electrophysiology, behavior and midbrain lesions in the hatchling *Xenopus laevis* tadpole, to dissect the midbrain contribution in the control of initiation and direction of swimming. We found that the severance of all connections between the tadpole's midbrain with hindbrain and spinal cord (midbrain-hindbrain border lesioned animals, MHB; n=14) led to increase in latency (median = 156 ms) to swim initiation following trunk skin stimulation, when compared to control animals (n=10; median = 105 ms; $p<0.0001$). Partial lesions to the MHB combined with lesions along the midline (ML) had similar effects to swim

latency (n=9; median = 126 ms; p=0.0082), in comparison to controls. We identified that the increase in latency is due to synchronous activation of antagonistic trunk muscles at initiation (15% increase in synchrony events in MHB *vs* controls). The tadpole initiates swimming predominantly on the contralateral side, away from the stimulus[3]. Control animals responded to trunk skin stimulation by initiating swimming at a ratio of 41% ipsilateral : 59% contralateral (n=10). While the ML lesion altered the swim initiation to a ratio of 63% ipsilateral : 37% contralateral (n=11). In addition, midbrain lesioned animals swam for longer due to following a circular swim trajectory ($p<0.0001$, ANOVA). In contrast, control animals were able to maintain the natural forward swimming. This change in swim trajectory led MHB lesioned animals (n=9) to stop in a position much closer to the starting point (median final displacement = 18 mm; p=0.0293), in contrast to controls (n=10) which moved the furthest away from the starting point due to forward swimming (median final displacement = 37 mm). Kruskal-Wallis test was used for all statistical comparisons unless otherwise stated. These results are in agreement with the role of midbrain in controlling gait, posture, body balance in older vertebrates[4]. 1. Grillner & El Manira 2020 *Physiol Rev* 100: 271-3202. Ryczko & Dubuc 2017 Front Neurosci 11: 2953. Buhl et al 2015 *J Physiol* 593: 4423-374. Thiele et al 2014 *Neuron* 83: 679-91 **Support:** The Physiological Society UK

Disclosures: **M. Larbi:** None. **G. Messa:** None. **H. Jalal:** None. **S. Koutsikou:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Chx10-positive reticulospinal neurons in the brainstem of hSyn:GFP salamanders.

Authors: *J. SWIEGERS[1], A. JOVEN[2], M. TOSCHES[3], A. J. IJSPEERT[4], A. SIMON[2], D. RYCZKO[1,5,6,7];
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Sherbrooke, Sherbrooke, QC, Canada; [7]Inst. de Pharmacologie de Sherbrooke, Sherbrooke, QC, Canada

Abstract: Salamanders recover voluntary locomotion after full spinal transection. The brainstem and spinal neurons involved are largely unknown. In mouse and zebrafish, excitatory reticulospinal neurons playing a role in locomotion initiation express the vesicular glutamate transporter 2 (Vglut2) and Chx10. However, there is little information regarding the presence of these neurons in salamanders. The genome of the Iberian newt (Pleurodeles waltl) was recently mapped, and this opens the door to the identification of such neurons within the species. Using in situ hybridization (RNAscope), we observed cells that were positive for Vglut2 mRNA and for Chx10 mRNA in the superior, middle and inferior reticular nuclei (known to contain reticulospinal neurons) as well as in the spinal cord grey matter. Many neurons positive for Chx10 mRNA were also positive for Vglut2 mRNA. Using RNAscope and immunofluorescence co-detection workflows, we showed that a large majority of reticular cells positive for Chx10 mRNA were also positive for an antibody against the Chx10 protein. In triple labelling experiments based on immunofluorescence detection coupled with tracer injection in the spinal cord of a new transgenic line of salamanders whose cells express a green fluorescent protein under control of a neuronal promoter (hSyn:GFP), we found many GFP-positive reticulospinal neurons that were immunopositive for Chx10 in the superior, middle and inferior reticular nuclei. Chx10 and glutamate colocalized in many reticulospinal neurons. To examine whether a descending glutamatergic reticulospinal drive could activate spinal motoneurons, we used calcium imaging in an ex vivo brainstem-spinal cord preparation, in which the motoneurons were loaded with a fluorescent calcium sensor. Electrical stimulation of the middle reticular nucleus evoked short latency excitatory responses in motoneurons on the same side. Increasing the stimulation intensity in the middle reticular nucleus increased the motoneuronal calcium responses. These responses were then blocked by glutamatergic antagonists. Altogether this suggests that the brainstem of the salamander houses reticulospinal neurons sending an ipsilateral descending excitatory drive, and these possibly share the discussed genetic markers found in zebrafish and mice. These neurons are good candidates to play a role in the control of locomotion before and after spinal cord regeneration in salamanders.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

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Program #/Poster #: 061.05

Topic: E.07. Rhythmic Motor Pattern Generation

Support: ERC-2020-SyG SALAMANDRA

Title: Multi-modal locomotion performance in a continuous closed-loop electromechanical model of the salamander spinal cord based on spiking neural networks

Authors: *A. PAZZAGLIA[1], A. BICANSKI[2], J. P. ARREGUIT O'NEILL[1], A. FERRARIO[1], A. SIMON[3], D. RYCZKO[4], A. IJSPEERT[1];
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Abstract: As amphibians, salamanders express a large variety of locomotor behaviours, including walking gaits on land with several different stepping patterns, underwater stepping and swimming. In vitro studies showed that the central pattern generators (CPGs) in the salamander spinal cord can generate many of the locomotor patterns observed in vivo. Moreover, stimulation of discrete brain stem targets can activate subparts of the spinal CPG and generate prototypical movements in semi-intact salamander preparations. Previous modelling studies focused on the two main gaits of the salamander, the walking trot and swimming, as well as the transition between the two. In this study we show that differential brain stem drive to a continuous spinal cord model can account for the brain stem stimulation experiments. The spiking neural network simulating the locomotor circuits of the salamander's spinal cord is linked to a mechanical model that simulates muscles and body properties as well as their interactions with water and ground. The inclusion of a simulated body allows us to compare the locomotion performance obtained in open loop and closed loop patterns where the network receives input from axial proprioceptive and limb exteroceptive sensory neurons, activated by the joints positions and the ground reaction forces respectively. The model is capable to centrally generate the CPG activity associated with the patterns of most known salamander motor behaviours, including lateral and diagonal sequential walking gaits, walking trot, underwater stepping, amble, isolated limb activation, swimming, and gait transitions between these modes. The inclusion of sensory feedback improves the properties of the generated locomotor patterns by increasing their stability while allowing to obtain more biologically-adherent intersegmental phase lags (IPLs). Contextually, the model preserves the flexibility of the open loop CPG network in terms of capability to modulate turning, frequency and IPL. The observed results pave the way for a systematic analysis of the interplay between open loop and sensory feedback-driven pattern generation in salamanders' locomotion.

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Poster

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Modeling gait selection of salamanders using exteroceptive sensory feedback

Authors: *J. ARREGUIT[1], S. SUZUKI[2], K. KASUI[3], A. PAZZAGLIA[1], A. FERRARIO[1], S. TATA RAMALINGASETTY[4], A. ISHIGURO[3], A. J. IJSPEERT[1];
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Abstract: Salamanders exhibit a wide range of locomotor skills due to their amphibious nature. When crossing between environmental media, they can switch between swimming, walking and other gait patterns, such as aquatic stepping. Previous works have proposed spinal network models based on central pattern generators, sensory feedback, or a combination of both, which lead to the emergence and coordination of gaits similar to those observed in the animals when tested in a physics-based simulation or on a robotic platform. However, while previously proposed networks have shown how these gaits could be achieved, there is very little known about the key principles that govern the gait selection mechanism to properly handle a given environment.
In this work, we investigate the role of exteroceptive feedback in gait selection and gait transitions when switching environments. In particular, we study different feedback rules from hydrodynamic sensors along the body and contact sensors in both the body and the limbs to test how the gait switching could be obtained by modulation of descending drive coming from higher centers, and additionally by using local sensory information to directly modulate the network. We evaluate our proposed models by setting up a 3D physics-based neuromechanical simulation environment with a salamander model that can walk on land and swim in water. This model incorporates a morphology based on the animal and includes a muscle model proposed by Ekeberg to actuate the body. We modeled the neural networks using abstract oscillators as they provide an ideal setting for modeling how sensory information yields coordinated gaits. Multiple experiments were run to evaluate the proposed control mechanisms in different scenarios where gait transitions between swimming and walking were needed. The results show that the proposed models can display robust gait switches that perform well in a variety of complex environments. Although the current models demonstrate qualitative similarities with the salamanders' kinematics, future work will validate these models against animal data.

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Poster

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Title: Recovery of walking and swimming after spinal cord transection in salamanders: movement analysis with deep learning.

Authors: *I. KHSIME[1], C. VAN DER ZOUWEN[1], A. JOVEN[2], A. SIMON[2], A. J. IJSPEERT[3], D. RYCZKO[1,4,5,6];
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Abstract: Salamanders swim underwater and walk on ground. They are an ideal animal model to study how the locomotor circuitry can generate two different behaviors. They are also able to recover voluntary locomotion after full spinal transection. However, a precise description of the gradual recovery of swimming and walking movements after full spinal transection is lacking. In order to do such analysis, here we used a deep-learning based software (DeepLabCut) to analyze the locomotor movements of the salamander Pleurodeles waltl. We recorded swimming and walking movements from below at 300 frames per second in a motorater. We tracked twenty-five user-defined anatomical points distributed on the body axis, limbs and head without the need to place any physical markers. To analyze axial movements, we measured the amplitude of the angular excursions between series of three anatomical points along the body axis as a function of time. During swimming, a traveling wave of axial movements was propagated from head to tail. The amplitude of the angular excursions increased from rostral to caudal body sites. During stepping, tracking of the limbs allowed us to measure the speed of limb movements, from which we evaluated the footfall pattern, swing and stance duration together with cycle duration. Salamanders mostly displayed a lateral sequence walk, and this was coordinated with a standing wave of axial movements at the level of the trunk. After spinal cord injury, the coordination between the body parts above the lesion and below the lesion was lost. After a few weeks, walking movements were recovered before swimming movements. Our study shows that the use of DeepLabCut provides a powerful approach to obtain a detailed analysis of salamander locomotion. This will allow us to study the recovery of swimming and walking movements during regeneration of the locomotor circuitry.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Intraspinal proprioceptors in a merged sensorimotor circuit for locomotion

Authors: *L. PICTON, M. BERTUZZI, R. BJÖRNFORS, P. FONTANEL, I. PALLUCCHI, A. EL MANIRA;
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Abstract: Animal behaviour is ultimately realised by patterned sequences of movement controlled by motor circuits in the brain and spinal cord. These movements rely on proprioception - sensory feedback on the position and motion of our bodies. Proprioceptors are thought to exist only in the periphery and to act in refining our movements. However, profound tension changes also occur in spinal cord tissue during locomotion. This raises the possibility that intraspinal proprioceptive neurons may exist that could directly and rapidly integrate movement information and thus act as a functionally integrated sensory component of the locomotor network. Here, we describe a segmental organ of proprioception in the spinal cord of adult zebrafish, which is embedded by intraspinal mechanosensory neurons expressing piezo2 channels. These specialized sensory neurons display piezo2-mediated mechanical currents in response to direct mechanical stimulation. We show that these cells are inhibitory, commissural neurons with unique molecular and physiological profiles. Upon bending of the spinal cord during swimming, intraspinal proprioceptors on one side fire to provide strong, selective, monosynaptic inhibition to the excitatory, rhythm-generating V2a interneurons responsible for the bending in that direction, highlighting a plausible locomotor burst-termination mechanism. Ablation of these neurons leads to slower and less efficient locomotion in vivo, demonstrating an important role for this feedback in the intact animal. Finally, we also elaborate on the powerful control these proprioceptors exert over the swim network. We describe entrainment of the fictive locomotor rhythm due to mechanically-applied rhythmic bending of spinal cord. We reveal the mechanism of entrainment by assessing how direct sensory feedback to V2a interneurons controls the timing of their activity during real ongoing swimming movements. This leads to a cascade of downstream effects on motoneurons and V0d interneurons, whose firing properties are ultimately dictated by the timing and strength of V2a excitatory drive. In summary, we describe a central proprioceptive organ that locally detects lateral body movements during locomotion and provides direct inhibitory feedback onto rhythm-generating interneurons responsible for the central motor program. This forms a unified sensorimotor network that dynamically aligns central pattern generation with movement outcome for efficient locomotion.

The circuit also provides a biological mechanism for automatic adjustments of motor network activity in response to environmental cues that affect body bending dynamics.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

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Program #/Poster #: 061.09

Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Brain circuits encoding start, duration and speed of swimming in adult zebrafish

Authors: *L. MROWKA, E. M. BERG, D. MADRID PULGARIN, L. D. PICTON, M. BERTUZZI, A. EL MANIRA;
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Abstract: The flexibility of locomotor movements requires an accurate control of its start and duration combined with the ability to execute prompt changes in vigor and speed. How brainstem circuits encode and convey these detailed locomotor parameters remains unclear. Here we have combined *in vivo* brainstem calcium imaging, whole-cell recordings, anatomical tracing and behavior in adult zebrafish to address these questions. We reveal that the detailed parameters of locomotor movements are encoded by two molecularly, topographically and functionally segregated glutamatergic neuron subpopulations within a restricted brainstem nucleus, the nMLF. The start, duration and changes speed of locomotion are encoded by vglut2^{+} neurons located in the medial nMLF, while sudden changes in vigor are encoded by vglut1^{+} neurons located in the lateral nMLF. Accordingly, ablation of the medial vglut2^{+} neurons compromised low speed explorative swimming while ablation of the lateral vglut1^{+} neurons impaired high speed fast swimming. Our results reveal two separate subpopulations of descending command neurons each encoding specific parameters of locomotor behavior showing that motor commands are elaborated and structured before they are conveyed to spinal networks. Our analysis thus provides mechanistic insights into how separate brainstem subpopulations implement flexible locomotor commands. These two brainstem command subpopulations are suitably organized to integrate environmental cues by processing different sensory modalities and hence generate swimming movement with flexible speed and vigor to match the behavioral needs of the animal.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Intracellular sodium dynamics contribute to episodic properties of swimming in larval zebrafish

Authors: B. MERCIER[1], S. M. KOROGOD[3], J. PARKER[3], G. S. CYMBALYUK[3], ***M. A. MASINO**[2];
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Abstract: Swimming in larval zebrafish consists of discrete episodes of activity separated by periods of inactivity. The dynamics of this multiscale behavior is not well understood. We propose that intracellular sodium concentration ($[Na^+]_i$) is a pivotal state variable governing the temporal properties of swimming. $[Na^+]_i$ is regulated by the Na^+/K^+ pump, which is instrumental in episodic and continuous bursting patterns of locomotor circuits on different timescales across species. Our goal was to determine how the dynamics of $[Na^+]_i$ and the Na^+/K^+ ATPase pump current (I_{pump}) impact locomotor activity at the burst and episode time scales. Thus, we utilized our recently developed optogenetic method to induce locomotor activity in spinalized transgenic zebrafish and quantified episodic and bursting locomotor properties in fictive swimming preparations. We tested two pharmacological agents; monensin was used to increase $[Na^+]_i$, which in turn led to activation of the I_{pump}, whereas strophanthidin was used to decrease or block I_{pump}. Application of monensin (10 μM) disrupted the organization of episodic activity, decreased the number of bursts per optogenetic stimulus (baseline: mean = 142.7 (SD 23.8) bursts; treatment: mean = 79.4 (SD 42.2) bursts; micron = 9, paired t-test, $t = 6.27$, $p < 0.001$), and decreased the duration of the first episode (baseline: mean = 4.0 (SD 1.4) s; treatment: mean = 1.1 (SD 1.2) s; n = 9, paired t-test, $t = 8.20$, $p < 0.001$). Application of strophanthidin (5 μM) disrupted the organization of episodic activity, increased the number of bursts per optogenetic stimulus (baseline: mean = 135.7 (SD 38.2) bursts; treatment: mean = 160.0 (SD 30.4) bursts; n = 7, paired t-test, $t = -3.72$, $p = 0.01$), and showed a trend toward increased duration of the first episode (baseline: mean = 3.6 (SD 1.4) s; treatment: mean = 4.6 (SD 2.4) s; n = 7, paired t-test, $t = -2.19$, $p = 0.07$). Finally, we developed a biophysical model of the zebrafish locomotor CPG, which was represented by cells connected through excitatory AMPA glutamatergic synapses. The model described the dynamics of $[Na^+]_i$ and the cellular membrane potential, including slowly inactivating persistent Na^+ current, h- current, low-threshold slowly inactivating Ca^{2+} current, and I_{pump}. It recapitulated key temporal characteristics of the evoked episodic patterns under baseline and both treatment conditions. Overall, these results demonstrate that intracellular

sodium dynamics offer a potential mechanism for the control of episodic activity produced by spinal locomotor circuits.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Sensory stimuli evoke calcium responses in glutamatergic and GABAergic neurons of the Mesencephalic Locomotor Region in freely behaving mice.

Authors: *C. VAN DER ZOUWEN[1], J. BOUTIN[1], L. DEJAS[1], A. FLAIVE[1], T. HSU[2], V. KONANUR[2], M. F. ROITMAN[2], D. RYCZKO[1,3,4,5];
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Abstract: Although the role of the Mesencephalic Locomotor Region (MLR) in locomotor control has been extensively studied, not much is known about the sensory events that influence MLR activity. Locomotion potentially needs to be initiated in various contexts, which could be channeled to the MLR through sensory modalities. Here we investigated whether MLR neurons show increases in activity in response to unconditioned sensory stimulations in freely moving mice. First, to validate our ability to target the glutamatergic ($Vglut2^+$) and GABAergic ($VGAT^+$) MLR neurons, we injected a virus encoding for channelrhodopsin in a Cre-dependent manner in the MLR of Vglut2-Cre and VGAT-Cre mice. Applying blue light to the MLR evoked locomotion in Vglut2-Cre mice and stopped locomotion or evoked backwards locomotion in VGAT-Cre mice. Then, we used a genetically encoded calcium sensor (GCaMP7f), expressed through virus injection in the MLR, to record activity of $Vglut2^+$ and $VGAT^+$ MLR neurons with fiber photometry. When Vglut2-Cre mice were made to walk on a motorized treadmill, calcium signals increased in the $Vglut2^+$ MLR neurons, confirming the locomotor status of these neurons.

Remarkably, calcium signals in the MLR also increased during treadmill locomotion in VGAT-Cre mice. An air puff applied to the mouse body, a brief unexpected sound, or a looming stimulus all evoked calcium increases in both Vglut2^{+} and VGAT^{+} MLR neurons, indicating that both types of neurons can encode sensory information. Together, results suggest that sensory stimuli corresponding to potentially harmful events are channeled to the MLR, where they can be integrated into an escape response by glutamatergic neurons or a stop and/or retreat response by GABAergic neurons.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Spinal inhibitory interneurons interposed in afferent pathways to Shox2 interneurons

Authors: *J. R. MCGRATH, D. GARCIA-RAMIREZ, K. J. DOUGHERTY;
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Abstract: Spinal cord injury (SCI) disrupts descending control of spinal circuitry, often leading to severe locomotor deficits. Essential locomotor circuits that generate both the rhythm and pattern of locomotion are located in the lumbar segments of the spinal cord; thus, below most SCIs, and relatively intact after injury. A population of locomotor circuit interneurons (INs) identified by the transcription factor Shox2 is involved in both rhythm generation and pattern formation, providing a possible access point to control locomotor function. Efforts to improve locomotor recovery after SCI, such as epidural stimulation (ES), target the locomotor circuits via the activation of sensory afferents. Afferent pathways to Shox2 INs have been shown to be either excitatory or inhibitory in near equivalent proportions. However, in chronic complete SCI, there is an excitatory shift in afferent input to Shox2 INs. Recent data from our lab demonstrates that treatment with sub-motor-threshold ES restores sensory-evoked inhibitory input to Shox2 INs. This suggests that a population of inhibitory INs involved in sensory afferent pathways to Shox2 INs are a novel point of plasticity, and a potential therapeutic target following SCI. In this study, we aim to identify inhibitory INs interposed in sensory pathways to Shox2 INs. We established 2 triple transgenic mouse lines by crossing mouse lines that label inhibitory neurons (either GAD67-GFP or GlyT2-GFP) with a mouse line that labels Shox2 INs (Shox2cre; R26-lsl-tdTomato). We used lumbar spinal slices from neonatal mice in each transgenic line, and further subdivided inhibitory neurons based on laminar location. To test for connections between

inhibitory neurons and Shox2 INs, inhibitory neurons were stimulated pharmacologically by picospritzing of kainate, during simultaneous whole cell patch clamp recordings of the response of single Shox2 INs. We found that glycinergic neurons are more likely to be connected to Shox2 INs than GABAergic neurons. The glycinergic neurons with connections to Shox2 INs are largely restricted to medial lamina IV/V. This region coincides with an area containing dense parvalbumin labeling, indicating that it is a termination zone of proprioceptive afferent terminals. These findings identify a population of glycinergic neurons that are presynaptic to Shox2 INs, and potentially involved in the inhibitory control of Shox2 INs by low threshold afferent pathways.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Topic: E.07. Rhythmic Motor Pattern Generation

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Title: Hindlimb stepping on different treadmill belt speeds in neonatal spinal-transected rats

Authors: *A. L. BOZEMAN, M. R. BRUMLEY;
Psychology, Idaho State Univ., Pocatello, ID

Abstract: Background: The purpose of this study was to better understand plasticity of the developing spinal cord by examining the effect of treadmill speed on hindlimb stepping in rats with a neonatal spinal cord transection. Previous research has shown that the neural circuitry supporting stepping is located in the spinal cord. This circuitry can be activated by serotonergic stimulation, and the motor output can be regulated by sensory stimuli. In the present study, stepping was induced with a serotonergic receptor agonist and we tested if stepping behavior was modulated by treadmill speed. **Methods:** On postnatal day 1 (P1), rats received a low-thoracic spinal cord transection surgery or sham operation. On P5, they were suspended over a miniature treadmill for a 30-minute test session. Subjects then received a 0.75 microliter intraperitoneal injection of 3.0 mg/kg quipazine (a 5-HT_{2A} receptor agonist) to induce stepping. There were four treadmill belt speed groups: slow (1.6 cm/s), medium (3.2 cm/s), fast (4.8 cm/s), and non-moving (control). There were six subjects per treadmill speed group and surgery condition. During video playback, interlimb coordination (hindlimb stepping) and intralimb coordination (hindlimb kinematic measurements) were scored. A repeated-measures ANOVA was used to analyze interlimb coordination, and a Mixed Models analysis was used to analyze intralimb coordination. **Results:** Spinal rats showed significantly more hindlimb steps and total hindlimb movements compared to shams, which is consistent with hindlimb supersensitivity to serotonin following

spinal injury. On the non-moving control belt, spinal rats had significantly longer step cycle durations whereas shams had shorter step durations. This was accounted for by changes in the swing phase of the step cycle. All subjects had significantly larger step areas on the non-moving belt compared to the moving belt speeds, which was due to longer steps in spinal subjects and higher steps in shams. **Conclusions**: The developing, isolated spinal cord *in vivo* can modulate hindlimb stepping in response to a moving treadmill belt following serotonergic activation. Although treadmill belt speed did not influence interlimb coordination (i.e., number of hindlimb steps), changes in intralimb coordination suggest significant adaptations due to sensory responsiveness of spinal circuitry. Adaptations to different speeds may be related to developmental changes in weight-bearing locomotion.

Disclosures: A.L. Bozeman: None. **M.R. Brumley:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Pyramidal activation of reticulospinal neurons in the neonatal mouse

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Abstract: Corticoreticular (**CR**) axons run together with corticospinal axons in the pyramids on each side of the midline. The CR projection, which includes collaterals of corticospinal axons, terminates bilaterally in the reticular formation where reticulospinal (**RS**) neurons controlling hindlimb movements reside. Electrophysiological evidence of functional connections between CR and RS neurons has been reported in adult mammals but it is not known if these connections are functional in young mammals. Here we investigate CR-RS connections in P0-P2 mice, i.e., at an age where corticospinal axons have not yet reached the lumbar spinal cord and therefore cannot directly recruit hindlimb motoneurons (**MNs**). To activate CR axons and assess the effect of this activation on RS neurons, we stimulated the pyramids unilaterally (bregma -7.23 mm; 5 x 200 µs, 10 Hz) and recorded synaptically evoked calcium transients from RS neurons previously labeled with Calcium Green-1 Dextran Amine (**CGDA**). We found that pyramidal stimulation selectively recruited RS neurons on the ipsilateral side (same side as the stimulation) and that this recruitment was eliminated by application of the ionotropic glutamate receptors antagonist kynurenic acid (5mM), consistent with blockade of excitatory transmission between CR axons and RS neurons. Finally, to test the impact of pyramidal stimulation on motor output, we

stimulated the pyramid unilaterally while we recorded calcium transients from L2 MNs from each side of the midline. We found that pyramidal stimulation led to the recruitment of contralateral MNs, and predominantly those located in the lateral motor column. Pyramidal recruitment of contralateral MNs was greatly reduced by a high cervical, contralateral hemisection (side opposite to the stimulation), compatible with a contribution from ipsilateral RS neurons with crossed descending axons.These results suggest that CR-RS connections are already functional at birth to allow for early cortical modulation of the reticulospinal pathways controlling hindlimb movements.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Only certain phase relationships of alpha-gamma coordination facilitate voluntary movement

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Abstract: It is still unknown in detail to what extent and in what way spinally-mediated sensorimotor mechanisms contribute to natural voluntary movement. Specifically, there is a need to disambiguate the functional contribution of descending alpha (α) and gamma ($\gamma_{dynamic}$, γ_{static}) cortico-spinal projections from propriospinal, sensory, and proprioceptive projections. As an extension of our neuromorphic computational approach in Jalaledini et al. (2017), Niu et al. (2017) and Nagamori et al. (2021), we actuated a tendon-driven single-joint robotic finger using motors programmed to act in real-time as an agonist-antagonist pair of Hill-Type muscles. In addition to modeling spiking neurons (Izhikevich, 2003), muscle spindles (Mileusnic et al., 2006) and Golgi tendon organs (Mileusnic and Loeb, 2006), their stretch reflex pathways were also innervated by descending α, $\gamma_{dynamic}$, and γ_{static} drives. The α drive (in pulses per second, pps)

was set to produce slow sinusoidal or point-to-point movements on the robotic finger's joint. By sweeping across various values of amplitude and relative phase of the $\gamma_{dynamic}$ and γ_{static} drives to the two muscles, we were able to quantify the effect of these various α-γ interactions on joint kinematics. 'Realism of movement' was quantified using three metrics: 1. *magnitude of voluntary movement*, measured in degrees; 2. deviation from *minimum jerk* to measure smoothness; and 3. *two-thirds power law* to compare movements with varying parameters. We saw that only a particular, typically phase-advanced family of γ drive profiles enables greater sinusoidal joint movements. Also, careful scheduling of γ drives during the ramp and hold phases is crucial to accurately start and stop point-to-point movement. Our results highlight that only certain families of task-specific amplitudes and phases of γ drives are sufficient (yet not necessary) to produce naturalistic motor action. This warrants further study in human neuromorphic models to validate the nuanced role of descending α-γ cortico-spinal commands in enabling spinal circuits to produce natural voluntary movement.

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Poster

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Title: How is voluntary movement disrupted in the presence of muscle afferentation?

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Abstract: Multiple computational models of neuromuscular control include cortico-spinal drive as the primary (or only) command signal to a muscle. However, actual α-motoneuron activation results from summation of excitatory and inhibitory descending, propriospinal, sensory and proprioceptive synaptic inputs. Muscle spindles provide homologous and heteronomous proprioceptive inputs encoding muscle fiber length and velocity. While γ-motoneurons can modulate these spindle outputs, the extent to which the spindle feedback inputs alter limb kinematics is unknown. Here we model the functional effects of excitatory spindle afferent signals on limb kinematics to quantify whether and how the open loop descending cortico-spinal

drive to α-motoneurons needs to be adjusted to counterbalance spindle afferent signals. Similar to (Hagen and Valero-Cuevas 2017), we used a 31-muscle Macaque arm model in MuJoCo and generated 100 open loop α-motoneuron commands that produced random free arm movements lasting 2 seconds starting from rest. We then systematically added excitatory monosynaptic spindle afferent to each muscle. We compared the baseline motion to the resulting disrupted trajectories and endpoint location after using five incremental feedback gains, proportional to the lengthening and eccentric velocity of each muscle. As expected, movements inducing greater fiber lengthening and eccentric velocities tended to be more disrupted as gain increased. However, these trajectory and endpoint disruption were neither linear nor necessarily kinematically significant. Our findings highlight that each arm movement must have a distinct, nonlinear compensatory interaction between α and γ motoneuron drives, which can range from subtle to strong. Moreover, our conceptual approach to computational neuromuscular control and learning should be broadened to encompass dynamic muscle afferentation.

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Poster

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Title: Chronic hypoxia rescues swallow-breathing coordination in a mouse model of Leigh Syndrome

Authors: *A. HUFF[1], M. K. AMARANTE[1], J. M. RAMIREZ[2,4], F. K. KALUME[3,4,5];
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Abstract: Difficulty swallowing, also known as dysphagia, hinders eating and is among the first clinical symptoms of Leigh Syndrome (LS). LS is a severe neurometabolic disorder and the most common form of mitochondrial disorder in the pediatric population. It has been linked to loss of function mutations in *Ndufs4*, the gene that codes for a subunit of the mitochondrial complex I. Mice lacking *Ndufs4* globally, constitutes as an excellent model of LS, mimicking several core symptoms of the disease in humans including seizures, ataxia, hypotonia, rigidity, weight loss,

and failure to thrive. In our previous work, we demonstrated that excitatory and inhibitory neurons drive distinct phenotypes in the mouse model. Knock out (KO) of *Ndufs4* in mice, specifically in glutamatergic (*Vglut2)* neurons, reproduces motor dysfunctions associated with LS. Whereas KO of the gene in GABAergic neurons primarily lead to epilepsy. Here we uncovered that KO of *Ndufs4* gene, specifically in the *Vglut2* neurons, mimics swallow dysfunction seen in LS. Using freely breathing urethane anesthetized adult Vglut2/Ndufs4 KO mice, swallow was evoked by injection of water into the oral cavity. Swallow respiratory activity was measured via monopolar suction electrodes of the hypoglossal (XII) and vagus (X) nerves as well as bipolar electromyogram (EMG) of the submental, laryngeal complex and costal diaphragm muscles. This protocol was performed in Vglut2/Ndufs4 KO mice 1) exposed to room air and 2) exposed to 11% oxygen for 6 months. We found, mice exposed to chronic hypoxia (CH) no longer presented with symptoms such as: ataxia, hunched posture, unbalanced, clasping, weight loss, etc, as seen in mice exposed to room air (RA). CH mice lived significantly longer than RA mice (225 ±6, 111±19, $p= 0.000$). CH mice weighed significantly more than RA mice (26 ±2g, 19±2g, $p= 0.000$). We also saw a discoordination in swallowing and breathing in RA mice with 1) swallows occurring during inspiration, 2) various abnormal swallow related phenotypes, 3) swallow induced apneas for upwards of 30s, and 4) variable reset of the respiratory rhythm. CH mice showed no evidence of the former occurring, indicating CH rescues swallow-breathing coordination. This study gives first insights into possible mechanisms of LS and treatment for dysphagia in LS.

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Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

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Title: Interdependent intrinsic and network properties for the emergence of rhythm in a model preBötC network

Authors: *R. S. PHILLIPS[1], N. A. BAERTSCH[2];
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Abstract: The preBötzinger complex (preBötC) produces respiratory rhythm in the mammalian brainstem and is perhaps one of the most extensively studied CPGs. Yet, despite decades of research aimed at defining the underlying biophysical mechanism of rhythmogensis, the relative roles of cellular and network properties remain controversial. Much of the contemporary debate

revolves around a persistent sodium current (I_{NaP}), which is widely expressed among preBötC neurons. In the absence of network interactions, a small fraction of preBötC neurons exhibit intrinsic rhythmic bursting that requires the voltage-dependent properties of I_{NaP}. This inspired the "pacemaker hypothesis" which posits that these intrinsic bursters or "pacemaker" neurons play a unique role in the genesis of the preBötC network rhythm. Subsequently, computational models of the preBötC network were developed and tuned such that, when synaptically coupled, I_{NaP}-dependent intrinsic bursters give rise to rhythm at the network level. As a result, the concept of pacemaker-driven rhythm generation has become somewhat synonymous with I_{NaP}-expression and vice versa. However, there are far more I_{NaP}-expressing neurons than intrinsic bursters in the preBötC. We hypothesized that other cellular characteristics among preBötC neurons, such as action potential shape, affect the expression of intrinsic bursting through interactions with the voltage-dependent properties of I_{NaP}. Using computational modeling and dynamical systems analysis, we demonstrate that modest increases in spike height or afterhyperpolarization (AHP) transform intrinsic bursting into a tonic spiking phenotype, even in neurons with high I_{NaP} expression. In a simulated network of neurons with distributed I_{NaP} conductances, increasing spike height or AHP, to abolish all intrinsic bursting properties, did not eliminate the network rhythm. Instead, these networks, comprised of intrinsically tonic and silent neurons, produced a low amplitude augmenting rhythm similar to "burstlets" produced by the preBötC under conditions of reduced excitability or following suppression of Ca^{2+} channels. Burstlet theory, presented as an alternative to the pacemaker hypothesis, posits that these low amplitude oscillations arise from feed-forward synaptic excitation among tonic spiking neurons that does not require intrinsic bursting or I_{NaP}. Our simulations clearly illustrate a middle ground where synaptic interactions among tonic spiking neurons and the voltage-dependent properties of I_{NaP}, but not intrinsic bursting, can be interdependent characteristics of the preBötC network that lead to an emergent respiratory rhythm.

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Poster

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Title: Using optogenetic approaches to unravel the role of preBötzinger complex and postinspiratory complex in swallow-breathing coordination

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Abstract: The coordination of swallowing with breathing, in particular inspiration and postinspiration, is essential for homeostasis in most organisms. While much has been learned about the neuronal network critical for inspiration, the preBötzinger complex (preBötC), little is known how this network interacts with swallowing. Even less is known about the neural network critical for postinspiration (PiCo) and its role in swallow-breathing coordination. Using a freely breathing anesthetized mouse model, swallows were stimulated by 1) water injected into the oral cavity, 2) simultaneously water injection without laser activation of preBötC, and 3) laser activation of PiCo using optogenetic techniques. Swallow and laryngeal activity were measured via monopolar suction electrodes of the hypoglossal (XII) and vagus (X) nerves as well as bipolar electromyogram (EMG) of the submental, laryngeal complex and costal diaphragm muscles. Optogenetic activation within the preBötC of Dbx1, *Vglut2, Sst,* and *Vgat* neurons, and inhibition of Dbx1 neurons as well as within PiCo of *ChAT*, *Vglut2*, and doubly labeled *ChAT/Vglut2* neurons will gain a first understanding of the coordination between the preBötC, PiCo and swallow behavior. We found that swallow-induced suppression of inspiratory activity is not directly mediated by the inhibitory neurons in the preBötC. Stimulation of preBötC *Dbx1* neurons delayed laryngeal closure of the swallow sequence. Inhibition of *Dbx1* neurons increased laryngeal closure duration and stimulation of *Sst* neurons pushed swallow occurrence to later in the respiratory cycle, suggesting that excitatory neurons from the preBötC connect to the laryngeal motoneurons and contribute to the timing of swallowing. Activation of PiCo neurons, both *ChAT, Vglut2*, and doubly labeled, triggered both swallow and laryngeal closure in a phase dependent manner. Stimulation of PiCo neurons during inspiration or immediately after, is significantly more likely to trigger a swallow then laryngeal closure which is more likely to be triggered toward the end of expiration. We then introduced chronic intermittent Hypoxia (CIH), a model for sleep apnea, known to 1) destabilize inspiratory activity and 2) is associated with dysphagia. Interestingly, the delayed swallow sequence, shown with *Dbx1* stimulation, was also caused by CIH, as well as a significant decrease in the probability of triggering a swallow when PiCo neurons were activated, regardless of respiratory phase. We propose that a stable preBötC and PiCo are essential for normal swallow pattern generation and disruption may contribute to dysphagia seen in obstructive sleep apnea.

Disclosures: A. Huff: None. **M.K. Amarante:** None. **L.M. Oliveira:** None. **J. Ramirez:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.20

Topic: E.07. Rhythmic Motor Pattern Generation

Support: CONACYT A1-S-14473
Intramural Research Program of the NIH, NINDS

Title: Effects of reducing excitatory synaptic strength by blockade of vesicular glutamate transporters on inspiratory burst frequency, amplitude, and duration in vitro

Authors: *C. MORGADO-VALLE[1], J. C. SMITH[2], E. VAZQUEZ-MENDOZA[1], H. KOIZUMI[2], L. LOPEZ-MERAZ[1], L. BELTRAN-PARRAZAL[1];
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Abstract: The inspiratory phase of the respiratory rhythm is generated in the brainstem preBötzinger complex (preBötC), containing a neuronal network of interacting glutamatergic, GABAergic and glycinergic neurons. The glutamatergic excitatory synaptic interactions in the preBötC are essential for inspiratory rhythm and burst pattern generation. PreBötC inspiratory neurons fire bursts of action potentials on top of a 10-15 mV depolarization dubbed the inspiratory drive potential. Synaptic currents underlying this excitatory drive are mainly driven by activation of glutamatergic non-NMDA receptors, since progressive blockade of these receptors by CNQX reduces excitatory synaptic currents and ultimately eliminates the inspiratory rhythm. Inspiratory burst frequency is also dependent on voltage-gated, subthreshold activating conductances in the excitatory neurons, such as persistent sodium current (I_{NaP}), whereas amplitude is strongly regulated by calcium-activated non-selective cationic current (I_{CAN}). Understanding the contributions of excitatory synaptic interactions, I_{NaP}, and I_{CAN} is a current problem that has been under intense investigation by experimental and modeling approaches. In this study, we focused on analyzing how the strength of excitatory synaptic transmission regulates inspiratory burst frequency, amplitude, and duration. We used a novel strategy in rhythmically active rat transverse slice preparations *in vitro*, in which we bath-applied the vesicular glutamate transporter (VGLUT) blocker Rose Bengal (RB), a fluorescein analog that is a known potent inhibitor of glutamate uptake into synaptic vesicles, to interfere with glutamatergic synaptic transmission. From bilateral recordings of preBötC population activity, we analyzed perturbations of the inspiratory rhythm and pattern, and we found a paradoxical increase of inspiratory burst frequency, accompanied by a reduction in the amplitude and duration of inspiratory bursts. Cellular-level recordings of synaptic drive potentials and other measurements of post-synaptic currents confirmed the efficacy of RB to interfere with excitatory synaptic transmission. These perturbations are consistent with recent neuronal biophysical network models that predict how the dynamical operation of preBötC excitatory networks depends on synaptic strength together with neuronal biophysical properties including I_{NaP} and I_{CAN}.

Disclosures: C. Morgado-Valle: None. **J.C. Smith:** None. **E. Vazquez-Mendoza:** None. **H. Koizumi:** None. **L. Lopez-Meraz:** None. **L. Beltran-Parrazal:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

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Program #/Poster #: 061.21

Topic: E.07. Rhythmic Motor Pattern Generation

Support: FAPESP GRANT 2018/15957-2
FAPESP GRANT 2019/11863-6
FAPESP GRANT 2021/06886-7

Title: Activation of astrocytes in the lateral parafacial region increases ventilation, expiration and sympathetic activity in mice

Authors: *D. MORAES[1], B. H. MACHADO[2], M. P. SILVA[3];
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Abstract: The lateral aspect of the parafacial (pFL) region, which contains expiratory neurons, controls pulmonary ventilation, expiratory abdominal muscles (active expiration) and sympathetic outflow during high chemical drive (hypercapnia/acidosis). Astrocytes modulate the neuronal activity/excitability and synaptic transmission as well as control the activity of motor circuits. Herein, we investigated the contribution of pFL local astrocytes in the control of pulmonary ventilation, expiratory neuronal and neural activities and their impact on the sympathetic outflow and cardiovascular function of mice (7 weeks old - C57Bl/6). The pFL astrocytes were unilaterally activated by virally driven expression of designer receptors exclusively activated by designer drugs (hM3Dq) using the glial fibrillary acidic protein (GFAP) promoter. The *in vivo* and *in situ* preparations of pFL-GFAP-hM3Dq mice received systemically clozapine N-oxide (90 µM) while the pulmonary ventilation, as well as the activities of pFL expiratory neurons, inspiratory (phrenic), expiratory (abdominal) and sympathetic (thoracic) nerves were recorded. We observed that GFAP-hM3Dq-signaling activation increases pulmonary ventilation, induces an active expiratory pattern, with increased abdominal activity at the end of expiration, and evokes the pFL expiratory neurons firing. GFAP-hM3Dq-signaling activation also enhanced the sympathetic activity and perfusion pressure levels, without affecting the heart rate. On the other hand, GFAP-hM3Dq-signaling activation did not affect the hypercapnia-evoked active expiration and the concomitant response of sympathetic outflow. Besides, clozapine N-oxide did not affect the respiratory or sympathetic outflows in the absence of hM3Dq expression. These findings demonstrate that astrocytes modulate the activity of pFL neuronal circuit controlling pulmonary ventilation, as well as the expiratory and sympathetic activities in mice.

Disclosures: D. Moraes: None. **B.H. Machado:** None. **M.P. Silva:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.22

Topic: E.09. Motor Neurons and Muscle

Support: NIH-NINDS R00NS114194

Title: A machine learning approach to simplify modeling of spinal locomotor circuitry

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Abstract: While much progress has been made identifying and characterizing cell types relevant for vertebrate locomotion, understanding the functional roles of connectivity patterns among subpopulations has remained a challenge. Biophysically detailed models can reproduce many of the main behavioral features of the spinal locomotor circuit, but the complexity of such models makes it difficult to determine what role is played by single-cell properties, and what is due to patterns of network connectivity. Here we use a rate-based model with homogeneous single-neuron parameters to explore the specific role of connectivity in the function of zebrafish spinal locomotor circuitry, trading biological realism for simplicity and interpretability. Including experimentally derived constraints on the connectivity between different interneuron subpopulations in zebrafish, and using machine learning to optimize the remaining free synaptic weights, the model reproduces two distinctive features of axial-based swimming: (i) inter-segmental phase lag that is independent of swim frequency and (ii) the progressive recruitment of speed modules with increasing swim frequency. Constant phase lag and modular recruitment remain possible even when all the neurons within each subpopulation are identical at the single-cell level and are distinguished only through their connectivity. This demonstrates that these and possibly other features do not necessarily rely on specific single-neuron properties, and instead can arise from connectivity alone. Our approach to spinal locomotor circuit modeling is a promising path for assessing functional connectivity, enabling predictions about the existence and relative strengths of synaptic connections between subpopulations in zebrafish, as well as in species with more complex spinal circuitry.

Disclosures: B. Lemberger: None. **D. McLean:** None. **J. Murray:** None.

Poster

061. Pattern Generation: Intrinsic, Afferent, and Descending Control

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 061.23

Topic: E.03. Basal Ganglia

Support: Novo Nordisk Foundation Laureate Grant NNF15OC0014186
Lundbeck Foundation R276-2018-183 and R345-2020-1769
Faculty of Health and Medical Sciences, University of Copenhagen

Title: Pedunculopontine neurons for global motor arrest

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[3]Dept. of Neurosci., Karolinska Institutet, Stockholm, Sweden

Abstract: The episodic nature of movement requires neural mechanisms for movement initiation, maintenance, and arrest. Motor arrest mechanisms are actively engaged in diverse contexts such as the termination of goal-directed movements, or as part of behavioral responses that require a global motor arrest with a specific purpose. Examples include fear-related defensive freezing to avoid being detected by predators, or behavioral interruption upon (a) detection of environmental cues to evaluate whether they represent a threat, or (b) to switch tasks if required for an adequate behavioral output adapted to external needs. Within global motor arrest, previous studies have mostly focused on the circuits mediating defensive freezing. However, the neuronal circuits that connect with the executive motor circuits to implement a global motor arrest in non-defensive contexts are poorly understood.
Using a combination of anatomical, physiological, and behavioral techniques, we report the discovery that glutamatergic $Chx10^+$ neurons in the pedunculopontine nucleus (PPN) evoke global motor arrest in mice. First, we describe a subpopulation of glutamatergic neurons within the PPN that expresses the transcription factor Chx10 (Chx10-PPN) and has a rostral bias, with higher neuron density within the rostral-half of the nucleus. Second, we show that optogenetic activation of Chx10-PPN neurons instantaneously interrupts all ongoing movements including locomotion, grooming, and rearing. The motor arrest is time-locked to the activation of Chx10-PPN neurons and, after stimulus offset, mice quickly resume the activity they were previously engaged in. Third, we characterize the context-dependent nature of the kinematic and muscle activation pattern observed upon motor interruption. Finally, we find that Chx10-PPN neurons also affect the respiratory and cardiac rhythms because the evoked motor arrest is reliably accompanied by apnea and bradycardia. In addition, we demonstrate that the global motor arrest evoked from Chx10-PPN neurons differs from the defensive freezing evoked from glutamatergic neurons in the ventrolateral periaqueductal gray (vlPAG), both in its motor and autonomic components.
Thus, our study (1) defines a group of excitatory neurons that triggers a global motor arrest different from defensive freezing, and (2) identifies a locomotor-opposing role for $Chx10^+$ glutamatergic neurons in the rostral PPN, contrary to the locomotor-promoting role of the (mainly non-Chx10) glutamatergic neurons in the caudal PPN.

Disclosures: H. Goñi-Erro: None. **R. Leiras:** None. **R. Selvan:** None. **O. Kiehn:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.01

Topic: F.03. Stress and the Brain

Support: NHMRC Ideas grant 2011753

Title: Nucleus of the solitary tract activation dynamically alters anxiety-like behavior in rats

Authors: *A. G. BUTLER[1], M. R. MELO[1], A. A. CONNELLY[1], J. K. BASSI[1], A. M. ALLEN[1], S. J. MCDOUGALL[2];
[1]Anat. and Physiol., The Univ. of Melbourne, Parkville, Australia; [2]Florey Inst. of Neurosci. and Mental Hlth., Melbourne, Australia

Abstract: Vagal nerve stimulation (VNS) affects multiple functions including memory and emotional states, such as anxiety. The nucleus of the solitary tract (NTS), which receives the vagal afferent input likely responsible for these effects of VNS and is essential for maintaining autonomic balance, contains multiple cell groups with heterogeneous neurochemical phenotypes and widespread axonal projections to subcortical autonomic, neuroendocrine and neuromodulatory systems - including the locus coeruleus (LC). Whilst this neuroanatomy provides a basis for the involvement of the NTS in anxiety-like behavior, the physiological evidence for this is lacking and the role of the NTS in modulating behavior is poorly understood. To determine if the NTS can influence anxiety-like behavior we mimicked VNS through optogenetic stimulation of the NTS. AAV2-CAG-ChR2-mCherry (n=13) or AAV2-CAG-mCherry (n=10) were injected into the NTS of ketamine/medetomidine anesthetized male Sprague Dawley rats (300 g). Two weeks later, the rats were instrumented to record the diaphragm electromyograph (breathing) and the electrocardiogram (heart rate) during optical activation and behavioral testing. Anxiety-like behavior was assessed using an elevated plus maze (EPM) and novelty suppressed feeding test (NSF). The NTS was optically activated for 5 min (10 mW, 10 ms duration at 20 Hz, 15 s on/5 s off), in an acclimatization box, prior to each test. On the last day of testing the animals received optogenetic or sham stimulation in the same box and were perfused 90 min later to measure Fos expression in the NTS and LC. Optogenetic stimulation of the NTS cells elicited an immediate, small tachycardia which was time locked to each 15 s on period and returned to base line during each 5 s off period of the 5 min duty cycle. In both the EPM and NSF optogenetic stimulation of the NTS induced an anxiogenic phenotype, characterized by less time spent in the open arms (EPM) and an increased latency to eat (NSF), relative to the shams. Interestingly, after the NSF, when reintroduced to the home cage, the NTS stimulated group ate less food, suggesting an effect on satiety. These data demonstrate a direct role for the NTS in anxiety-like behavior and provide a foundation for identifying the neural pathways responsible for this effect and their role in VNS.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.02

Topic: F.03. Stress and the Brain

Support: R21 DA052815

Title: A role for delta opioid receptors in the locus coeruleus in mediating the behavioral response to stress

Authors: *J. TKACZYNSKI, O. BORODOVITSYNA, D. J. CHANDLER;
Cell Biol. and Neurosci., Rowan Univ., Stratford, NJ

Abstract: Stress is a physiological state that occurs in response to threatening stimuli that is characterized by specific adaptations to aid in survival. The noradrenergic locus coeruleus (LC) is a structure in the brain that has been shown to be stress-responsive and contribute to anxiety-like behaviors. Previous work from our laboratory has shown that acute exposure to predator odor and restraint stress promotes long-term increases in anxiety-like behaviors and LC excitability that correlate with downregulation of the δ opioid receptor (DOR) in adolescent male rats. Because of the well established relationship between LC activity and anxiety-like behavior, and the inhibitory role of opioidergic neurotransmission within the LC, we sought to further clarify the role for DORs in the LC in mediating the behavioral response to stress. To this end, we induced overexpression of DORs within the LC with the viral construct AAV-PRSX8-oprd1 or induced expression of the red fluorescent protein mCherry as a control. Rats were then subjected to control conditions or stressor exposure and anxiety-like and coping behaviors were measured. Immediately after stressor exposure, DOR overexpressing animals spent significantly more time in the open arms of the elevated plus maze when compared to the mCherry-expressing stress group. One week later, there was a significant difference in time in the center of the open field test between the mCherry control and stress groups, but not the DOR overexpressing control and stress groups, indicating a protective effect of DOR overexpression on anxiety-like behavior. In addition, a separate group of animals overexpressing DORs in LC underwent testing in the defensive shock probe burying task. Preliminary findings suggest that DOR overexpression reduces burying behavior in this task, although further studies are needed to determine if this represents active or passive coping. Collectively, these findings suggest that increased DOR signaling in the LC occludes the effects of stress on anxiety-like behavior and promotes stress resilience.

Disclosures: J. Tkaczynski: None. **O. Borodovitsyna:** None. **D.J. Chandler:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.03

Topic: F.03. Stress and the Brain

Support: DOD USAMRAA Grant W81XWH2110877
Kerman Family Fund for Parkinson's Research
Selma Schottenstein Harris Lab for Research in Parkinson's

Gardner Family Center for Parkinson's Disease and Movement Disorders
Parkinson's Disease Support Network of Ohio, Kentucky & Indiana
Cohen Veterans Bioscience

Title: Sex differences in glucocorticoid regulation of midbrain dopamine neurons in rat

Authors: J. P. HERMAN[1], S. E. MARTELLE[1], J. B. CHAMBERS[1], K. H. LUNDGREN[2], ***K. B. SEROOGY**[2];
[1]Pharmacol. and Systems Physiol., [2]Neurol., Univ. of Cincinnati, Cincinnati, OH

Abstract: Glucocorticoid receptors (GR) are expressed in midbrain dopamine neurons and are thought to be involved in transducing negative effects of glucocorticoids on cell survival in Parkinson's disease (PD) models. To test involvement of GR in mediating motor and non-motor behaviors relevant to PD, we generated a rat GR knockout in dopamine neurons by crossing GR flox rats with a DAT1-Cre driver line. Gene deletion in midbrain dopamine neurons was confirmed by immunohistochemistry, demonstrating lack of GR in midbrain neurons expressing tyrosine hydroxylase. We then performed analyses of locomotor and cognitive behaviors in cohorts of adult male and female DAT1-Cre-directed GR deletion (DAT1-$Cre^{+/-}$:$NR3C1^{fl/fl}$) rats and Cre-negative controls (DAT1-$Cre^{-/-}$:$NR3C1^{fl/fl}$). Our data indicate that females with GR deletion show reduced locomotor activity in the open field relative to controls, suggestive of a mild locomotor phenotype. Locomotor activity was not reduced in male DAT1-Cre GR knockout rats, possibly due to enhanced variability in this group. No effects of DAT1-Cre deletion were observed in novel object recognition in either sex. Examination of emotional memory was tested using auditory fear conditioning. We did not observe changes in acquisition of conditioned fear. However, retention of fear was impaired in male (but not female) DAT1-Cre knockout animals, suggesting a role for GR in initial expression of conditioned fear via dopamine neurons. There was no effect of GR deletion on extinction or reinstatement of fear conditioning. Together, these results provide important data indicating that DAT1-Cre-directed GR deletion produces functional changes in dopamine-related motor and non-motor behaviors, including locomotion and fear memory. Our data implicate GR-dopamine interaction in mediating behaviors relevant to motor and non-motor symptoms in our stress/PD model.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 062.04

Topic: F.03. Stress and the Brain

Support: Canadian Institute for Health Research 201903PJT-419517-PT

Title: The role of noradrenergic transmission in resilience to unavoidable stress.

Authors: *M. BAIRACHNAYA[1], E. ISINGRINI[2], B. GIROS[1,2];
[1]Psychiatry - The Douglas Res. Ctr., McGill Univ., Montreal, QC, Canada; [2]CNRS UMR 8002 - INCC, Univ. de Paris-Cité, Paris, France

Abstract: Major depressive disorder is a stress-related disorder associated with a significant impairment of functioning and quality of life. It is well documented that although existing therapeutical approaches could reduce manifestation of clinically important symptoms, a substantial proportion of patients show low remission rate, indicating the urgent need for new effective treatment strategies. In recent decades, progress has been made regarding the understanding of the causal psychological and molecular processes, nonetheless, coping mechanisms underlying individual differences in stress-response, as well as predisposition to stress resilience or vulnerability remain open questions. Moreover, little is known about the contribution of noradrenergic transmission to resilience to stress-related depressive disorders. In the current study, we used a unique transgenic mouse model of conditional KO for vesicular monoamine transporter type-2 (VMAT2) in dopamine beta hydroxylase (DBH) neuron population allowing central depletion of noradrenaline release ($VMAT2^{DBHcre}$ KO mice). 90 male mice (WT n = 39, KO n = 51) were tested using learned helplessness (LH) protocol, a common stress-related animal model of depression-like behavior. The footshock escape behavior (number of failures and latency to escape) was measured after 2 days of inescapable shock exposure and the data was analyzed using k-means clustering algorithm for further classification into stress-resilient (non-helpless) and stress-susceptible (helpless) cohorts. In addition, fiber photometry was used to record Ca^{2+} signals and to track the activity change of noradrenergic neurons throughout the LH test. We found that 10 days after the last session of inescapable footshock (TD10) susceptible KO mice showed a marked decrease in both number of failures and latency to escape as opposed to their WT littermates. Remarkably, by TD10 the number of susceptible KO animals decreased by 36.4%, while susceptible WTs only by 7.4%. In addition, the change in the squared mahalanobis distances to the centroid of the stress-resilient cohort was significantly reduced in susceptible KO mice (t-test, $p<0.05$) in comparison to WT, but was not significantly modified between resilient WT and KO mice. We also observed that the footshock rapidly induced an increase of GCaMP signals, indicating a strong activation of noradrenergic neurons in locus coeruleus, and thus providing a physiological correlate for aversive stimuli response.Taken together, our results confirm that depletion of noradrenaline in the brain leads to more pronounced extinction of helpless behavior.

Disclosures: **M. Bairachnaya:** None. **E. Isingrini:** None. **B. Giros:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.05

Topic: F.03. Stress and the Brain

Title: Giving up or waiting it out? Predicting stress resilience with ultrasonic vocalizations.

Authors: *N. STAFFORD[1], C. DONOVAN[2], R. CHASSE[2], C. PILGRAM[2], R. DRUGAN[3]; [1]Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [3]Psychology, [2]Univ. of New Hampshire, Durham, NH

Abstract: Rodent stress paradigms classify stress resilience/vulnerability in terms of coping behaviors. Adaptive coping associated with resilience is modeled by proactive control responses to escape a stressor. Inability to escape is considered passive coping, analogous to "giving up," and is implicated in anxiety or depressive-like behavior. Active coping blunts serotonergic (5HT) activity within the midrostrocaudal dorsal raphe (DR) that drives post-stress impairment. Recent challenges to the interpretation of passive strategies suggest it may be adaptive, yet, DR 5HT activity during adaptive passive coping is not well understood.

The rat 22-kHz ultrasonic vocalizations (USVs) are associated with adaptive passive behaviors, and recent studies identified 22-kHz USVs predicted stress resilience during an intermittent swim stress (ISS). Non-vocalizing rats exhibited negative post-ISS outcomes, while vocalizing rats appeared unaffected. USV emission was interpreted as a novel ethologically relevant predictor of post-stress functioning. It remains unclear if USVs are associated with active or passive coping during intermittent swim stress. The current study investigated coping behaviors, 5HT activity, and anxiety-like behaviors between vocalizing and non-vocalizing rats exposed to ISS.

Experiment 1 found differences between vocalizing and non-vocalizing rats on social anxiety and coping behaviors during ISS. ISS behavior was scored as passive (immobility) or active (swimming, climbing) per forced swim test conventions. Vocalizing rats engaged in consistent passive behaviors, while non-vocalizing rats engaged in consistent active behaviors. Experiment 2 replicated experiment 1, except brains were extracted 90min post-ISS for immunohistochemistry of cFos expression in 5HT cells within DR. Double-immunostaining to visualize cFos in 5HT cells found a consistent pattern of greater DR 5HT activity in vocalizing rats compared with non-vocalizing and control rats in dorsal, ventrolateral, and ventral subregions of dorsal raphe. These data may reflect a novel interpretation of passive coping that does not reflect "giving up." Here, an initial stress-susceptible organism that when faced with an inescapable stressor adopts a "wait it out" approach.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.06

Topic: F.03. Stress and the Brain

Support: NIH Grant R03HD097085
MSU Funds to JSL

Title: Sex differences in Midbrain Dorsal Raphe TPH and CRFR2 expression

Authors: *T. A. MEINHARDT[1], J. S. LONSTEIN[2];
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Abstract: Women are twice as likely to suffer from affective disorders than men, but the neurobiological etiology underlying this sex difference is not well understood. The midbrain dorsal raphe (DR), which provides a majority of the forebrain's serotonin, is linked to the expression of affective states and behaviors. The DR is sensitive to basal and stress-induced release of CRF and the related Urocortins through the expression of CRF receptors (CRFRs) that modulate DR serotonergic activity and behavioral responses to stress. There is little research directly comparing the sexes in their DR serotonin and CRF systems. Therefore, the goal of the current study was to quantify the expression of CRFR2 within serotonin and GABA cells of the DR in adult male and female rats. Using dual fluorescent *in-situ* hybridization (RNAscope, ACD Bio), the number of cells expressing *CRFR2* mRNA with either GAD_{65} or *TPH2* mRNA were quantified in four major subregions of the DR (rostral DR, dorsal DR, ventral DR, and lateral wings of the DR). The expression intensity of *TPH* and *CRFR2* fluorescence was also measured via thresholding analysis. Preliminary analyses revealed that males have more *TPH2*-expressing cells also expressing *CRFR2* in the lateral wings of the DR than do females. There was also a sex difference in the total number of *TPH2*-expressing cells in the ventral DR (M > F). These data suggest that serotonin cells in the male DR lateral wings are more sensitive to CRF and Urocortins than are those cells in females, which may allow males to have faster and/or more 5-HT release in response to stress. These differences may contribute to sex-based differences in forebrain serotonergic signaling and subsequent affective behaviors. In addition, these results could have implications for sex-based susceptibility to developing stress-related affective disorders.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

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Program #/Poster #: 062.07

Topic: F.03. Stress and the Brain

Support: NIMH grant R01MH122844-02

Title: 2-arachidonoylglycerol signaling in the lateral habenula: impacts on stress coping behavior and downstream immediate early gene expression

Authors: *H. R. WRIGHT[1], Z. D. G. FISHER[1], R. URRUTIA-CARMAGO[1], D. E. GINDER[2], A. M. BROWN[1], H. S. AREY[1], J. L. RITCHIE[1], R. A. FUCHS[1], R. J. MCLAUGHLIN[1,2];
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Abstract: Differences in stress coping strategies impact the development of stress-related disorders and are orchestrated in part by activity of dopamine (DA) neurons in the ventral tegmental area (VTA) and serotonin (5-HT) neurons in the dorsal raphe nucleus (DRN). The lateral habenula (LHb) regulates both DA and 5-HT activity via inhibitory neurons within the rostromedial tegmental nucleus that project to the VTA and DRN. Our data indicate that the endocannabinoid 2-arachidonoylglycerol (2-AG) is recruited in the LHb during stress, and intra-LHb cannabinoid type-1 receptor activation/blockade increases avoidance and approach behaviors respectively, suggesting that interfering with the synthesis or metabolism of 2-AG may affect LHb-mediated regulation of behavioral responses. However, the role of LHb 2-AG in the expression of stress coping strategies and the downstream mechanisms underlying these effects remain unknown. Thus, we examined whether altering LHb 2-AG concentration during acute or chronic social defeat stress (SDS) affects coping behaviors and downstream expression of the immediate early gene c-fos in the VTA and DRN. Bilateral cannula were aimed at the LHb in male Sprague Dawley rats (n=7-9/group). In exp. 1, rats received a microinfusion of the 2-AG hydrolysis inhibitor MJN110 (0, 0.5, or 1 μg/side) prior to acute SDS exposure. In exp. 2, rats underwent 7 daily SDS sessions and received a microinfusion of the 2-AG synthesis inhibitor DO34 (0, 0.07, or 0.7 μg/side) prior to the last session. Behavior was analyzed with pose estimation and predictive classifier software. In exp. 2, VTA and DRN brain slices were stained for fos, and DA or 5-HT markers for immunofluorescence analysis. Results indicate that rats receiving 1μg MJN110 during acute SDS traveled less distance, and trended toward being attacked less than vehicle-treated rats. In exp. 2, all rats traveled less on day 7 than day 1. However, rats receiving 0.7 μg DO34 before the final session trended towards moving less, being attacked less and investigated more by the resident rat. Thus, both LHb 2-AG manipulations resulted in passive behaviors consistent with LHb activation, without affecting approach or avoidance behaviors per se. Notably, only 28% of 0.7 μg DO34 rats were defeated compared to 44% of 0.07μg DO34 and 71% of VEH. Furthermore, intra-LHb DO34 treatment dose-dependently reduced fos colocalization with DRN 5-HT (but not VTA DA) neurons compared to VEH, suggesting a 5-HT-mediated mechanism of action. Altogether, these results support a role for 2-AG in regulating LHb activity and further our understanding of brain systems that regulate behavioral responses to stress.

Disclosures: H.R. Wright: None. **Z.D.G. Fisher:** None. **R. Urrutia-Carmago:** None. **D.E. Ginder:** None. **A.M. Brown:** None. **H.S. Arey:** None. **J.L. Ritchie:** None. **R.A. Fuchs:** None. **R.J. McLaughlin:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.08

Topic: F.03. Stress and the Brain

Support: CIHR PJT-168855

Title: Convergence of monosynaptic inputs from neurons in the brainstem and forebrain on parabrachial neurons that project to the paraventricular nucleus of the thalamus

Authors: S. LI, S. LI, ***G. J. KIROUAC**;
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Abstract: Neurons in the paraventricular nucleus of the thalamus (PVT) send divergent projections to many areas of the forebrain including dense projections to the shell of the nucleus accumbens, dorsolateral region of the bed nucleus of the stria terminalis (BSTDL) and the lateral region of the central nucleus of the amygdala (CeL). Experimental evidence indicates that the PVT integrates emotionally salient signals to regulate appetitive and aversive behavioral responses. The lateral parabrachial nucleus (LPB), a well-known relay for emotionally salient signals to the forebrain, is an important source of lower brainstem afferents to the PVT. The present study was done to further characterize the LPB projection to the PVT. Retrograde cholera toxin B (CTB) tracing experiments demonstrate that the LPB is the main source of PVT projecting neurons in the pons and medulla. An intersectional rabies tracing approach was used to map and quantify the sources of monosynaptic inputs to LPB-PVT projecting neurons. Major sources of inputs to LPB-PVT neurons included the reticular formation; periaqueductal gray (PAG); nucleus cuneiformis; and the superior and inferior colliculi. Moreover, distinctive clusters of input cells to LPB-PVT neurons were also found in the BSTDL and CeL. An intersectional anterograde viral tracing approach demonstrates that LPB-PVT projecting neurons densely innervate all of the PVT in addition to providing collateral innervation to the preoptic area, lateral hypothalamus, zona incerta and PAG but not the BSTDL and CeL. The results of the tracing experiments reported here indicate that LPB-PVT projecting neurons are part of a network of interconnected neurons involved in arousal, homeostasis, and the regulation of behavioral states. In addition, populations of neurons in the BSTDL and CeL are anatomically positioned to provide feedforward or feedback signals to LPB-PVT projecting neurons.

Disclosures: S. Li: None. **S. Li:** None. **G.J. Kirouac:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.09

Topic: F.03. Stress and the Brain

Title: Neural correlates of open-label placebo effects

Authors: *M. SCHAEFER, A. KÜHNEL, F. SCHWEITZER, S. ENGE, M. GÄRTNER;
Med. Sch. Berlin, Med. Sch. Berlin, Berlin, Germany

Abstract: While placebo effects are well-known, research in the last decade revealed intriguing effects that placebos may have beneficial effects even when given without deception. At first glance this seems paradoxical, but several studies have reported improvements in chronic pain,

depression, anxiety, or emotional distress. However, since open-label placebos can only be administered in an unblinded manner, it cannot be ruled out that open-label placebo effects may merely represent a response bias. Thus, objective biological or psychophysiological markers are needed to help understanding open-label placebo effects. The present study aims to fill this gap by showing neural underpinnings for open-label placebo effects. Participants watched highly arousing negative pictures while lying in the fMRI scanner. Results showed reduced emotional distress when receiving an open-label placebo before. This effect was associated with an activation of the periaqueductal grey, anterior cingulate cortex, and bilateral hippocampi, which regulated the affective states in the open placebo group. Remarkably, we did not find any prefrontal brain activation, suggesting that expectations of placebo effects do not explain open-label placebo responses. Our results provide first insights in the neural underpinnings of the open-label placebo effect and suggest that placebos without deception may offer a feasible, cost-effective, and ethically justifiable new way to address both clinical and nonclinical symptoms.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 062.10

Topic: F.03. Stress and the Brain

Support: KBRI Basic Research Program through the Korea Brain Research Institute funded by the Ministry of Science and ICT of Korea / grant #22-BR-02-03

Title: Skeletal muscle-derived target-X attenuated central fatigue in C57BL/6 mice

Authors: *S. LEE, J. YOON;
Korea Brain Res. Inst., Daegu, Korea, Republic of

Abstract: Prolonged exercise induces central fatigue related to neurochemical changes involving dopamine, serotonin, and noradrenaline and also athletic dysfunction. However, the precise mechanism of central fatigue is still unclear. According to the reliable secretomics approach, we had discovered target-X from the skeletal muscle secretome and its expression level is reduced in an age-dependent manner. Here, we hypothesized that skeletal muscle-derived target-X is associated with central fatigue and investigated whether target-X is effective in the improvement of central fatigue or not. Old male mice (13-month-old) were intracerebroventricular (ICV) injected with target-X, then the mice ran on a treadmill to induce central fatigue. As a result of behavioral tests, running distances significantly increased in the target-X-treated group than in the control group, indicating mice improved physical endurance by target-X. In the forced swimming test, target-X-treated mice showed relatively shorter immobility time than the control group, resulting in improved depression. Next, neurotransmitter measurement analysis observed

that target-X significantly decreased the prolonged exercise-induced excessive levels of 5-HIAA and 5-HT, suggesting that target-X reduced central fatigue. Finally, global proteomics analysis discovered the molecular signatures involved in target-X mediated neural events. We studied the molecular mechanism according to the subsequent experiments. These findings suggest the new molecular mechanism of central fatigue and propose the target-X pathway as the regulatory target of central fatigue including hippocampal neuronal activity.

Disclosures: **S. Lee:** None. **J. Yoon:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.11

Topic: F.03. Stress and the Brain

Title: Multi-region response to threat conditioning resolved by spatial transcriptomics

Authors: *J. OTTEN[1], S. DAN[2], R. LARDENOIJE[1], T. KLENGEL[3];
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[3]Harvard medical Sch., McLean Hosp., Belmont, MA

Abstract: Background: Maladaptive transcriptional alterations within a complex neurocircuitry are central to many stress- and trauma-related disorders. However, how different brain regions, their subdivisions and nuclei interact influencing behavioral outcomes remains largely unknown. Methods:We generated spatial transcriptomics data in C57BL/6 mice exposed to a standard auditory threat conditioning paradigm (n=8 per group). Brains were harvested in the early memory consolidation period and sections were placed on 10X Visium chips. We developed custom scripts to process sequencing data and perform downstream analysis. Results:As expected, unsupervised clustering analysis of the obtained expression data reveals anatomically correct clustering into 11 broad regions and further sub clustering resulted in 35 subregions. We performed differential expression and regional enrichment analysis (p.FDR < 0.05) using data from the Allen Brain Atlas confirming the identity of the identified clusters. Differential expression analysis between threat conditioned and control animals resulted in a total of 351 DEGs (p.FDR < 0.05) across subregions. Furthermore, our results indicate the highest ratio of DEGs per region for the retrosplenial cortex, CA2/CA3 of the hippocampus and the lateral and medial habenula. Network analyses further delineate the coordinated transcriptional response to fear conditioning across multiple brain regions. Conclusions:
Our results show broad, brain-wide differential gene expression in response to fear conditioning. We provide for the first-time evidence for a concerted and extensive transcriptional response in most of the brain regions investigated

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 062.12

Topic: F.03. Stress and the Brain

Support: JSPS KAKENHI Grant Number 21H03785
JSPS KAKENHI Grant Number 21K12797

Title: Stress reduction effects of mindfulness meditation; Examination based on heart rate variability and electroencephalography

Authors: *I. KONDO, N. HASHIMOTO, S. SHIMADA;
Meiji Univ., Meiji Univ., Kawasaki, Japan

Abstract: Recently, the effectiveness of mindfulness meditation in reducing stress and anxiety has been reported, and many studies have used subjective evaluations based on questionnaires. In contrast, several other studies have examined the effects of mindfulness meditation training using objective assessments at rest. A 4-week mindfulness meditation training program was conducted in this study. Following this, an experiment involving heart rate variability measurement and electroencephalography (EEG) analysis was performed for the participants before and after the training. A series of physiological changes during the first rest, stress task, meditation, and second rest were assessed in the experiment for an objective evaluation of the effects of the 4-week mindfulness meditation training on stress reduction. This study enrolled 24 healthy university students. However, due to measurement errors, the low frequency/high frequency (LF/HF) ratio was analyzed in 19 participants and EEG in 23 participants. Three-factor mixed ANOVA was performed for each scale of Group (meditation group and control group), Phase (first rest, stress task, meditation, and second rest), and Session (pre-training session and post-training session). We observed significant results for the LF/HF ratio in the two-way interaction effect between Group and Session ($F(1,17) = 7.773$, $p = 0.013$, *partial* $\eta^2 = 0.082$). Simple main effect showed that the LF/HF ratio decreased more significantly in the meditation group than in the control group in the post-training session ($F(1,34) = 11.70$, $p = 0.002$). Two-factor mixed ANOVA for each scale of Group and Session showed the interaction between Group and Session for the functional connectivity values of the left and right anterior cingulate cortices in the θ-wave band ($F(1,19) = 4.764$, $p = 0.034$, *partial* $\eta^2 = 0.021$). Simple main effect showed a significant decrease in the meditation group than in the control group in the post-training session ($F(1,19) = 4.404$, $p = 0.049$). There was also a significant decrease in the meditation group during the post-training session as compared to the pre-training session ($F(1,19) = 5.588$, $p = 0.029$). The LF/HF ratio of the meditation group was significantly lower than that of control group in the post-training session, suggesting that the 4-week mindfulness meditation training program used in this study enhances stress reduction and reduces physiological stress changes. Furthermore, the functional connectivity values in the left and right anterior cingulate cortices in the θ-wave band reduced significantly, indicating that meditation practice reduces the activity of the default mode network in the resting state.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 062.13

Topic: F.03. Stress and the Brain

Support: NIH Grant F30 OD032120 to C. Dearing
NIH Grant R01 HL150559 to B. Myers

Title: Prefrontal-medullary circuit inhibition dysregulates physiological responses to metabolic stress

Authors: *C. DEARING, C. MCCARTNEY, E. LUKINIC, S. A. PACE, B. MYERS; Colorado State Univ., Fort Collins, CO

Abstract: Chronic stress increases risk for metabolic disorders, including diabetes. However, the neurobiological basis of chronic stress impacts on glucose homeostasis has not been defined. The current study tested the hypothesis that the prefrontal infralimbic cortex (IL) - rostral ventrolateral medullary (RVLM) circuit is necessary to prevent glucose intolerance. To this end, female rats with Cre-dependent expression of tetanus toxin in RVLM-projecting IL neurons were chronically stressed for 2 weeks or remained unstressed. These rats were then acutely challenged with a fasted glucose tolerance test (GTT). Endocrine metabolic function was evaluated during GTT by measuring blood glucose, insulin, glucagon, and corticosterone, the primary rodent glucocorticoid. Following chronic stress, circuit-intact females had impaired glucoregulation characterized by decreased glucose clearance, elevated corticosterone, and insulin insensitivity. Inhibition of the IL-RVLM circuit also impaired glucose tolerance regardless of stress status. However, in unstressed animals with circuit inhibition, this impairment was characterized by elevated glucagon with no compensatory insulin response. Chronically stressed females with circuit inhibition showed broader autonomic dysregulation and disruption of counter-regulatory mechanisms involved in glucose homeostasis. Studies in males are ongoing but indicate that chronic stress exposure improves glucose clearance, while circuit inhibition during chronic stress leads to glucose intolerance. Collectively, these data indicate the IL-RVLM circuit is necessary for maintaining glucose homeostasis following chronic stress.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

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Program #/Poster #: 062.14

Topic: F.03. Stress and the Brain

Support: NIH F31 HL162571
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NIH R01 HL150559

Title: Prefrontal-medullary circuit activation attenuates stress reactivity

Authors: *S. A. PACE, E. LUKINIC, T. WALLACE, D. SCHAEUBLE, J. MOORE, B. MYERS;
Biomed. Sci., Colorado State Univ., Fort Collins, CO

Abstract: Organismal survival and adaptation to stress rely on brainstem catecholaminergic neurons. Notably, catecholaminergic neurons in the rostral ventrolateral medulla (RVLM) drive sympathetic activity and enable physiological adaptations, including vasoconstriction, corticosterone release, and glycemic mobilization. However, it is unclear how brain regions involved in the cognitive appraisal of stress regulate the activity of RVLM neurons. Our prior studies found that the rodent ventromedial prefrontal cortex (vmPFC) integrates behavioral and physiological responses to stress. Thus, a potential vmPFC-to-RVLM connection would represent a crucial link between stress appraisal and sympathetic reactivity. In the current study, we investigated a direct vmPFC-to-RVLM circuit by utilizing anterograde and retrograde tract tracers. Together, these studies demonstrated that stress-reactive glutamatergic vmPFC neurons project to catecholaminergic RVLM neurons in male and female rats. To understand the function of this vmPFC-to-RVLM circuit, we injected a viral vector coding for channelrhodopsin-2 (ChR2) in the vmPFC of males and females. Next, a fiber optic cannula was implanted dorsal to the RVLM to evoke vmPFC synaptic glutamate release. Animals then received photostimulation during restraint stress with blood sampled to determine stress reactivity. Compared to controls, male rats expressing ChR2 on vmPFC terminals had suppressed glycemic stress responses ($p < 0.05$). In contrast, stimulation of the vmPFC-to-RVLM circuit in females did not affect glucose mobilization ($p < 0.05$). However, ChR2 decreased corticosterone responses to stress relative to control rats in both sexes (males, $p < 0.01$; females, $p < 0.05$). Thus, both male and female rats have a direct circuit from the vmPFC to the RVLM that limits glucocorticoid stress responses. Tissue analysis post-experiment revealed that vmPFC-to-RVLM stimulation preferentially activated non-catecholaminergic RVLM neurons in both sexes (males, $p < 0.05$; females, $p < 0.01$). Moreover, vmPFC appositions were identified onto GABAergic and glycinergic RVLM neurons in both sexes. Therefore, vmPFC projections may activate local RVLM inhibitory cells to limit stress reactivity. Ultimately, excitatory/inhibitory balance at vmPFC synapses in the RVLM may be critical for the health consequences of stress.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

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Program #/Poster #: 062.15

Topic: F.03. Stress and the Brain

Support: NIH R01 HL150559 to B. Myers

Title: Infralimbic prefrontal cortical projections to the autonomic brainstem: quantification of inputs to cholinergic and adrenergic/noradrenergic nuclei

Authors: T. WALLACE, **C. MCCARTNEY**, *B. MYERS;
Colorado State Univ., Fort Collins, CO

Abstract: The ventromedial prefrontal cortex regulates both emotional and physiological processes. In particular, the infralimbic cortex (IL) integrates behavioral, neuroendocrine, and autonomic responses to stress. However, the organization of cortical inputs to brainstem nuclei that regulate homeostatic responses are not well defined. Therefore, we hypothesized that IL projections differentially target pre-ganglionic parasympathetic neurons and adrenergic/noradrenergic nuclei. To quantify IL projections to autonomic brainstem nuclei in male rats we utilized viral-mediated gene transfer to express yellow fluorescent protein (YFP) in IL glutamatergic neurons. YFP-positive projections to cholinergic and adrenergic/noradrenergic nuclei were then imaged and quantified. Cholinergic neurons were visualized by immunohistochemistry for choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine. Adrenergic/noradrenergic neurons were visualized with immunohistochemistry for dopamine beta hydroxylase (DBH). DBH converts dopamine to norepinephrine, which also serves as a precursor for epinephrine. Our results indicate that IL glutamate neurons innervate the cholinergic dorsal motor nucleus of the vagus with greater density than the nucleus ambiguus. Furthermore, numerous DBH-positive cell groups receive IL inputs. The greatest density was to the C2 and A2 regions of the nucleus of the solitary tract with intermediate levels of input to A6 locus coeruleus and throughout the C1 and A1 regions of the ventrolateral medulla. Minimal input was present in the pontine A5. Collectively, our results indicate that IL projection neurons target vagal preganglionic parasympathetic neurons, presympathetic neurons of the ventrolateral medulla, as well as diffuse modulators of homeostatic function that arise from the nucleus of the solitary tract and locus coeruleus. Ultimately, these findings provide a roadmap for determining circuit-level mechanisms for neural control of homeostasis and autonomic balance.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Topic: F.03. Stress and the Brain

Support: R01MH053851
I01BX003512

Title: Antidepressant-like effects of extinction learning as an animal model of behavioral therapy

Authors: *S. E. BULIN[1], J. LIU[2], D. A. MORILAK[3];
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Abstract: Exposure-based behavioral therapy, the most effective treatment for posttraumatic stress disorder (PTSD), also reduces depressive symptoms. However, neurobiological mechanisms underlying the beneficial effects of exposure-based behavioral therapy on depression remain unknown. Our lab has established fear extinction as a rat model of exposure therapy to investigate the mechanisms underlying its therapeutic behavioral effects in chronically stressed rats. In this study, we demonstrated that extinction learning reduced immobility in the forced-swim test and reversed chronic stress-induced reduction in sucrose preference. Chemogenetic inactivation of pyramidal neurons in the ventral medial prefrontal cortex (vmPFC) prevented the antidepressant-like effects of extinction. Extinction learning enhanced synaptic plasticity induced by in vivo optogenetic long-term potentiation in the pathway from mediodorsal thalamus (MDT) to vmPFC, and restored spine density in layers V and II/III in vmPFC of chronically stressed rats. These results suggest that activity-dependent neuroplasticity induced by extinction learning in vmPFC may contribute to its antidepressant-like effects after chronic stress.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

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Program #/Poster #: 062.17

Topic: F.03. Stress and the Brain

Support: Grant from National Science Centre, Poland, no. 2017/25/B/NZ7/00638

Title: Bidirectional noradrenergic response to the low-frequency electromagnetic field (50 Hz) exposure - adaptation or sensitization

Authors: A. KLIMEK, H. KLETKIEWICZ, A. SIEJKA, J. MALISZEWSKA, M. KLIMIUK, J. WYSZKOWSKA, M. JANKOWSKA, A. NOWAKOWSKA, M. STANKIEWICZ, ***J. ROGALSKA***;
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Abstract: Recent studies indicated that electromagnetic field (EMF) exposure can act as a stress factor. In many cases, pathophysiological complications or diseases arise from stress. Nowadays the number of artificial sources of EMF is huge and still is increasing. It raises concerns about its unfavourable effects on organisms mainly the disruptions of the functions of the nervous system. EMF exposure is often repeated or prolonged thus it is important to consider the EMF exposure as a cumulative process. We put forward the hypothesis that repeated exposure to EMF might affect the set-point of noradrenergic system activity (involved in stress response). Therefore, the research aimed at determining whether 1) EMF causes bidirectional changes (hormesis effect) in the noradrenergic system depending on EMF intensity (1 or 7 mT), and 2) repeated EMF exposure changes behavioural response to subsequent stress factors. Adult (3-month-old) Wistar male rats were treated with EMF (50 Hz) of 1 mT or 7 mT 3 times (3x 7-day) every 3 weeks. The NA concentration was analysed after each period of exposure (3 times) to evaluate the direction and dynamics of changes in its level. Control animals were subjected to the same experimental procedure as the respective animals exposed to EMF except for magnetic field exposure. Moreover, we evaluated the impact of EMF exposure on behavioural changes occurring in response to subsequent stress factor - plus-maze test. We have found the hormetic (bidirectional) effect of EMF which results in different activation of the noradrenergic system. A single exposure to EMF with a value of 1 mT resulted in a slight increase in noradrenergic system activity (NA level in the hypothalamus, the locus coeruleus and plasma). After each subsequent exposure, the level of NA was lower or not different from the control level. However, EMF of 7 mT led to sustained stimulation of noradrenergic system activity which was higher with each next exposure. Moreover, rats exposed to EMF of 7 mT showed less anxiety-related behaviour than that noticed in control and exposed to 1 mT EMF rats. Our data suggest that the exposure to EMF can establish a new “set-point” for noradrenergic activity and the direction and dynamics of this process depend on the intensity of the field. The EMF of 1 mT induced some endogenous adaptive processes, but 7 mT EMF caused sensitization. Consequently, the stronger field - 7 mT can be recognised as harmful to the organism. The research ensures a new view on possible therapeutic properties of electromagnetic fields and provides new data for reliable risk assessment of the exposure to EMF, which is of crucial importance for the health of society.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

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Program #/Poster #: 062.18

Topic: F.03. Stress and the Brain

Support: Anonymous donor from a Benefunder program

Title: Effects of Mycobacterium aurum DSM 33539 on biological signatures of stress-induced neuroinflammation and stress resilience in adult male rats

Authors: *S. A. SAGO[1], A. CLIFTON[2], C. A. ZAMBRANO[2], K. M. LOUPY[2], B. M. MARQUART[2], H. M. D'ANGELO[3], L. K. FONKEN[4], M. G. FRANK[3], S. F. MAIER[3], C. A. LOWRY[2];
[1]Integrative Physiol., Univ. of Colorado At Boulder, Boulder, CO; [2]Integrative Physiol., [3]Psychology and Neurosci., Univ. of Colorado Boulder, Boulder, CO; [4]Pharmacol. and Toxicology, Univ. of Texas at Austin, Austin, TX

Abstract: Stress-related psychiatric disorders, such as anxiety disorders, affective disorders, and trauma and stressor-related disorders, such as posttraumatic stress disorder (PTSD), are becoming more prevalent and are associated with significant individual and socioeconomic costs. Although the etiology and pathophysiology of stress-related psychiatric disorders is complex, emerging evidence suggests that increased inflammation is a risk factor for each of these disorders. This is supported by rodent studies, which have shown that peripheral inflammation, particularly stimulated release of the proinflammatory cytokine interleukin 6 (IL-6) from leukocytes, predicts individual variability in susceptibility to development of exaggerated anxiety-like defensive behavioral responses to subsequent stress exposure. In contrast, immunization with whole cell, heat-killed preparations of *Mycobacterium vaccae* NCTC 11659 (recently reclassified as *M. kyogaense* sp. nov. NCTC 11659) or *M. vaccae* ATCC 15483, bacterial strains with anti-inflammatory and immunoregulatory properties, have been shown to prevent stress-induced exaggeration of peripheral inflammation, neuroinflammation, and anxiety-like defensive behavioral responses. However, the extent to which the anti-inflammatory and stress resilience effects of *M. vaccae* strains generalize to other mycobacterial strains is not known. In the current study, we set out to determine whether treatment with *M. aurum* DSM 33539, a mycobacterial strain that is believed to be phylogenetically related to *M. vaccae* 15483 [Dai et al., 2011, *J Clin Microbiol*, 49(6): 2296-2303] would also prevent stress-induced exaggeration of neuroinflammation and promote stress resilience in adult male rats. Here we show that immunization with *M. aurum* DSM 33539 prevented inescapable stress- (IS-) induced increases in hippocampal *Il6* mRNA expression and prevented IS-induced exaggeration of anxiety-like defensive behavioral responses in the juvenile social exploration paradigm, assessed 24 h after exposure to IS in adult male rats. Future studies are required to fully characterize the mechanisms through which *M. aurum* DSM 33539 and closely related mycobacterial strains prevent stress-induced exaggeration of neuroinflammation and promote stress resilience.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.19

Topic: F.03. Stress and the Brain

Support: NIH Grant R01DA052465

Title: Modified pair housing lowers common stress markers and effects cocaine self-administration

Authors: *K. CZARNECKI[1], E. EKOBENI[1], N. HARRINGTON[1], E. A. HELLER[2]; [2]Dept. of Systems Pharmacol. and Translational Therapeut., [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Relapse is a pernicious feature of substance use disorder (SUD)[1]. Fully elucidating the mechanisms that lead to relapse requires studying long term abstinence in drug exposed animals. This presents a challenge as many model organisms used in such research are isolated, confounding their natural social structures[2]. Concurrently, there is evidence that long term isolation is stressful in rodent models and thus isolation further confounds studies of long-term drug abstinence and relapse[2]. This leads to the question of what effect does housing have on stress levels, and given that stress correlates with increased drug intake, is there a subsequent effect on rates of self-administration? To investigate this, an initial cohort of male and female mice were separated into one of three housing schemes: isolated, paired or a modified pair cage wherein they were separated with a clear plastic partition that allowed for visual, auditory, olfactory, and limited tactile interaction. Stress was measured by blood corticosterone (CORT) levels as well as two common behavioral assays, the open field test and light -dark test. Initial findings were that CORT levels were lowest in modified pair housing and that both modified, and pair housed animals demonstrated less anxiety in an open field test. Following this assessment, a second group of animals underwent cocaine IVSA housed in the modified structures. Our findings revealed that while stress levels remained low during cocaine self-administration, there was a noted increase in saline intake compared to prior studies. Particularly interesting was that cocaine subjects demonstrated a high discrimination between an active and inactive operants, a distinction lacking in their saline counterparts. These results suggest that housing influences learning and reward motivation and should be a carefully considered component of any rodent behavioral studies. 1 Venniro, M., Banks, M.L., Heilig, M. *et al.* Improving translation of animal models of addiction and relapse by reverse translation. *Nat Rev Neurosci* **21,** 625-643 (2020). https://doi.org/10.1038/s41583-020-0378-z2 Engeln, M., Fox, M.E. & Lobo, M.K. Housing conditions during self-administration determine motivation for cocaine in mice following chronic social defeat stress. *Psychopharmacology* **238,** 41-54 (2021). https://doi.org/10.1007/s00213-020-05657-y3 Newman, E., Leonard, Michael. Social Defeat Stress and Escalation of Alcohol Consumption: Focus on CRF, *Neurobiology of Stress* **9,** 151-165 (2018). https://doi.org/10.1016/j.ynstr.2018.09.007

Disclosures: K. Czarnecki: None. **E. Ekobeni:** None. **N. Harrington:** None. **E.A. Heller:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.20

Topic: G.05. Mood Disorders

Support: PhRMA Foundation Starter Grant
DMU IOER Research & Grant Award #03-20-08

Title: Nlrp3 mediates brain-kidney inflammatory responses in a chronic pain model

Authors: J. FOLTZ[1], V. BABICH[2], L. WINTER[1], B. DUONG[1], A. GARMAN[1], F. DI SOLE[1], ***V. DURIC**[1];
[1]Physiol. and Pharmacol., Des Moines Univ., Des Moines, IA; [2]Mercy Col. of Hlth. Sci., Des Moines, IA

Abstract: Stress associated with physical and emotional aspects of chronic pain has been previously linked to the development of mood disorders and dysfunction of multiple organs including the kidney. While the underlying neurophysiological mechanisms remain elusive, here we examined the effects of chronic pain on activation of immune-inflammatory responses in the brain and kidney. Male rats were exposed to chronic inflammatory pain for up to 42 days [multiple injections of Complete Freund's Adjuvant (CFA) into the hind paw], which produced a state of chronic allodynia and enhanced behavioral emotionality. Biochemical analysis of the hippocampus, a limbic region that regulates mood and stress responses, showed that CFA evoked increases in expression of ionized calcium binding adaptor molecule 1 (IBA1) and NLRP3 inflammasome proteins, known markers of microglial activation and neuroinflammatory responses, respectively. NLRP3 is also a key player in renal inflammatory responses and acts via activation of pro-inflammatory cytokine such as interleukin-18 (IL-18). Analysis of NLRP3 and IL-18 protein levels via immunocytochemistry demonstrated significant increase levels of these proteins in the renal glomeruli and tubules induced by CFA. This suggests that chronic pain and related stress effects induce a neuronal and renal inflammatory response via NLRP3 activation. The neutrophil gelatinase-associated lipocalin (NGAL) is recognized as early biomarker of renal injury and an increase in NGAL levels in the circulation and urine associates to an inflammatory response in disease states such as chronic kidney disease, neoplasia, and atherosclerosis. CFA induced an increase in the renal protein expression of NGAL and in the serum and urine levels of NGAL. These results suggest the development of renal injury in conjunction with inflammatory responses in an enhanced stress state associated with chronic pain. Together, these findings provide new evidence to support a mechanistical understanding of a bidirectional relationship between chronic pain-related stress and development of renal dysfunction via an activation of the NLRP3 inflammasome. Further understanding of this relationship could contribute to the identification of novel treatment and strategies to diminish both mental health and renal physiological consequences of chronic pain.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.21

Topic: G.05. Mood Disorders

Support: NIMH R01 MH111604

Title: Androgen receptors in ventral hippocampal neurons projecting to nucleus accumbens regulate sex-specific responses to stress

Authors: *I. LAKIC[1], E. S. WILLIAMS[2], R. M. BASTLE[3], I. S. MAZE[4], A. J. ROBISON[1]; [1]Physiol., Michigan State Univ., East Lansing, MI; [2]Michigan State Univ., Freeland, MI; [3]Mount Sinai Sch. of Med., New York, NY; [4]Dept. of Neurosci., Icahn Sch. of Med. At Mount Sinai, New York, NY

Abstract: Depression is a leading cause of disability in the United States and is nearly twice as prevalent in females as it is in males, but the molecular underpinnings of this discrepancy remain unclear. We previously showed that female mice have higher baseline excitability in ventral hippocampal (vHPC) neurons projecting to the nucleus accumbens (NAc), and that this higher excitability causes susceptibility to stress-induced anhedonic behavior. We also showed that the sex differences in both excitability and anhedonic responses to stress are dependent on adult testosterone, but the mechanism of these testosterone effects is unknown. Neurons in the vHPC express high levels of androgen receptor (AR) which when activated are capable of affecting cell excitability. Thus, the present study examined whether testosterone-mediated resilience to stress-induced anhedonia in mice is dependent on AR activation specifically in the vHPC-NAc circuit. Using a novel intersecting viral strategy, we knocked out AR expression specifically in vHPC cells projecting to NAc in transgenic Cre-inducible Rosa-eGFP-L10a male mice floxxed for AR, then exposed them to subchronic variable stress or chronic unpredictable stress (CUS). We then used a battery of behavioral tests to examine the role of vHPC-NAc AR expression in responses to stress, including sucrose preference as a measure of anhedonic responses. Circuit-specific AR knockout was validated using dual label immunofluorescence. We found that knocking out the AR in the vHPC-NAc produced a decreased sucrose preference compared to AR intact controls in stressed males. Additionally, circuit-specific TRAP and regional real-time polymerase chain reaction (rt-PCR) on tissue punches taken from the vHPC were used to examine possible baseline differences in gene expression in male and female mice, both in whole vHPC and specifically in vHPC-NAc neurons. These results provide exciting new possibilities to study sex-specific hormone-driven changes in brain circuit function driving behaviors central to depression and may provide insight for future gene targets for therapeutic intervention.

Disclosures: I. Lakic: None. **E.S. Williams:** None. **R.M. Bastle:** None. **I.S. Maze:** None. **A.J. Robison:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.22

Topic: G.05. Mood Disorders

Support: NIH RO1-MH115900

Title: Chronic, predictable stress during adolescence increases resilience in female mice via α4δδGABAA receptors

Authors: *L. KENNEY, S. SMITH;
SUNY Downstate Med. Ctr., NEW YORK, NY

Abstract: Many experiments investigating stress during adolescence use male rodents, focus on its negative effects, and lack mechanistic explanations for the development of mood disorders. This study explores the effect of chronic, predictable stress during puberty in male and female mice on a measure of depression, to explore potential benefits of stress. Mice were restrained for 2 h/day over 2 weeks, beginning at puberty's onset, followed by the forced swim test (FST) to measure a depressive phenotype. Restrained female mice were significantly less immobile compared to controls (R: 37% ± 0.04, C: 56% ± 0.04, t(26)= 3.6, p= 0.001), an effect maintained in adulthood (R: 43% ± 0.03 C: 50% ± 0.03, t(49)=1.79, p=0.04), suggesting the development of resilience to depressive behavior. In contrast, restrained male mice were not different from controls (R: 58% ± 0.12, C: 52.6% ± 0.17, t(17)= 0.69, p=0.50), indicating resilience is selective for females. Allopregnanolone (THP) is a neurosteriod which is released in the brain during stress and is a potent positive modulator of extrasynaptic α4βδ GABAA receptors (GABARs). Restrained female mice were administered finasteride to block THP production over the 2 week period following puberty onset (P35-49). This increased immobile time compared to unrestrained mice (vehicle: 36% ± 0.08, finasteride: 55% ± 0.04), confirming THP's importance for resilience. Additionally, α4GABAR knockout mice showed no differences in immobility (C: 62% ± 0.04, R: 60% ± 0.04, t(13)= -0.08, p=0.94), implicating α4βδ GABARs in the development of resilience. Altogether, results suggest that chronic, predictable stress during puberty improves resilience in female mice through a mechanism involving THP and α4βδ GABARs.

Disclosures: L. Kenney: None. **S. Smith:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.23

Title: WITHDRAWN

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.24

Topic: G.05. Mood Disorders

Support: MITACS research training award
NSERC discovery grant
CIHR-CRC

Title: Chronic stress induced by repeated corticosterone injections has sexually dimorphic effects on hypothalamic reelin levels that are reversed with peripheral administration of recombinant reelin in rats.

Authors: *C. LIRIA SANCHEZ-LAFUENTE[1], J. ALLEN[1], R. ROMAY-TALLON[2], J. JOHNSTON[1], L. E. KALYNCHUK[1], H. J. CARUNCHO[1];
[1]Med. Sci., Univ. of Victoria, Victoria, BC, Canada; [2]Univ. of Illinois Chicago, Chicago, IL

Abstract: Stress-related disorders like depression affect more women than men and sex differences exist in the regulation of stress but how these contribute to the development of stress-related disorders is not fully understood. Previously, we have seen that rats exposed to several weeks of the stress hormone corticosterone (CORT) show behavioural alterations paralleled by decreases in reelin in male hippocampi that can be restored with repeated and single peripheral reelin injections. Since reelin is a protein involved in synaptic plasticity we hypothesized that it might be important for the sex-specific detrimental effects of chronic stress. Therefore, we examined reelin expression in different neuronal populations involved in the stress response, during basal and stressed induced conditions. We found that the basal density of reelin-positive cells in males was significantly higher in the hypothalamic paraventricular nucleus and medial preoptic area, compared to females. In males, chronic CORT injections for 21 days caused a significant decrease in reelin protein levels and reelin-positive cells in the hypothalamus and were reversed with peripheral administration of recombinant reelin. Meanwhile, reelin levels in females were not affected by CORT or reelin injections. Basal hypothalamic MeCP2 protein levels were higher in males and inversely paralleled reelin levels in the hypothalamus. Basal DNMT3a protein levels did not differ, but they decreased more in males after chronic CORT treatment. For the first time, this study shows that hypothalamic reelin is sexually dimorphic and can be differentially affected by chronic stress in rats. Moreover, that epigenetic mechanisms

might be behind sexually dimorphic reelin levels in the hypothalamus, but further studies should be conducted to fully ascertain this.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.25

Topic: G.05. Mood Disorders

Support: R01MH072908
R01MH120514
R01MH120637
F31MH114624

Title: Crf neurons establish resilience via stress-history-dependent modulation

Authors: *S. HAYNES[1], H.-S. SEONG[4], A. LACAGNINA[2], M. AFZAL[1], C. MOREL[1], K. RAJAN[5], R. L. CLEM[6], L. J. YOUNG[7], M.-H. HAN[3];
[2]Neurosci., [3]Mental Hlth. and Publ. Hlth., [1]Icahn Sch. of Med. at Mount Sinai, New York, NY; [4]USC, Los Angeles, CA; [5]Neurosci., Icahn Sch. of Med. At Mount Sinai Grad. Training Program In Neurosci., New York, NY; [6]Neurosci., Mount Sinai Sch. of Med., New York, NY; [7]Ctr. for Translational Social Neurosci., Emory Univ., Decatur, GA

Abstract: Background: Major Depressive Disorder (MDD) is a debilitating mental disorder with a lifetime prevalence in the U.S. of 25%. Despite extensive research exploring the etiology of MDD, an urgent question remains as to what governs the exact moment of its emergence. Using Chronic Social Defeat Stress, we asked the question of at which point we see the earliest emergence of what would become a durable depressive-like (DEP) phenotype in c57bl/6j mice. The Bed nucleus of the stria terminalis (BNST) plays a pivotal role in stress-related psychopathology, aided in part by CRF neurons.
Methods: Mice subjected to 10 days of CSDS were assessed via social interaction (SI) and sucrose-preference tests. Cell-attached electrophysiology in *Crf-Cre*::*TdTomato* mice to record BNSTovCRF cells. To simultaneously manipulate and record the activity of $BNSTov^{CRF}$ neurons *in vivo*, *Crf-Cre* mice were injected with viral constructs (cre-dependent DREADDs and gCAMP7f calcium-indicator).
Results: We observed that depressive-like behavior emerges between 7 and 10 days of CSDS as indicated by a marked change in mean social interaction score (day 7: 1.96 vs day 10: 0.61, N= 216 mice, $p<0.0001$, 2-way ANOVA). The depressive phenotype occurred exclusively after at least seven stress episodes, directly related to a stress accumulation effect. A decrease in spontaneous firing rate in $BNSTov^{CRF}$ neurons was observed selectively in DEP mice compared

to mice that underwent only 7 defeat episodes (denoted STR mice) and had not yet gone on to develop a depressive-like phenotype (5.194 hz vs 1.210 hz; $p<0.0001$, N = 17-19 neurons per group, one-way ANOVA). hM3Dq DREADDs resulted in (0/7 mice, 0%) of STR becoming DEP mice, compared with the hM4Di condition where 1/8 mice, 87.5%, and control-mCherry (9/13 mice, 70%) of STR became DEP mice respectively. Notably, this effect occurred only when CNO was administered between 7-10 stress exposures, not between 4-7 or 11-13 ($p>0.05$, N = 14-20 mice/group). Fiber photometry supported this finding in hM3Dq/GCAMP7f vs in the hM4Di/GCamp7f or mCherry/gCamp7f respectively. Using RNAscope, we uncovered that STR vs DEP mice displayed a greater degree of crhr1 mRNA was present in crf neurons.
Conclusions: Here we reveal surprising results that, in contrast to its well-studied role in promoting negative aversive states, CRF neurons play a direct role in promoting durable resiliency to depression. This opens an exciting avenue for translationally relevant treatments that take into account the subject's stress history in providing crucial components to which drugs may work

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.26

Topic: G.05. Mood Disorders

Support: Brain and Behavior Research Foundation Young Investigator Grant
University of Florida, College of Pharmacy

Title: Characterizing putative neuronal ensembles after acute and chronic social defeat stress

Authors: *S. RAKELA, B. W. SORTMAN, B. CERCI, B. L. WARREN;
Pharmacodynamics, Univ. of Florida, Gainesville, FL

Abstract: Many psychiatric disorders have been conceptualized toinvolve aberrant formation and recollection of learned associations. Anxietydisorders, for example, are thought to involve learned associations betweenstressful events and stress-paired stimuli that become abnormally activated todrive expression of maladaptive behaviors. Stress plays an established role inmodulating learning and memory, and is an important risk factor for thedevelopment of many depressive and anxiogenic psychiatric disorders. Acute andchronic stress exposure are known to have differential and often contradictoryeffects—while acute stress can enhance learning and memory, chronic stressexposure is associated with overall memory deficits and initiation ofmaladaptive behavioral patterns. Chronic social defeat stress is known toinduce a depressive-like phenotype in rodents, including increased sucrose andsocial avoidance, whereas typical behavioral preferences for sucrose andsociability remain intact following acute social defeat

stress. Though a largebody of work has focused on characterizing neurocircuitry involved indeleterious stress effects, mechanistic understanding of neurobiologicalchanges that underlie the progression from acute to chronic stress effects islacking. Furthermore, past work has often examined whole brain regions orcircuits, which may obscure changes occurring in the much smaller subset ofneurons thought to store learned associations: neuronal ensembles. Here, weutilize the social defeat procedure in male FosTRAP mice to compare neuronsactivated by initial social defeat stress exposure to neurons activated by thetenth social defeat stress exposure within the same subject. This techniqueallows for selective targeting of putative ensemble neurons, which would beactivated by stress-paired stimuli and the stress event. In this procedure, anexperimental FosTRAP mouse is placed into the home cage of a territorial maleCD-1 mouse for 10min daily across 10d. The CD-1 exhibits aggression towards theexperimental mouse until the latter exhibits a submissive behavior known associal defeat. We extrapolated the FosTRAP system to label neurons activated byeither the first defeat session (Acute) or novel context exposure (NC) withTdTomato, and performed immunohistochemistry for the immediate early geneprotein product, c-Fos, to identify neurons activated by the tenth defeatsession with GFP. We found significantly greater colocalization of fluorophoreswithin the prefrontal cortex of mice in the Acute group than the NC group,suggesting that a similar and specific population of neurons continues tomediate stress effects after initial stress exposure.

Disclosures: **S. Rakela:** None. **B.W. Sortman:** None. **B. Cerci:** None. **B.L. Warren:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.27

Topic: G.05. Mood Disorders

Title: Reduced excitability in hippocampal CA1 neurons induces stress-susceptible behavior in Igsf9b-deficient mice

Authors: *S. YOON[1,2], W. SONG[1,2], S. OH[2], Y. KIM[2], M.-H. KIM[1,2];
[1]Neurosci. Res. Institute, Med. Res. Ctr., Seoul Natl. Univ., Seoul, Korea, Republic of; [2]Biomed. Sci. and Physiol., Seoul Natl. Univ. Col. of Med., Seoul, Korea, Republic of

Abstract: Stress is a potent environmental risk factor for a depressive disorder which is thought to be caused by interactions between genetic and environmental factors. Genetic factors contribute to stress response and inter-individual differences in stress vulnerability. However, little is known about how genetic factors make the nervous system more susceptible to environmental risk factors. Here we show that immunoglobulin superfamily member 9b (Igsf9b) confers stress susceptibility in mice by modulating the excitability of hippocampal CA1 neurons. Igsf9b deficient ($Igsf9b^{-/-}$) mice were more susceptible to chronic social isolation or restraint stress compared to WT mice and exhibited enhanced depression-like behaviors following exposure to chronic stress. $Igsf9b^{-/-}$ mice exhibited significantly higher levels of serum

corticosterone than WT controls during restraint stress. Electrophysiological and biochemical analyses revealed that CA1 neurons of Igsf9b$^{-/-}$ mice are less excitable than those of WT mice due to an enhanced hyperpolarization and cyclic nucleotide-activated (HCN) current (I_h) and HCN2 channel expression. Igsf9b was co-immunoprecipitated with HCN2 isoform, and overexpression of Igsf9b decreased the protein levels of HCN2 in a dose-related manner. Virus-mediated knockdown of HCN2 in the CA1 neurons of Igsf9b$^{-/-}$ mice normalized CA1 neuron excitability and stress susceptibility. In addition, overexpression of HCN2 in the CA1 neurons of WT mice phenocopied enhanced stress susceptibility and reduced CA1 neuron excitability of Igsf9b$^{-/-}$ mice. Collectively, these results suggest that Igsf9b regulates stress responses and stress susceptibility through the modulation of neuronal excitability and HCN2 expression in the hippocampal CA1 neurons.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.28

Topic: G.05. Mood Disorders

Support: R01 MH121829
T32 GM142521
R01 MH111604

Title: Characterization of oxytocin neurons in circuits affecting social behaviors

Authors: *C. SUGIMOTO[1], A. L. EAGLE[1], N. DUQUE-WILCKENS[1], B. C. TRAINOR[2], A. J. ROBISON[1];
[1]Physiol., Michigan State Univ., East Lansing, MI; [2]Psychology, Univ. of California Davis, Davis, CA

Abstract: Exposure to psychosocial stress can contribute to development of multiple mood-related disorders including major depressive disorder (MDD), anxiety, and post-traumatic disorders. MDD is driven by complex genetic and environmental factors, but the biological etiology of MDD remains unknown. About half of MDD patients do not respond fully to existing treatments, and therefore there is a critical need for further understanding MDD etiology and development of new therapeutic targets. Additionally, MDD disproportionally affects women, with the female prevalence rate almost double that of men, but the biological basis of this sex difference is not fully understood. Chronic social defeat stress induces behaviors in mice that are relevant to disorders like MDD, including increased social vigilance and decreased social interaction. Oxytocin (OT) is a well-known modulator of social behaviors, and the bed nucleus of the stria terminalis (BNST) and paraventricular nucleus (PVN) of the hypothalamus are important coordinators of social behavior and anxiety. We previously found that a novel group of

OT-producing neurons within the BNST are more sensitive to social defeat stress in females than males, and that blocking OT synthesis within the BNST is sufficient to prevent stress-induced changes in social approach and vigilance behaviors (Steinman et al., 2016, Duque-Wilckens et al., 2020). However, the physiological properties of these OT neurons and how they are affected by stress, as well as the neural circuits upstream and downstream of these OT neurons, remain unclear. We used *ex vivo* whole cell slice electrophysiology on OT-cre::L-10 GFP mice to examine the physiological properties of unstressed male and female adult mice. BNST- and PVN-OT neurons had increased sEPSC frequency, but not amplitude, compared to non-OT neurons in the same region or slice (cortex), indicating an extremely high excitatory drive on these neurons. No sex differences were found in either OT neuron population. Current studies are determining 1) the effects of stress on these properties; and 2) the spine density of these BNST- and PVN-OT neurons and the source(s) of their glutamatergic inputs to uncover the circuit(s) that may contribute to OT neuron effects on chronic stress responses.

Disclosures: C. Sugimoto: None. **A.L. Eagle:** None. **N. Duque-Wilckens:** None. **B.C. Trainor:** None. **A.J. Robison:** None.

Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.29

Topic: G.05. Mood Disorders

Support: NIH MH077681
NIH MH105824
NIH DA033945

Title: Ach signaling modulates activity of the gabaergic signaling network in the basolateral amygdala and behavior in stress-relevant paradigms

Authors: *Y. MINEUR[1], T. N. MOSE[2], K. L. MAIBOM1[2], S. T. PITTENGER[4], A. R. SOARES[5], H. WU[6], S. R. TAYLOR[7], Y. HUANG[3], M. PICCIOTTO[8];
[1]Yale Univ. Sch. Med., New Haven, CT; [2]Yale Univ. Sch. of Med., North Haven, CT; [3]Yale Univ. Sch. of Med., New Haven, CT; [4]Yale Sch. of Med., New Haven, CT; [5]Interdepartmental Neurosci. Program, [6]Yale Univ., New Haven, CT; [7]Neurobio. Section, Biol. Sci., Yale Univ., North Haven, CT; [8]Dept Pyschiat, Yale Univ., New Haven, CT

Abstract: Balance between excitatory and inhibitory (E/I) signaling is important for maintaining homeostatic function in the brain. Indeed, dysregulation of inhibitory GABA interneurons in the amygdala has been implicated in human mood disorders. We hypothesized that acetylcholine (ACh) signaling in the basolateral amygdala (BLA) might alter E/I balance resulting in changes in stress-sensitive behaviors. We therefore measured ACh release as well as activity of calmodulin-dependent protein kinase II (CAMKII)-, parvalbumin (PV)-, somatostatin (SOM)-

and vasoactive intestinal protein (VIP)-expressing neurons in the BLA of awake, behaving male mice. ACh levels and activity of both excitatory and inhibitory BLA neurons increased when animals were actively coping, and decreased during passive coping, in the light-dark box, tail suspension and social defeat. Changes in neuronal activity preceded behavioral state transitions, suggesting that BLA activity may drive the shift in coping strategy. In contrast to exposure to escapable stressors, prolonging ACh signaling with a cholinesterase antagonist changed the balance of activity among BLA cell types, significantly increasing activity of VIP neurons and decreasing activity of SOM cells, with little effect on CaMKII or PV neurons. Knockdown of α7 or β2-containing nAChR subtypes in PV and SOM, but not CaMKII or VIP, BLA neurons altered behavioral responses to stressors, suggesting that ACh signaling through nAChRs on GABA neuron subtypes contributes to stress-induced changes in behavior. These studies show that ACh modulates the GABAergic signaling network in the BLA, shifting the balance between SOM, PV, VIP and CaMKII neurons, which are normally activated coordinately during active coping in response to stress. Thus, prolonging ACh signaling, as occurs in response to chronic stress, may contribute to maladaptive behaviors by shifting the balance of inhibitory signaling in the BLA.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.30

Topic: F.03. Stress and the Brain

Support: ERC

Title: The activity of the paraventricular thalamic nucleus' calretinin expressing neurons after acute stress underlies long term behavioral changes

Authors: *A. JÁSZ[1], L. BIRO[2], B. KIRALY[1], B. HANGYA[3], L. ACSADY[4];
[2]Thalamus Res. Group, [3]Dept. of Cell. and Network Neurosci., [1]Inst. of Exptl. Med., Budapest, Hungary; [4]Inst. Exp. Med. Hung Acad Sci., Budapest, Hungary

Abstract: Behavioral disorders caused by stress affect millions of people around the world, but its neurobiological bases are still unclear. The calretinin-positive neurons of the paraventricular thalamus (PVT/CR+) are in a unique position to participate in stress induced sleep disturbances since their activity is significantly affected by stress and by sleep-wake transitions. In the accompanying study, using optogenetic inhibition, we found that the post-stress activity of PVT/CR+ cells is critical to establish the acute stress induced behavioral changes. Thus, in this study we aimed to determine the activity of PVT/CR+ cells before and after the exposure to a

natural stressor (2MT, 10 min). We also tested whether stress induced long term changes in firing activity can be reversed by post-stress photoinhibition. Recordings involved 3 hours sessions for five days before and after stress using movable optrodes.During pre-stress days PVT/CR+ cells displayed strongly state dependent activity. Unit activity within the nest during wakefulness was lower than outside the nest and further decreased during sleep. Following the exposure to 2MT firing rate was elevated for four days with strongest increase in the nest. Both high frequency spike clusters and synchrony among PVT/CR+ cells significantly increased in a state dependent manner. Photoinhibition of PVT/CR+ neurons after the 2MT presentation prevented altered firing rate, increase in high frequency clusters and cross correlations on the poststress days. These data together strongly suggest that altered post-stress activity of PVT/CR+ cells is crucial to establish the neuronal network responsible for the emergence of stress induced behavioral phenotype.

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Poster

062. Neural Mechanisms of Stress Responsivity and Related Behaviors

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 062.31

Topic: F.03. Stress and the Brain

Support: ERC

Title: The role of calretinin-positive midline thalamic neurons in stress induced behavioral alterations

Authors: *L. BIRO[1], A. JÁSZ[2], Z. BUDAY[1], G. S. KOMLÓSI[3], R. BÓDIZS[4], L. ACSADY[5]; [2]Thalamus Res. Group, [1]Inst. of Exptl. Med., Budapest, Hungary; [3]Neurosci. Inst., NYU Langone Hlth., New York, NY; [4]Semmelweis Univ., Budapest, Hungary; [5]Inst. Exp. Med. Hung Acad Sci., Budapest, Hungary

Abstract: Severe acute stress could induce the emergence of psychiatric disorders, although the underlying neuronal mechanisms are presently unresolved. Calretinin expressing neurons in the paraventricular nucleus of the thalamus (PVT/CR+) form a critical hub between brainstem and forebrain and play essential roles in arousal, anxiety and stress regulating circuit operation. Thus, in this study we tested whether the activity of PVT/CR+ neurons, following an exposure to a natural stressor (fox odour, 2MT), contributes to acute stress-induced behavioral changes. We inhibited PVT/CR+ neurons after the stress event using optogenetic inhibition (SwiChR) and measured nesting behavior, locomotion, sleep, and stress hormone levels. We also quantified c-Fos expression in the PVT/CR+ cells and their projection areas. EYFP injected animals served as control.During the stress exposure, both groups showed similar levels of defensive behaviours. Following the stress exposure, the control EYFP group displayed increased EMG activity,

disturbed nesting behavior, and elevated corticosterone level. c-Fos expression was increased both in the PVT/CR+ cells and their projection areas. The behavioral changes persisted for five days following the stress exposure. Photoinhibition of PVT/CR+ cells after the stress exposure prevented all these changes, with the exception of acute hormonal stress response remained. This suggests that the post-stress activity of PVT/CR+ cells shapes stress induced behaviour, independently from the hormonal stress response.Collectively, our findings indicate that post-stress activity of PVT/CR+ neurons plays a fundamental role in the emergence of stress-induced behavioral changes, and post-stress inhibition of PVT/CR+ cells is sufficient to prevent these changes.

Disclosures: L. Biro: None. **A. Jász:** None. **Z. Buday:** None. **G.S. Komlósi:** None. **R. Bódizs:** None. **L. Acsady:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.01

Topic: F.03. Stress and the Brain

Support: R01-MH119670
R56-MH116670

Title: Tagging, tracing, and capturing neurons in the prefrontal cortex and hippocampus reveals a unique pattern of activation after social stress

Authors: *R. G. BILTZ[1], W. YIN[2], B. T. OLIVER[2], J. F. SHERIDAN[3], J. P. GODBOUT[4]; [1]The Ohio State Univ., COLUMBUS, OH; [2]The Ohio State Univ., Columbus, OH; [3]Inst. for Behavioral Med. Res., Ohio State Univ., Columbus, OH; [4]Neurosci., Ohio State Univ. Dept. of Neurosci., Columbus, OH

Abstract: Chronic stress may lead to the development of psychiatric disorders including anxiety and depression. Repeated Social Defeat (RSD) in mice recapitulates several key physiological and behavioral changes evident in humans. This includes activation of the sympathetic nervous system, HPA-axis activation, production and release of inflammatory monocytes, and anxiety-like behavior. Neurons in regions of the brain involved in fear and anxiety (e.g., prefrontal-cortex, hippocampus) are activated following RSD. Therefore, the purpose of this study was to determine the transcriptional signature of neurons within these regions after RSD. To examine region specific neural circuitry, a retrograde-adeno-associated-virus (rAAV) expressing Cre-recombinase was injected into the hippocampus of RiboTag mice. This induced an expression of a hemagglutinin-epitope within neurons of the prelimbic cortex and amygdala after four weeks. Next, circuit specific Ribotag mice (4 weeks after rAAV) were subjected to RSD and ribosomal bound mRNA (i.e., actively being translated) was collected from prefrontal-cortex and amygdala for RNA-sequencing. RSD induced 1,132 differentially expressed genes (DEGs) in prefrontal-

cortex neurons that project (directly or indirectly) into the hippocampus. These DEGs were associated with a variety of different pathways including synaptogenesis (e.g., *Atf4, Syngap1*) and neuroinflammation associated with Nf-KB signaling (e.g., *Jun, Rel, Itgb3*). As a follow up, a pan neuronal (*Baf53b)* RiboTag mouse was generated to tag and capture neurons activated by RSD. Pan-neuronal RiboTag mice were exposed RSD, and the hippocampus was collected for RNA-sequencing. There were 1,694 DEGs in hippocampal neurons after RSD. These genes were associated with oxidative stress (e.g., *ATP5e, Cox6c, Hsp90b1*), glutamatergic signaling (e.g., *Hspa8, Prkcz, Cacna1b*), and neuroinflammation associated with complement (e.g., *C4a, C1qb, C1qa).* Pathway analyses in the hippocampus identified an increase in numerous upstream regulators including *Interferon-y*. Overall, social stress induced a unique pattern of gene expression within neurons of the hippocampus and prefrontal cortex associated with synaptogenesis, oxidative stress, glutamatergic, and inflammatory related signaling.

Disclosures: R.G. Biltz: None. **W. Yin:** None. **B.T. Oliver:** None. **J.F. Sheridan:** None. **J.P. Godbout:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.02

Topic: F.03. Stress and the Brain

Title: Il1r1 knockout on glutamatergic neurons prevents chronic stress-induced enhancement of conditioned fear response

Authors: *E. GOODMAN[1], S. SWANSON[1], D. J. DISABATO[2], B. OLIVER[2], N. QUAN[3], J. F. SHERIDAN[4], J. P. GODBOUT[5];
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Abstract: Chronic stress may precipitate psychiatric disorders including anxiety and cognitive deficits. We reported that Repeated Social Defeat (RSD) in mice caused long-term sensitization associated with increased inflammatory and Interleukin 1 signaling. In stress sensitization, individuals become more vulnerable to subsequent adversity, and this may be linked with fear memory. The mechanisms underlying stress sensitization, however, are unknown. The purpose of this study was to begin to investigate the role of increased IL-1R1 signaling in neurons and the development of increased fear memory after RSD. In the first experiment, C57Bl/6 mice were exposed to RSD and conditioned fear memory/behavior was assessed 14 hours later. Fear memory is dependent on both hippocampal and amygdala processes. As expected, RSD influenced multiple phases of fear conditioning. For instance RSD induced accelerated fear acquisition, delayed fear extinction, and increased cued-based freezing. Based on our previous work, we next assessed how this fear memory was influenced by either microglia or IL-1R1

signaling in neurons. In the next experiment, PLX5622 (csf1r antagonist) was used to eliminate microglia prior to RSD and conditioned fear memory/behavior was assessed 14 hours later. As expect, RSD delayed fear extinction as above. Microglial depletion, however, had no effect on accelerated fear acquisition, contextual or cued-based freezing. Thus, fear conditioned behavior after RSD was microglia independent. We next assessed IL-R1 signaling in neurons using wild type and vglut2-IL1R1^{ko} mice. These mice have IL-1R1 knocked out selectively in glutamatergic neurons that are densely expressed in the hippocampus. Again RSD delayed fear extinction and this was IL-1R1 dependent. The vglut2-IL1R1^{ko} mice had complete prevention of all fear conditioned freezing after RSD compared to controls. Furthermore, there was increased ΔFosB expression after fear acquisition that was attenuated in vglut2-IL1R1^{ko} mice. Thus, the source of IL-1 in this aspect of stress sensitization is not from microglia. Taken together, these data are interpreted to indicate that increased IL1R1 mediated signaling in glutamatergic neurons, independent of microglia, augments sensitization and fear memory following RSD.

Disclosures: E. Goodman: None. **S. Swanson:** None. **D.J. Disabato:** None. **B. Oliver:** None. **N. Quan:** None. **J.F. Sheridan:** None. **J.P. Godbout:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.03

Topic: F.03. Stress and the Brain

Title: Effects of 10(Z)-hexadecenoic acid, a lipid isolated from Mycobacterium vaccae, a bacterium with anti-inflammatory, immunoregulatory, and stress resilience properties, on murine bone marrow-derived dendritic cells (BMDCs) following lipopolysaccharide (E. coli 0111: B4) challenge or control conditions

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Abstract: Increased inflammation is thought to be a risk factor for development of anxiety disorders, mood disorders, and trauma- and stressor-related disorders, such as posttraumatic stress disorder (PTSD). Meanwhile, evidence suggests that both inflammation and the prevalence of stress-related psychiatric disorders are increasing in modern urban societies. A possible explanation for the rise of inflammation in modern urban societies is the lack of exposure to certain microbes, “Old Friends,” that humans have coevolved with that have the capability of inducing immunoregulatory effects [Lowry et al., 2013, *Evolution, Medicine, & Public Health*, 2013(1): 46-64]. In this study we investigated the effects 10(*Z*)-hexadecenoic acid, a lipid isolated from a soil-derived “Old Friend”, *Mycobacterium vaccae* NCTC 11659, with anti-inflammatory, immunoregulatory, and stress resilience properties, on the lipopolysaccharide (LPS; *E. coli* 0111: B4)-induced inflammatory response and the LPS-induced intracellular signaling cascades associated with dendritic cell (DC) polarization in bone marrow-derived

dendritic cells (BMDCs) using the murine NanoString Inflammation V2 platform, which allows quantification of expression of 254 inflammation-related genes. Briefly, cells were exposed to 500 µM 10(*Z*)-hexadecenoic acid or vehicle followed, 1 h later, with 0.250 µg/ml LPS or vehicle challenge. Each treatment group had a sample size of (n = 6). Twenty-four hours after LPS or vehicle challenge, cells were harvested to isolate total RNA. Data analysis was performed using Rosalind (https://rosalind.onramp.bio/), with a HyperScale architecture developed by OnRamp BioInformatics, Inc. (San Diego, CA). Analysis revealed that exposure to LPS increased the expression of mRNAs encoding proinflammatory signaling including *Ccl5*, *Cxcl10*, *Il1a*, *Il1b*, *Il6*, *Il12a*, *Il12b*, *Irf7*, and *Ptgs2*. 10(*Z*)-hexadecenoic acid attenuated expression of several of these LPS-induced inflammatory genes, including *Cxcl10*, *Il1a*, *Il1b*, *Il12a*, *Il12b*, *Il6*, and *Ptgs2*. These data are consistent with the hypothesis that 10(*Z*)-hexadecenoic acid attenuates LPS induced inflammation in BMDCs. Together, these data are consistent with the hypothesis that 10(*Z*)-hexadecenoic acid is a potential intervention for prevention of anxiety disorders, mood disorders, and trauma and stressor-related disorders by reducing stress- or trauma-induced exaggeration of neuroinflammation.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.04

Topic: F.03. Stress and the Brain

Support: NIAAA Grant T32AA014127
NIH Grant AA022534
NIH Grant AA025967

Title: Prenatal alcohol exposure (PAE) generates sex-specific glucocorticoid receptor insensitivity in adult mouse offspring

Authors: *J. ZIMMERLY, A. K. FERNANDEZ OROPEZA, M. S. SUN, S. NOOR, A. PASMAY, A. PRITHA, C. F. VALENZUELA, D. D. SAVAGE II, E. D. MILLIGAN; Univ. of New Mexico Dept. of Neurosciences, Albuquerque, NM

Abstract: Prenatal alcohol exposure (PAE) results in a constellation of negative consequences clinically known as fetal alcohol spectrum disorders (FASD), including dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis stress response. The amygdala (AMG) can influence the HPA axis. Following HPA axis activation from a stressor, glucocorticoids (CORT) are

released into circulation and signal to cells by binding the key regulatory CORT receptor (GR). GR activation suppresses further CORT production though negative feedback, preventing further HPA activation. Endogenous FK506-binding-protein-51 (FKBP51) competes with CORT and interacts with GR, blocking GR activity, potentially inhibiting HPA activation. Stress-induced production of corticotropin-releasing hormone (CRH) in the hypothalamus initiates HPA activation and is associated with the induction of toll-like receptor 4 (TLR4). We hypothesize that PAE desensitizes GR signaling and increases neuroimmune responses to acute restraint stress in adulthood. Dexamethasone (DEX), a synthetic GR agonist, was administered to mimic CORT negative feedback causing blunted CORT levels. 1.5 hr prior to a 30-min restraint stress, subcutaneous vehicle (Veh, DMSO; 1:100, sterile phosphate buffered saline, (PBS), pH 7.4) or DEX (25 or 50 µg in DMSO:PBS) was given to 4 mo. male and female typical C57BL/6 mice. In a separate experiment, 5-6 mo. mouse offspring that underwent prenatal control exposure (saccharine; SAC) or PAE (10% EtOH) received Veh or DEX (25 µg) as described above. For both experiments, tail vein blood collection occurred immediately after stress. The female hypothalamus and AMG were collected 24 hr after stress. Messenger RNA (mRNA) for CRH, FKBP5, and TLR4 were assessed by RT-qPCR. Blood plasma CORT was quantified via enzyme-linked immunosorbent assay. In typical C57BL/6 mice, basal CORT levels were similar across all groups. DEX (25 and 50µg) blunted the stress-induced elevation in CORT in both males and females. In a separate experiment, CORT levels in non-stressed Veh-pretreated SAC and PAE were significantly lower than CORT levels from Veh-pretreated, stressed SAC and PAE male and female mice. But, DEX (25 µg) treatment in SAC-stressed female mice revealed blunted CORT but failed to suppress stress-induced CORT elevations from female PAE mice. DEX given to stressed male Sac mice failed to suppress CORT elevations while pretreatment in PAE male mice blunted CORT levels. Compared to SAC AMG, CRF mRNA expression was blunted in stress mice pretreated with DEX. No other significant mRNA changes were measured. PAE may create GR insensitivity to acute stress and alters amygdala CRH expression.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.05

Topic: F.03. Stress and the Brain

Support: NSERC
Ontario Veterinary College

Title: Role of endogenous androgens in mediating stress and neuroinflammation within the male rat hippocampus

Authors: *L. K. ISAACS, S. BHULLAR, A. PATEL, K. C. NICHOLSON, G. DESCALZI, N. J. MACLUSKY;
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Abstract: Neuroinflammation induces profound changes in regions of the brain, including the hippocampus (HC). Painful stimuli can lead to a rise in circulating glucocorticoids that can propagate systemic and neuroinflammatory processes, accompanied by stress-induced decreases in circulating gonadal steroids. Androgens display both anti-inflammatory and neuroprotective effects, such as attenuating microglia overactivation and promoting HC dendritic spine formation. Cellular signalling mechanisms including the ERK-directed phosphatase, DUSP6/MKP3 are known to be dysregulated during stress and neuroinflammation and modulated by androgens. However, the role of endogenous androgens in regulating the molecular and morphological changes following pain-induced neuroinflammation remains unclear. Adult male Sprague-Dawley rats were injected with either saline or a 0.1, 0.3, or 0.5mg/ml dose (n=6/group) of complete Freund's adjuvant (CFA) into the hind footpad to induce mild inflammatory pain and inflammation. Even at the highest CFA dose, none of the animals exhibited any motor deficits. On day 7, the Von Frey filament test was used to determine the presence of allodynia. CFA resulted in no differences in mechanical thresholds compared to saline controls indicating no changes in pain sensitivity. All rats were then sacrificed on day 10 post-injection. By day 10, CFA resulted in a small increase in serum corticosterone, while serum testosterone of all groups had recovered back to control levels. While the recovery of testosterone may have prevented prolonged stress and the development of allodynia, lasting morphological effects were seen. CFA resulted in a reduction in CA3 basal and apical dendritic branching and length in a dose-dependent manner compared to saline controls. To determine the impact of the early stress and neuroinflammatory processes, rats were either injected with saline or 0.5mg/mL CFA (n=4-6/group) and sacrificed 3-days later. While serum corticosterone had returned to baseline, CFA increased DUSP6/MKP3 protein expression within the dorsal CA3 HC. These results suggest that while the recovery of endogenous androgen secretion may help to prevent the development of allodynia, there are early molecular and lasting morphological changes resulting from the induction of mild peripheral inflammation. Ongoing experiments aim to further investigate the role of different androgens in modulating stress and inflammation. This research provides insights into the androgenic regulation and mechanisms of inflammatory processes.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.06

Topic: F.03. Stress and the Brain

Support: Busch Biomedical Grant
Rutgers Brain Health Institute

Title: Role of Nociceptin/Orphanin FQ in the regulation of inflammation-induced sickness behavior

Authors: *M. NISSENBAUM[1], C. CARDINALE[1], S. MOLESKO[1], S. WALPOLE[1], J. E. PINTAR[2], A. W. KUSNECOV[1];
[1]Psychology, Rutgers Univ., Piscataway, NJ; [2]Neurosci. & Cell Biol., Robert Wood Johnson Med. Sch., Piscataway, NJ

Abstract: The precursor propeptide Nociceptin/Orphanin FQ (N/OFQ) peptide is linked to behavioral changes, including anxiety, reward, and nociception. Activation of the nociceptin receptor (NOP) has been argued to oppose behavioral deficits due to stress, analgesic effects of mu-opioid receptor stimulation and systemic inflammation. Here we have examined the role of NOP in regulating the behavioral response to a proinflammatory stimulus, LPS. Behavioral testing examined a variant of a food or taste aversion paradigm in which C57BL/6 background NOP-deficient mice [NOP KO] (23F; 31M) and WT controls (20F; 22M) were treated with LPS (200μg/kg and 600μg/kg) and immediately exposed (for 90 mins) to Prosobee (a baby formula mice voluntarily consume without food deprivation).Consumption around the time of injection allowed for assessment of immediate illness effects, while recovery of consumption (or retention of aversion) was measured 24h later. Animals were also monitored for overnight food intake and body weight. After 24h, the 600μg/kg LPS group still exhibited taste aversion ($p<0.05$), while the low 200μg/kg LPS dose showed variable recovery. Interestingly, NOP KO mice treated with LPS consumed significantly less Prosobee than NOP KO mice treated with saline, and WT mice treated with LPS ($p<0.05$). For males, LPS-treated WT mice exhibited a significant recovery in consumption 24h after treatment ($p<0.01$), but LPS-treated NOP KO mice did not. In contrast, when compared to WT females given LPS, NOP KO females showed significant recovery in consumption 24h after LPS exposure ($p<0.01$). To measure the spleen cytokine response, WT and NOP KO mice were killed 90min and 3h after injection with LPS (200 μg/Kg) or Saline. At 90 mins, when compared to NOP KO saline animals, both male and female NOP KO mice showed a greater splenic IL-1β response (pg/μg protein) than the splenic IL-1β response of LPS-treated WT mice compared to WT saline control (NOP KO increase: $p<0.0001$; WT increase: $p<0.01$). At 3h after treatment, LPS-treated NOP KO females still showed exaggerated IL-1β output compared to saline-treated NOP KO females, while males did not ($p<0.01$). These data suggest that in the absence of N/OFQ signaling, the systemic IL-1β and sickness response to LPS is more pronounced, and subject to sex effects. Overall, it is suggested that N/OFQ may be important for restraining inflammatory responses and attending behavioral adjustments.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.07

Topic: F.03. Stress and the Brain

Support: Grant from the National Institute of Mental Health (NIMH) MH097988

Title: Inhibition of PAC1 receptor-expressing neurons in the bed nucleus of the stria terminalis (BNST) reduces anxiety like behavior

Authors: *M. AKTAR[1], A. CARDARELLI[1], V. MAY[2], S. E. HAMMACK[3];
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Abstract: Pituitary adenylate cyclase activating polypeptide (PACAP) and its cogent receptor PAC1 play pivotal roles in myriad physiological functions. We have demonstrated that chronic stress increases PACAP and PAC1 receptor expression in the bed nucleus of the stria terminalis (BNST), and our pharmacological studies suggest that BNST PACAP receptor activation is necessary and sufficient for many of the behavioral and physiological consequences of stressor exposure. Here we use chemogenetic approaches in PAC1-ires-Cre mice, in which Cre recombinase expression is downstream of the PAC1 receptor gene promoter to specifically inhibit the activity of PAC1 expressing cells in the BNST. Using a designer receptor exclusively activated by designer drugs (DREADD) strategy and the PAC1-Cre mice, we injected a viral vector to elicit cre-dependent expression of the inhibitory hM4Di gene, or a control reporter gene, in BNST PAC1-expressing neurons. After recovery, administered systemic injections of clozapine N-oxide (CNO) and assessed anxiety-like behavior on an elevated plus maze. CNO significantly increased open-arm exploration without reducing total locomotor activity, suggesting that the inhibition of PAC1-expressing neurons in the BNST is anxiolytic, and consistent with our prior work suggesting a key role for BNST PACAP receptor activation in anxiety and stress responding.

Disclosures: M. Aktar: None. **A. Cardarelli:** None. **V. May:** None. **S.E. Hammack:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

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Program #/Poster #: 063.08

Topic: F.03. Stress and the Brain

Support: Department of Veterans Affairs Grant I21 BX002085
Department of Veterans Affairs Grant IO1 BX001804
NSF Grant IOS-1656626
NIH Grant R01AG050518
NIH Grant F31DK131773

Title: Effect of pyridostigmine bromide, repeated restraint stress and an immune challenge on hippocampal-dependent learning and memory

Authors: *H. E. BURZYNSKI[1,2], K. E. AYALA[1,2], M. A. FRICK[2], V. A. MACHT[2], J. L. WOODRUFF[1,2], C. A. GRILLO[1,2], J. R. FADEL[1,2], L. P. REAGAN[1,2];
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Abstract: Gulf War Illness (GWI) is a multi-symptom illness that continues to affect over 25% of Gulf War veterans. While soldiers were exposed to several hazards in the Gulf, the prophylactic use of the acetylcholinesterase inhibitor, pyridostigmine bromide (PB) and war-related stress have been identified as chief factors in GWI pathology. As one of the most insidious aspects of GWI is the cognitive impairments that continue to worsen as veterans age, we hypothesized that the combination of PB and repeated restraint stress (RRS) impairs hippocampal-dependent learning and memory over time. Using our previously established rat model of GWI, we conducted novel object recognition (NOR) testing at both an early (10 days post treatment) and delayed (3 months post treatment) time point in the same cohort. Interestingly, many clinical studies have found that GWI patients exhibit exaggerated cognitive deficits following a stressful stimulus such as an exercise challenge. Thus, we also investigated how an innate immune or stress challenge affects spatial and non-spatial hippocampal-dependent learning and memory in our rat model of GWI. Specifically, adult male rats underwent a second NOR session at each time point in which they were challenged with a low dose (30 µg/kg) of intraperitoneal lipopolysaccharide (LPS). To mimic clinical testing, hippocampal-dependent learning and memory was also assessed with the Morris Water Maze (MWM) at the delayed time point as this test is considered physically demanding. We found that rats with a history of PB treatment 3 months prior exhibited hippocampal-dependent learning and memory deficits when challenged with LPS, but not saline, in the NOR task. Similarly, in the same cohort, PB-treated rats showed deficits in the MWM task, irrespective of stress history. Ultimately, these studies, in combination with our previous findings, highlight the long-term effects of PB treatment on hippocampal function and the impact of a new challenge/stressor before behavioral testing in GWI studies.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

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Program #/Poster #: 063.09

Topic: F.03. Stress and the Brain

Support: DoD grant W81XWH-19-1-0802

Title: Effects of Probiotic Therapy on Exercise Endurance and Post-Exercise Behavior in a Mouse Model of Gulf War Illness

Authors: *E. V. KOZLOVA[1], A. E. BISHAY[1], M. E. DENYS[1], C. V. BERDASCO[1], B. CARABELLI[1], V. PIAMTHAI[2], A. HSIAO[2], M. C. CURRAS-COLLAZO[1];
[1]Molecular, Cell and Systems Biol., [2]Dept. of Microbiology and Plant Pathology, Univ. of California Riverside, Riverside, CA

Abstract: Gulf War Illness (GWI) is a chronic multi-symptom condition that persists among veterans of the 1991 Persian Gulf War (GW). Latently-emerging and persistent symptoms fall into 5 general domains including cognitive and fatigue categories. Our group and others have indicated a possible role of the gut-brain axis in GWI pathology (DOI: 10.1016/j.lfs.2021.120153), which may be a major contributor to the neurotoxicity, inflammation and persistent exercise fatigue phenotypes (https://doi.org/10.1016/j.lfs.2021.120094). The current study examines the therapeutic effects of readily available probiotic supplements which could serve as an alternative treatment desperately needed in the rapidly aging GW veteran population. We have previously shown that chronic exposure to GW agents reduces latency to enhaustion on an exercise endurance test (EE). We investigated the potential benefit of probiotic (PR) therapy on the GWI behavioral phenotypes. Adult male C57Bl6/N and C57Bl6/J mice were exposed to GW agents 5d/wk for 28 days: PB (8.7 mg/kg/d; po), PER (1.3 mg/kg/d; top) in 70% ETOH, 33% DEET in 70% EtOH (75 µl/30 g bw; top) and 5min/d stress (n=8-16; 4 mo of age). A probiotic cocktail of L. reuteri ATCC 23272 (gift of Biogaia), L. rhamnosus GG ATCC 53103, L.casei ATCC 393 and B.longum DSM 20219 was administered p.o. 3 times/week (10^8 CFU/mL) during GW agent exposure and until behavioral testing. RT-qPCR analysis showed colonization in gut after administration of 6-7 doses of all strains except L. rhamnosus. For EE, which involves 3 training days and 2 testing days,the latter used for mean latency to exhaustion. All GW mice tired faster on EE relative to CON/S (n=26-28/group, $p<.05$). PR treatment produced an apparent reduction in exhaustion. Latency scores on EE were not due to differential body weight or lean mass in GW relative to CON/S. To examine whether GW mice showed post exertional malaise (PEM), the effect of exercise on other measures of fatigue and stress responses was examined. Compared to CON/S, mean values for latency to fall on hanging wire test were apparently greater for GW + PR vs GW in 8 of 12 mice/group tested. Mean values for percent time spent mobile on tail suspension test were significantly lower for GW (n=7-8/group, $p<.001$) but not GW + PR. These results show that our GW mouse model displays exaggerated exhaustion during exercise that may aggravate other behavioral manifestations of GWI pathophysiology such as depression-related behaviors. Probiotic therapy may prove beneficial as an alternative method of treatment providing relief for GWI-related fatigue.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

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Program #/Poster #: 063.10

Topic: F.03. Stress and the Brain

Support: K01 MH117343
Tulane University Start-up Package

Title: Cognitive and neuroinflammatory impacts of B lymphocyte deficiency and chronic restraint in male mice

Authors: *I. PURSELL, K. MCDONALD, H. WANG, R. FREITAS, L. GARFINKEL, A. TRINH, E. ENGLER-CHIURAZZI;
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Abstract: Innate immunity-related molecules like cytokines, as well as acquired immunity-related molecules like MHC and antibody receptors, are known to be expressed in the brain and play key roles in brain development, cognition, and neural circuit formation. B lymphocytes (B cells) are emerging as important modulators of brain function. For example, B cells migrate to the brain parenchyma after a brain injury (such as stroke) and may support recovery/repair. New evidence suggests that the peripheral immune system is a crucial mood regulator, revealing new therapeutic options for chronic stress disorders like anxiety and major depression. Evidence of a brain-spleen communication pathway and shifts in splenic B cells compartment profiles following exposure to stress supports this possibility. The goal of this study was to explore the extent to which the presence of B cells altered the physiological, behavioral, and neuroinflammatory response to chronic stress. We hypothesize that B cells promote a resilience phenotype thus our MuMT B cell deficient mice are more susceptible to cognitive impairment and neurological impacts of long-term exposure to stress. Using a 2x2 factorial design (N=20), we examined the effect of B cell deficiency induced by genetic (muMT) manipulation on behavioural responses to chronic restraint stress (CRS) among male 3-month-old mice. CRS mice were placed into restraint for 6 hours for 32 consecutive days. Control animals underwent brief insertion (10sec) into restraint device with immediate withdrawal. Throughout the stress induction period, significant main effects of Genotype and Stress Experience for percent body weight change from baseline were observed. On Morris Water Maze (MWM) test of spatial reference memory, we reported an early and transient impairment (day 1) in swim distance (m) among muMT B cell deficient mice ($p = 0.05$). Other physiological and behavioral measures, such as terminal adrenal weights, passive avoidance, hot plate, and y-maze, revealed no statistically significant differences between groups. Even in the control wild type mice, blood corticosterone protein assays revealed elevated levels, suggesting that all mice displayed a stressed phenotype regardless of stress condition. Neuroinflammatory markers being tested using qPCR, Western Blot, and immunohistochemistry are ongoing; initial analyses suggest that B cell deficiency promotes enhanced expression of Iba1 and GFAP hippocampal inflammatory markers. Taken together, though our hypothesis was not supported, but data suggest further evaluation of B lymphocyte deficiency contributions to brain inflammation and behavior is warranted.

Disclosures: I. Pursell: None. **K. McDonald:** None. **H. Wang:** None. **R. Freitas:** None. **L. Garfinkel:** None. **A. Trinh:** None. **E. Engler-Chiurazzi:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.11

Topic: F.03. Stress and the Brain

Support: DEFENSE RESEARCH AND DEVELOPMENT ORGANIZATION FUNDS

Title: Total Sleep deprivation enhances microglial-mediated neuroinflammation: adenosine A1 receptor antagonism- a potential therapeutic target mechanism

Authors: *B. THONDALA[1], G. CHAUHAN[2], K. RAY[2], H. PAWAR[2], K. KISHORE[2], S. KUMAR[2], S. GAUR[2], U. PANJWANI[2];
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Abstract: Sleep deprivation is a biological stress that engenders neuroinflammation. It is still unclear how the causal relationship between immune dysregulation and sleep deprivation leads to chronic inflammatory conditions eventuating in psychiatric disorders. We hypothesized that total sleep deprivation (TSD) for 48 hours will induce microglial mediated neuroinflammation via TLR4 receptor and antagonism of Adenosine A1 receptor may rescue TSD induced impairments on fear extinction recall. Animals were randomized into 3 groups; Cage Controls (CC), TSD + Vehicle (20% Dimethyl Sulfoxide) and TSD+ 8-Cyclopentyltheophylline (8-CPT; Adenosine A1 Receptor Antagonist, 10mg/kg, intraperitoneal, twice daily). We used a customized rodent sleep deprivation system to deprive male Sprague Dawley rats (n=11, 8 to 10 weeks old) of total sleep for 48 hours after Cued fear extinction training on day 2 (74.4±2.4, $p<0.001$) (Freezing scores are represented as mean±SEM). Appropriate ANOVA measures and Tukey's and Bartlett's multiple comparison tests were used for post-hoc analysis of data. We found that 48h TSD impaired extinction memory recall on day 4 (100.3±2.4, $p<0.001$) whereas, 8-CPT administration ameliorated the fear extinction recall (69.2±2.4, $p<0.01$). Immunofluorescence analysis (n=5, 8 to 10 weeks old; values expressed are mean differences ± standard error of difference) showed increased co-expression of TLR4 (-22.1±2.4, $p<0.03$) with Iba1 (-11.1±1.8, $p<0.04$) and increased expression of neuroinflammatory markers like IL1β (-16.1±2.2, $p<0.01$), phospho-NFκB (-15.8±2.1, $p<0.03$), TNFα (-10.8±1.72, $p<0.01$) in the dorsal hippocampus of TSD animals as compared to CC. 8-CPT administration decreased the expression of TLR4 (10.7±2.3, $p<0.003$), Iba1 (7.8±1.76, $p<0.004$), TNFα (8.5±1.7, $p<0.01$), phospho-NFκB (13.1±2.1, $p<0.03$), IL1β (7.9±2.2, $p<0.01$) compared to TSD. Reduced expression of PSD-95 (20.8±2.9, $p<0.02$) and synaptophysin (21.3±1.9, $p<0.001$) in TSD animals was improved by 8-CPT (-8.4±3.1, $p<0.02$). These results correlate with the detrimental effect of TSD on extinction memory recall. Sleep recordings showed that the percentage of NREM and REM sleep was reduced in TSD+8-CPT animals (12.1; 2.1) as compared to CC (69.5; 11.33) and TSD (13.4; 4.6). In conclusion, we present data on microglial mediated neuroinflammation which paves the way to understanding receptor biology of neuroinflammation induced by TSD in rodents. Antagonism of Adenosine A1 receptor rescues

neuroinflammation and synaptic plasticity. Our study recognizes Adenosine A1 receptor as a potential therapeutic target for pathological conditions of memory recall impairments.

Disclosures: **B. Thondala:** None. **G. Chauhan:** None. **K. Ray:** None. **H. Pawar:** None. **K. Kishore:** None. **S. Kumar:** None. **S. Gaur:** None. **U. Panjwani:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.12

Topic: F.03. Stress and the Brain

Title: Dysfunctional behaviors, physiology, and microglial response in a single prolonged stress mouse model of PTSD

Authors: *J. HOLSTEN, K. PRECOTT, S. BEUSCHEL, A. SALIHU, T. PETERSON; Psychology, Univ. of North Carolina Wilmingon, Wilmington, NC

Abstract: Nearly 8 million people are diagnosed with Post-Traumatic Stress Disorder (PTSD) in the US yearly. The disorder is precipitated by a traumatic event that manifests into dysfunctional behaviors like anxiety and hypervigilance, ultimately disrupting a person's well-being. Physiological and pathological alterations occur immediately following a traumatic event and are associated with dysfunctional behaviors but are poorly understood. Recent data suggests microglia, alter their typical ramified, branch-like morphology in the prefrontal cortex (PFC) and hippocampus into a hyper-ramified subtype over a month following a traumatic event. However, the morphology of microglia have primarily been assessed on a 2-dimensional (2D) scale in a variety of PTSD animal models. Moreover, microglia morphology has not been quantified in a single prolonged stress (SPS) model of PTSD. This model has been shown to mimic behavioral and physiological deficits of the human condition. The current study utilized 41 male and female C57/6J mice to measure behavior (elevated plus maze, open field, marble burying, and social interaction), physiology (urinary corticosterone), and microglia morphology in a SPS mouse model. This model was comprised of a series of five consecutive stimuli: 2 hr restraint stress, 20 min forced swim, 15 min recuperation in soiled rat bedding, exposure to diethyl ether until unconscious, and single housed for 7 days. The SPS group displayed significant differences in anxiety- and hypervigilant-like behaviors ($p < .05$). Urinary corticosterone levels of female SPS mice were elevated at the acute time point ($p < .001$) but returned to similar levels at the delayed time point compared to naïve mice. Microglia were fluorescently labeled with Iba1 and imaged with a confocal microscope using z-stacks for 3D image reconstruction of the PFC, hippocampus, and amygdala. Microglia were assessed by the number of cells, processes, end points, and sphericity. We found female SPS mice had an increased number of microglia in the PFC compared to naïve females ($p < .05$), but no other brain regions. There were no differences in morphology between SPS and Naïve mice. We also found decreased urinary corticosterone and increased number of microglia were associated with increased hypervigilant-like behavior in

male mice ($p < .001$). There were no associations found for female mice. Establishing the microglia response and morphological changes of microglia in a valid model of PTSD is crucial to understand the different subtypes microglia adopt in PTSD. By determining the morphological response, researchers can gain insight into their functionality and develop future treatments.

Disclosures: J. Holsten: None. **K. Precott:** None. **S. Beuschel:** None. **A. Salihu:** None. **T. Peterson:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.13

Topic: F.03. Stress and the Brain

Title: Neutrophil elastase mediates alcohol induced stress susceptibility in adult male mice

Authors: *L. PARISE[1], F. CATHOMAS[3], K. L. CHAN[2], L. LI[4], H.-Y. LIN[2], R. DURAND-DE CUTTOLI[5], A. V. AUBRY[6], S. J. RUSSO[7];
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Abstract: In the past year there has been a sharp increase in both alcohol use and major depression, which is concerning considering the high rates of comorbidity between mood disorders and substance abuse. While there are similarities in the presentation of peripheral markers after both stress and alcohol exposure, the underlying mechanisms and whether they interact, specifically at the level of the blood brain barrier (BBB), remains unclear. The BBB is critical for maintaining proper homeostatic balance within the central nervous system and acts to selectively regulate the molecules that are trafficked into the brain. Using a model of moderate binge drinking, we investigated how alcohol exposure influences stress-related pathology, focusing on peripheral dysregulation and BBB-related alterations. To this end, adult mice were given intermittent access to 20% alcohol. After 4 weeks, blood plasma and nucleus accumbens tissue punches were collected from all groups to assess changes in peripheral inflammatory markers and BBB-related targets, respectively. A separate group of mice were given a retro-orbital injection of a 10 kDa dextran to test BBB permeability. Mice exposed to 4 weeks of alcohol had increased circulating neutrophils and showed social avoidance after exposure to subthreshold stressor, which does not elicit a stress response in control mice. Further investigation revealed an increase in neutrophil elastase (NE), a serine protease released by neutrophils which can target tight-junction proteins on endothelial cells. Interestingly, only male mice with a history of alcohol exposure showed exaggerated expression of NE after a stress challenge. Alcohol-exposed male mice also had increased brain-permeability to a peripherally-

injected dye, Evans' Blue (70 kDa) . Increased permeability is likely due to the reduction of tight junction proteins (Claudin 5) within the nucleus accumbens and leakage was confirmed via immunohistological staining of blood vessels (tomato lectin) and a peripherally-injected 10 kDa dextran.Taken together these data suggest that chronic, intermittent exposure to alcohol changes the peripheral landscape to a pro-inflammatory state, promoting stress susceptibility. Further, this work implicates that serine proteases could be a novel target for managing alcohol-induced deficits to stress-responding.

Disclosures: L. Parise: None. **F. Cathomas:** None. **K.L. chan:** None. **L. Li:** None. **H. Lin:** None. **R. Durand-De Cuttoli:** None. **A.V. Aubry:** None. **S.J. Russo:** None.

Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.14

Topic: F.03. Stress and the Brain

Support: R01 MH127850-01

Title: Microglia depletion alters the impact of a two-hit model of early adversity on cognitive behaviors and perineuronal net formation

Authors: *M. FANIKOS[1], K. R. GILDAWIE[2], A. A. VALENTINE[3], A. PARAKOYI[1], H. C. BRENHOUSE[1];
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Abstract: The dynamic relationship between the nervous and immune systems plays an integral part in the long-term, sex-dependent impacts of adversity. Microglia are the primary neuroimmune cell and are known for their role in the brain's response to stress as well as their tendency to cause neuronal damage when overactivated. Functional and morphological activation states have been found to differ in male and female animals exposed to multiple early adverse events. Microglia alter neurons and their activity through the release of cytokines, elimination of synapses, and phagocytosis of debris. For example, microglia are known to drive degradation and regulation of perineuronal nets (PNNs). PNNs are specialized structures of the extracellular matrix that preferentially enwrap parvalbumin (PV) expressing interneurons. Furthermore, early-life adversity (ELA) reduces adolescent and adult PV expression in the prefrontal cortex (PFC) which may play a role in the adversity-induced deficits in cognition. We have shown that multiple occurrences of early adverse experiences induce a long-lasting reduction to PV cell count and PV+ PNN structural integrity in females only.
To address if microglial activity in the early postnatal period mediates the sex-dependent response to multiple adverse experiences early in life, microglia were transiently depleted during postnatal ELA, from postnatal day (P)2 to P10, using liposomal clodronate. Rats underwent two

forms of ELA: maternal separation from P2 to P20, and juvenile social isolation (SI) from P21 to P35. On P35 all SI rats were housed with a condition- and sex- matched conspecific and were left undisturbed until behavioral testing on P70. Rats were tested in the spontaneous alternation and social recognition tasks, which test working memory and social behaviors respectively. Following these behavioral paradigms, the density and intensity of PV neurons and PNNs in the PFC were quantified.
These results will elucidate the role microglia play in modulating the sex-specific impacts of multiple adverse early life experiences on cognitive and social behaviors and PNN formation in the PFC.

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Poster

063. Neuroinflammation and Neuroimmunology in Stress

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 063.15

Topic: F.03. Stress and the Brain

Support: Rowan University School of Osteopathic Medicine, Department of Cell Biology and Neuroscience

Title: Chronic stress induces neuroinflammation and alters locus coeruleus physiology

Authors: *A. A. REYES, D. J. CHANDLER;
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Abstract: Chronic stress exposure is a risk factor in developing neuropsychiatric disorders such as anxiety and depression. The locus coeruleus (LC), the primary site of brain norepinephrine (NE), is a key anatomical brain region implicated in stress response and the development of pathological anxiety. Recent studies have demonstrated a role of chronic stress in the induction of neuroinflammation. However, the link between chronic stress, neuroinflammation, and LC has not been established. In this study, we demonstrate the role of chronic stress in LC using elevated plus maze (EPM), ex-vivo electrophysiology, immunohistochemistry (IHC), and quantitative real-time-PCR (qRT-PCR). To model chronic stress, adolescent (5-6-week-old) male (control n=16, stress n=16) and female (control n=7, stress n=7) Sprague Dawley rats were exposed to chronic stress by physical restraint with predator odorant 2,4,5-Trimethylthiazole (TMT) two hours per day over ten days for two consecutive weeks. Chronic stress exposure significantly reduced average weight gain in male stress rats (p=0.0002), with no difference in females. However, anxiety-like phenotype testing using EPM did not reveal any differences in key anxiety-like measures such as open arm time, distance traveled, and time immobile between stress and control rats in both sexes. Despite the lack of anxiety-like behaviors, IHC, qRT-PCR, and electrophysiology reveal chronic stress-induced changes in LC. IHC staining using Tyrosine

Hydroxylase (TH), a noradrenergic marker, Iba-1, a microglial marker, and MHCII, an activation marker, revealed a significant increase in activated microglia in LC of male stress rats (p=0.0435). Interestingly, female stress rats show an opposite trend, with chronic stressed female rats showing a significant reduction in LC microglia (p=0.007). Analysis of pro- and anti-inflammatory genetic markers in male LC tissue punches using qRT-PCR (control n=5, stress n=5) revealed increased pro-inflammatory *cd74* (p=0.0159) and *il6* (p=0.0845) expression. *Ex-vivo* electrophysiology recordings of LC slices in male rats (control n=11 cells from 2 rats, stress n=16 cells from 3 rats) showed increased LC firing, increased resting membrane potential, decreased action potential threshold, and decreased activation gap in stress rats. Our results suggest that although chronic stress does not promote changes in anxiety-like behavior, it induces LC neuroinflammation through increased activated pro-inflammatory microglia and alters LC neuronal physiology. The potential link between these two chronic stress-induced changes in LC remains to be elucidated.

Disclosures: A.A. Reyes: None. **D.J. Chandler:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.01

Topic: F.07. Biological Rhythms and Sleep

Support: R01 HD100580
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K99 NS119291
F32 HD098805
K12 GM068524
R01 HD082567
R01 HD072754

Title: Circadian behavior in mice with conditional deletion of Six3 in Neuromedin-S neurons

Authors: *K. TONSFELDT[1], L. E. CHUN[2], M. J. PATEL[3], N. P. NAING[3], J. CASSIN[3], M. GORMAN[4], P. L. MELLON[3];
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Abstract: The hypothalamic suprachiasmatic nucleus (SCN) regulates circadian rhythms in mammals. The Six family of homeodomains proteins is critical for eye and forebrain development, and *Six3* retains expression in the adult SCN and has been used as a marker for the SCN. Recently, we demonstrated that loss of *Six3* in mature neurons using a Synapsin-cre driver disrupts circadian rhythmicity and female fertility. Here, we narrowed our study to understand the role of *Six3* in Neuromedin-S (NMS)-containing neurons, which represent approximately half

of SCN neurons. Using NMS^{cre} and $Six3^{flox/flox}$ mice, we created $Six3^{NMS}$ mice to explore the role of *Six3* in NMS neurons. While the mice had normal circadian wheel running behavior in light-dark conditions, we found that $Six3^{NMS}$ males, but not females, had significantly shorter periods in constant darkness compared to control $Six3^{flox}$ males. We found no differences in puberty onset as measured by preputial separation or vaginal opening compared to $Six3^{flox}$ mice. In females, we found no effect of genotype on time to first litter, average number of pups per litter, or estrus cycle length. We also found that all mice exhibited normal corticosterone surges in light-dark, and these rhythms persisted in constant darkness. Overall, our findings demonstrate that loss of *Six3* in NMS neurons has a much milder phenotype than loss of *Six3* in synapsin neurons. Future studies will determine if this effect is due to the timing of cre expression (and loss of *Six3*) or neuron populations targeted within the SCN.

Disclosures: K. Tonsfeldt: None. **L.E. Chun:** None. **M.J. Patel:** None. **N.P. Naing:** None. **J. Cassin:** None. **M. Gorman:** None. **P.L. Mellon:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.02

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant 1R15GM134528

Title: Exploring the daily expression patterns of Fgfr1 and Klb in the SCN

Authors: *E. N. WELCH[1], T. D. NIEPOKNY[2], E. M. MINTZ[3];
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Abstract: Fibroblast growth factor 21 (FGF21) is an endocrine-acting signaling molecule that is a member of the fibroblast growth factor family of proteins. Functionally, FGF21 plays a role in signaling metabolic and nutritional stress to peripheral and central tissues and aids in maintaining metabolic homeostasis. FGF21 acts on the receptor FGFR1 and the obligate co-receptor, β-klotho (KLB), which is localized to FGF21 target tissues. Centrally, the suprachiasmatic nucleus of the hypothalamus (SCN) has been implicated as an important region for mediating changes to systemic metabolic activity and circadian behavior induced by FGF21 signaling. The SCN has localized expression of *Klb* mRNA, elevated relative to other regions of the brain. To further explore the role of FGF21 signaling in the SCN, we sought to characterize the daily expression patterns of *Fgfr1* and *Klb* mRNA in the SCN of C57BL/6J mice. Male and female animals were individually housed on a 12:12 LD light cycle with food available *ad libitum* for two weeks and sacrificed at 3-hour intervals over 24 hours. Tissue from the SCN was then excised, and RNA samples were reverse transcribed into cDNA for analysis using quantitative real-time PCR. Rhythmicity of mRNA expression was measured using cosinor analysis. Statistically significant rhythms were detected in the expression of *Fgfr1* and *Klb* mRNA in the SCN of both sexes.

Additionally, peak expression of *Klb* mRNA was delayed by about 1.5 hours in males compared to females. The amplitude and robustness of rhythmic expression of *Fgfr1* and *Klb* in the SCN suggests that these rhythms are functionally important and that FGF21 signaling is likely being modulated in a time-dependent manner.

Disclosures: E.N. Welch: None. **T.D. Niepokny:** None. **E.M. Mintz:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.03

Topic: F.07. Biological Rhythms and Sleep

Title: Behavioral and molecular consequences of a habenula specific deletion of Bmal1

Authors: *C. GOLDFARB[1], K. SCHÖTTNER[2], A. BERGDAHL[4], S. AMIR[3];
[2]Ctr. for Studies in Behavioral Neurobio. (CSBN), [3]Psychology, [1]Concordia Univ., Montreal, QC, Canada; [4]Health, Kinesiology & Applied Physiol., Concordia, Montreal, QC, Canada

Abstract: Circadian clocks keep temporal order of molecular, physiological, and behavioral processes with respect to the cyclic environment - enabling systemic homeostasis and proper function of the organism. The circadian timing system is comprised of a central pacemaker within the suprachiasmatic nucleus of the hypothalamus (SCN) and peripheral clocks throughout the brain and body. The circadian clock in the habenula, however, represent a unique characteristic as its time-setting appears to happen independently from the central circadian pacemaker in the SCN. Uniquely positioned to act on a large source of dopaminergic neurons, the habenula sends inhibitory signals to the ventral tegmental area (VTA) and the substantia nigra (SN). Alterations to these regions, and to the dopaminergic system, can have various behavioral consequences, potentially leading to psychological disorders and diseases such as addiction, schizophrenia, and Parkinson's disease. We are investigating the role of the habenula as a pacemaker for the production and release of dopaminergic signals along the nigrostriatal pathway. Using male and female Bmal1 floxed mice, we microinjected AAV-2/9 Cre-eGFP and AAV-2/9-eGF viruses into the lateral habenula to selectively knockout Bmal1 The impact of this knockout was investigated through a battery of tests that assess dopamine-related behaviors. We found that a habenular-specific deletion of *Bmal1* led to a significant impact on motor functioning in both male and female mice; whereas affective behaviour was only mildly impacted. Daily rhythms of gene expression of the SN and dorsal striatum (DS), primarily targeting the circadian clockwork and cell signalling pathways, were also assessed. Results indicate changes in molecular processes in knockout animals, that may contribute to the observed behavioral phenotypes. As proper functioning of these regions is presumably maintained by a mutual interaction of components of the circadian clock and the dopamine signalling pathway, these findings support that disrupting clock functioning in the habenula can impact functioning of downstream targets.

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Poster

064. Biological Clocks and Rhythms

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.04

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI 18K19330
JSPS KAKENHI 19K16185
JSPS KAKENHI 21K06263
JSPS KAKENHI 22K06311

Title: Differential GABAergic Ca^{2+} responses in the hypothalamic ventral subparaventricular zone of the diurnal grass rat, Arvicanthis niloticus

Authors: S. TAMOGAMI[1,2], S. NAKAGAWA[1], Y. KASUGA[1], E. MORIOKA[1], T. YOSHIKAWA[3], *T. MOCHIZUKI[1], M. IKEDA[2,3];
[1]Grad. Sch. of Sci. and Engin., [2]Grad. Sch. of Innovative Life Sci., [3]Organization for Intl. Educ. and Exchange, Univ. of Toyama, Toyama, Japan

Abstract: GABA is the major neurotransmitter contained in the hypothalamic suprachiasmatic nucleus (SCN), the central circadian pacemaker in mammals. Although the GABAergic projections from the SCN are dense in the sub-paraventricular zone (SPZ) and paraventricular nucleus (PVN) of the hypothalamus, their physiological roles have not been systematically compared between nocturnal and diurnal rodents. Here, we used *fura-2* based Ca^{2+} imaging technique for acutely-isolated brain slices of juvenile Nile grass rats (*Arvicanthis niloticus*) and C57 BL/6J mice, and compared GABA responses in the ventral SPZ (vSPZ) and the PVN. Upon a pulsate stimulation with GABA (200 μM) under treatment of tetrodotoxin (0.5 μM), vSPZ and PVN cells elevated intracellular Ca^{2+} concentrations depending on the brain loci, time of day and species. In the vSPZ, day-night variations in Ca^{2+} responses were lacking in both species, whereas the size of Ca^{2+} response was significantly smaller in grass rat slices and number of cells responding to GABA in Nile grass rats (37.3%) was less than half of that in mice (77.5 %). In the PVN, number of cells responding to GABA during the night (49% for Nile grass rats and 70% for mice) was significantly larger than those during the day (33% for Nile grass rats and 49% for mice). These results suggest differential GABA functions in the vSPZ and PVN between diurnal and nocturnal species.

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Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

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Program #/Poster #: 064.05

Topic: F.07. Biological Rhythms and Sleep

Support: PRSTRT 2022-00001
USDA-NIFA 2021-67014-34999
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NSF-PRCEN 1736019

Title: The development of circadian behavior is associated with changes in the expression of IGFBP-ASL in the brain of honey bees Apis mellifera

Authors: *E. AVILÉS RÍOS[1], E. COURTNEY[2], Y. B. KORU[1], Y. ORTIZ ALVARADO[1], M. DÖKE[1], A. MONTES[1], A. A. RUGGIERI[1], N. RODRIGUEZ[1], R. GIORDANO[3], D. RAVI[4], J. LEON[1], A. GHEZZI[1], T. GIRAY[1], J. L. AGOSTO RIVERA[1];
[1]Univ. de Puerto Rico, San Juan, PR; [2]Minerva Univ., San Francisco, CA; [3]Florida Intl. Univ., Miami, FL; [4]Know your bee, San Juan, PR

Abstract: Honey bees, like most living organisms, show a circadian rhythm that influences processes in their behavior and metabolism. In accordance with this we have seen in previous studies that bees's gut microbiota can affect the way some cells are expressed. Recent studies have shown that the development of circadian behavior is associated with an increase in the number of brain circadian pacemaker cells called Pigment-dispersing factor (PDF) cells. Studies in our laboratory indicate that the development of circadian behavior is also regulated by gut microbes. However the mechanisms by which gut microbes regulate circadian rhythm development and PDF cell number remains to be elucidated. A transcriptomic study in our laboratory indicated that a gene called Insulin- Like Growth Factor Binding Protein Acid Labile Subunit (IGFBP-ALS) was one of the top 5 differentially expressed genes when comparing brain gene expression patterns of rhythmic and arrhythmic bees with and without antibiotics. Since IGFBP-ALS is involved in brain developmental processes in other organisms, this study aims to further characterize the expression of IGFBP-ALS in relation to the development of circadian behavior, PDF cells and gut microbes. Our preliminary data indicates that brain IGFBP-ALS expression decreases when honey bees develop circadian rhythms and that antibiotic treatment prevents this decrease. Our studies not only will provide insights into the mechanisms underlying the development of circadian rhythms but also will shine light into how gut microbes regulate brain development.

Disclosures: E. Avilés Ríos: None. **E. Courtney:** None. **Y.B. Koru:** None. **Y. Ortiz Alvarado:** None. **M. Döke:** None. **A. Montes:** None. **A.A. Ruggieri:** None. **N. Rodriguez:** None. **R. Giordano:** None. **D. Ravi:** None. **J. Leon:** None. **A. Ghezzi:** None. **T. Giray:** None. **J.L. Agosto Rivera:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.06

Topic: F.07. Biological Rhythms and Sleep

Support: NSF-DBI 2150326

Title: A Genome Analysis of IGFALS in Honey Bees: Understanding the Allele Frequency of Insulin-Like Growth Factor Binding Protein Acid Labile Subunit in Apis mellifera Foragers in Puerto Rico

Authors: *E. COURTNEY[1], A. AVALOS[2], Y. KORU[3], E. AVILES[5], J. L. AGOSTO[6], T. GIRAY[4];
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Abstract: Honey bees have strong endogenous circadian clocks that relate to behavioral development, which is critical for colony survival. Forager bees, responsible for gathering resources for the colony, have well-developed circadian rhythms that allow them to effectively time foraging to maximize their efficiency, whereas nurse bees, who do not need to leave the hive, can accomplish tasks without fully expressing circadian rhythms. Like other neurological circuits, circadian circuits are regulated by environmental, genetic, neuroplastic, and epigenetic factors. Throughout bee colonies, there exists variation in genotypes that regulate the polyethism that is characteristic of the division of labor, with some bees becoming foragers at an earlier age than other bees. Aside from genetic factors, preliminary data has demonstrated a relationship between gut microbes, the ontogeny of circadian rhythm, and the expression of Insulin-Like Growth Factor Binding Protein Acid Labile Subunit (IGFALS). To further characterize this relationship and its potential adaptive value, we will explore allele frequencies of IGFALS in honey bee populations throughout Puerto Rico using existing whole-genome data of 270 individuals. Additionally, with existing data spanning from before Hurricane Maria to after, the effect of resource availability on the allele frequency of IGFALS will be investigated based on preliminary findings that allele frequencies of genes responsible for foraging behavior were selected for following mass disturbance. In this investigation, we will quantify the relative frequencies of various alleles of IGFALS using the Genome Analysis Toolkit (GATK) version 3.8 HaplotypeCaller, examining 270 samples of queen and foraging bees from the Apis mellifera population of Puerto Rico. We hypothesize that there will be a change in the allele frequency of IGFALS in foraging bee populations following Hurricane Maria. This exploratory analysis will add to existing knowledge on the role of climate-driven events in population dynamics, and help to elucidate the role of IGFALS in promoting circadian rhythms in honey bees.

Disclosures: E. Courtney: None. **A. Avalos:** None. **Y. Koru:** None. **E. Aviles:** None. **J.L. Agosto:** None. **T. Giray:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

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Program #/Poster #: 064.07

Topic: F.07. Biological Rhythms and Sleep

Support: NIH grant NS092388
T32 GM132494 from NIH NIGMS

Title: Disrupted Cardiovascular Function and Metabolism by Exposure to Artificial Light at Night

Authors: *O. MELÉNDEZ-FERÁNDEZ[1], J. A. LIU[1], J. C. WALTON[1], P. D. CHANTLER[2], A. DEVRIES[1], R. J. NELSON[1];
[1]Neurosci., [2]Exercise Physiol., West Virginia Univ., Morgantown, WV

Abstract: Circadian rhythms (CRs) are internal cycles of ~24 hours driving physiological and behavioral processes. These rhythms are set to precisely 24 hours every day by exposure to light early during the day. Light information is transmitted from the eyes to the master circadian clock, the suprachiasmatic nucleus (SCN) of the hypothalamus, that maintains optimal temporal function of physiology and behavior. Exposure to artificial light at night (ALAN) disrupts CRs and is associated with several health challenges including cardiovascular disease. Specifically, endothelial function (EF), a clinically-relevant indicator of cardiovascular health, is impaired in obesity and other metabolic diseases; EF is also impaired in night shift workers. However, few controlled laboratory studies focus on ALAN exposure as an environmental mediator of circadian disruption in EF. Our lab has demonstrated that exposure to ecologically-relevant levels of ALAN disrupts rhythms in metabolism and immune function. Further, genetic manipulations of core clock genes in cardiovascular tissue reveal impairments in the endogenous daily variation in blood pressure, heart rate, growth and endothelial factor production, among other cardiovascular measures. These data led to the hypothesis that disrupted circadian rhythms by ALAN perturbs EF in rodents, and impairs energy utilization efficiency. To test this hypothesis, we performed vascular reactivity assays on male and female Swiss Webster mice following 8 weeks of ALAN exposure to determine endothelial-dependent and -independent changes in reactivity across 24 hours. Concurrently, energy metabolism was assessed through indirect calorimetry. The data suggest that (1) changes in endothelial-dependent function exist throughout the day, (2) sex differences exist, (3) exposure to ALAN blunts 24 hour variations in reactivity, and (4) ALAN elevates fat accumulation, despite elevated heat production. Together, these data suggest that ALAN impairs homeostatic aortic response and metabolism, which can lead to cardiovascular dysfunction. Next questions to be addressed are (1) whether bioavailability of nitric oxide or production of reactive oxygen species mediate the observed effects, and (2) whether the observed changes in vascular function correlate with changes in circadian clock gene expression in the aorta.

Disclosures: O. Meléndez-Ferández: None. **J.A. Liu:** None. **J.C. Walton:** None. **P.D. Chantler:** None. **A. DeVries:** None. **R.J. Nelson:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.08

Topic: F.07. Biological Rhythms and Sleep

Support: 5T32HD087194-05
5P50HD096723-02
1K01DK121734-01A1

Title: Sex Differences in the Microbiome-Gut-Brain Axis are Time-of-Day Dependent

Authors: *S. MUNYOKI[1], J. GOFF[2], C. A. MCCLUNG[3], K. E. MORRISON[4], E. JAŠAREVIC[5];
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Abstract: Circadian rhythms orchestrate a wide range of homeostatic processes, including metabolism, immune function, and the bidirectional communication between the gut and brain. Emerging evidence points to the gut microbiome and its metabolites as a novel class of entraintment signals, but the precise molecular mechanisms by which diurnal rhythms in microbial metabolites contribute to these processes remain unknown. Moreover, circadian rhythms are influenced by sex, suggesting that microbiome-gut-brain axis function is sex-specific and influenced by time-of-day. To examine the hypothesis that sex differences in the microbiome-gut-brain axis are time-of-day dependent, we leveraged a novel systems biology approach by combining longitudinal analyses with dietary manipulation, metabolomics, bulk and single-cell transcriptomics of gut and brain, single-cell immunophenotyping, and machine learning. We harvested tissues from C57Bl/6J and BALB/c male and female mice every 4 hours across the day-night cycle. Bulk transcriptomic analyses of the intestinal tract revealed that transcriptional signatures were sex-specific and dependent on time-of-day. Diurnal gene expression patterns in the gut were synchronized with the production and peripheral availability of microbial-derived short-chain fatty acids (SCFAs). Integrated single-cell immunophenotyping, metabolomics, and transcriptomics revealed that time-of-day shifts in the peripheral availability of SCFAs were associated with modifications to circulating immune composition and transcriptional signatures in key neural regions controlling whole-body metabolism, including the arcuate nucleus of the hypothalamus. These time-of-day and sex-specific patterns were completely abolished in germ-free mice, suggesting that an intact gut microbiome is necessary for the synchronization of sex differences across the microbiome-gut-brain axis. Further, biosynthesis of SCFAs requires dietary availability of soluble fibers. Thus, to determine whether

dietary availability of soluble fibers and subsequent availability of SCFAs is a necessary time-of-day sex differences in the microbiome-gut-brain axis, C57BL/6 mice were fed a refined high-fat low-fiber diet (HFD) or a grain-based chow (GBC) control for six weeks. Time-of-day effects on SCFA availability were disrupted in the HFD animals, suggesting that dietary availability of soluble fiber is essential for daily oscillations in cecal weights and SCFA abundance. Our findings highlight the need to consider sex and timing of sample collection in neuroscience experiments.

Disclosures: S. Munyoki: None. **J. Goff:** None. **C.A. McClung:** None. **K.E. Morrison:** None. **E. Jašarevic:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.09

Topic: F.07. Biological Rhythms and Sleep

Support: NS109916-03S1

Title: Activity-dependent clock gene regulation in mouse neocortex

Authors: *E. BARRIOS[1], M. SHA[1], S. XIAO[1], B. HORSFALL[2], D. WISE[3], S. B. NELSON[4]; [1]Biol., [2]Neurosci., Brandeis Univ., Waltham, MA; [3]Neurosci., Brandeis Univ., Somerville, MA; [4]Dept Biol MS#008, Brandeis Univ., Waltham, MA

Abstract: We recently found that the PARbZIP transcription factor family (TEF, HLF, DBP) regulates homeostatic plasticity following activity deprivation in excitatory cortical neurons (bioRxiv 2021.10.20.465163). Members of this family are also known regulators of the circadian clock. Because of the PARbZIP TFs strong transcriptional induction following activity deprivation, we performed RNAseq following induction of hyperactivity and activity deprivation and found that activity also bidirectionally regulated other clock genes. CLOCK and NPAS2 were regulated in opposite directions which is unexpected since they compensate for each other in circadian contexts and so might be expected to respond identically. We also knocked out BMAL1, a binding partner of CLOCK and NPAS2, and found a similar, but less powerful, homeostatic phenotype as the PARbZIP transcription factor knockout. Currently, we are performing knockdown of CLOCK and NPAS2 via CRISPRi in excitatory cortical neurons in these different activity paradigms to determine how they regulate activity dependent transcription and homeostatic plasticity.

Disclosures: E. Barrios: None. **M. Sha:** None. **S. Xiao:** None. **B. Horsfall:** None. **D. Wise:** None. **S.B. Nelson:** None.

Poster

064. Biological Clocks and Rhythms

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Program #/Poster #: 064.10

Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant 1R15GM134528

Title: Endocannabinoid system gene expression in the suprachiasmatic nucleus of male and female mice

Authors: *T. D. NIEPOKNY, E. M. MINTZ;
Kent State Univ., Kent State Univ., Kent, OH

Abstract: Circadian rhythms in physiology and behavior, generated by the suprachiasmatic nucleus of the hypothalamus (SCN), help organisms anticipate events in their environment. The daily light/dark cycle (LD) is the main environmental stimulus that synchronizes SCN output to the proper time of day. Minute adjustments are necessary to properly shift clock timing with the changing environment, and the endocannabinoid system (ECS) acts as a neuromodulatory signaling mechanism in the SCN. The endocannabinoid 2-arachidonoylglycerol is involved in the clock's responses to light, and cannabinoid receptor agonists can inhibit the phase-shifting effects of light. Some endocannabinoids, their receptors, and synthesis and degradation enzymes are expressed rhythmically in various parts of the brain and body, including the SCN. Still, limited knowledge exists about the role of endocannabinoids in circadian rhythm regulation. In this study, we have utilized laser capture microdissection and quantitative PCR to investigate ECS mRNA gene expression at two opposing timepoints in the SCN. We detected 13 of the 19 ECS components we examined and followed up with a 24-hour time-course experiment on a 12:12 LD cycle to test the rhythmic expression of ECS genes. Of the tested components, peroxisome proliferator activated receptor gamma *(Pparg)*, fatty acid binding protein 7 *(Fabp7)*, glycerophosphodiester phosphodiesterase 1 *(Gde1)*, abhydrolase domain-containing 4 *(Abhd4)*, abhydrolase domain-containing 6 *(Abhd6)*, and abhydrolase domain-containing *(Abhd12)* were rhythmic in both sexes, peroxisome proliferator activated receptor alpha *(Ppara)*, N-acyl phosphatidylethanolamine phospholipase D *(Nape-PLD)*, diacylglycerol lipase beta *(Daglb)*, and monoglyceride lipase *(Mgll)* were rhythmic in one sex, and cannabinoid receptor 1 *(Cnr1)*, diacylglycerol lipase alpha *(Dagla)*, and fatty acid amide hydrolase *(Faah)* were not rhythmically expressed as assessed by cosinor analysis. We hypothesize that some components will be rhythmic in constant conditions, and that they may have different spatial distributions within the SCN. Rhythmic expression among synthesis and degradation enzymes in the ECS suggests that circadian regulation of endocannabinoid activity in the SCN should be investigated as an important regulatory mechanism.

Disclosures: T.D. Niepokny: None. **E.M. Mintz:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.11

Topic: F.07. Biological Rhythms and Sleep

Title: Identifying central mechanisms of glucocorticoid circadian rhythm dysfunction in breast cancer

Authors: *A. M. GOMEZ, A. BERISHA, L. BOYD, A. M. KAUFMANN, J. C. BORNIGER; Cold Spring Harbor Lab., Cold Spring Harbor, NY

Abstract: The circadian release of endogenous glucocorticoids is essential in preparing and synchronizing the body's daily physiological needs. Disruption in the rhythmic activity of glucocorticoids has been observed in individuals with a variety of cancer types, as well as blunting of this rhythm has been shown to predict cancer mortality and quality of life. This suggests that a disrupted glucocorticoid rhythm is potentially a shared phenotype across cancers. However, how this disrupted rhythm extrapolates to rodent models, and the causal mechanisms that link glucocorticoid rhythm dysfunction and cancer outcomes remain preliminary at best. The regulation of daily glucocorticoid activity is maintained, in part, by the coordinated response of the hypothalamic-pituitary-adrenal (HPA) axis, consisting of the suprachiasmatic nucleus (SCN) and corticotropin-releasing hormone-expressing neurons of the paraventricular nucleus of the hypothalamus (PVN^{CRH}). Consequently, we set out to examine if cancer-induced glucocorticoid dysfunction is regulated by disruptions within these hypothalamic nuclei. In comparison to their tumor-free baseline, mammary tumor-bearing mice exhibited a blunting of glucocorticoid rhythms across multiple timepoints throughout the day, as measured by fecal corticosterone rhythms, during tumor progression. We further examined how peripheral tumors shape hypothalamic activity within the brain. Preliminary serial two-photon tomography for whole-brain cFOS imaging suggests a disrupted activation of the PVN in mice with tumors. Additionally, we found GFP labeled neurons within the PVN after injection of pseudorabies virus expressing GFP into the tumor, pointing to the PVN as a primary target disrupted by mammary tumors. Preliminary *in vivo* fiber photometry data show that the activity of PVN^{CRH} neurons exhibit enhanced calcium activity during tumor progression, as compared to baseline (no tumor) activity. Taken together, this suggests that there may be an overactive HPA response during tumor progression, which in turn, may result in a subsequent negative feedback on glucocorticoid rhythms. Current studies are examining whether tumor progression modulates SCN calcium activity, as well as if manipulation of the neurocircuitry surrounding glucocorticoid rhythmicity alters tumor characteristics.

Disclosures: A.M. Gomez: None. **A. Berisha:** None. **L. Boyd:** None. **A.M. Kaufmann:** None. **J.C. Borniger:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

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Program #/Poster #: 064.12

Topic: F.07. Biological Rhythms and Sleep

Support: MOST-111-2636-B-002-021

Title: Investigating the circatidal rhythm in vertebrates using Periophthalmus modestus (shuttles hoppfish)

Authors: *T.-H. LIANG, Y.-M. CHIU, S.-K. CHEN;
Dept. of Life Sci., Natl. Taiwan Univ., Taipei City, Taiwan

Abstract: The behavior of intertidal organisms may be affected by the sun or the moon. For example, circadian rhythm, annual rhythm, and seasonal rhythm are modulated by the sun. In contrast, circalunar rhythm, circasemilunar rhythm, and circatidal rhythm may be regulated by the moon. Current research primarily focuses on the circatidal rhythm of invertebrates such as crustaceans, mollusks, and insects. However, whether circatidal rhythm exists in other intertidal vertebrates is unclear. Thus, we investigate the circatidal rhythm of an intertidal vertebrate *Periophthalmus modestus* in a stimulated tidal environment. Using video cameras to monitor and analyze the activity individually, we found that *P. modestus* has tidal rhythmic behaviors such as movement time, dry, and wet zone location preference under both 6.5:6.5 high-low tide cycle and constant conditions. Furthermore, the circadian rhythm is relatively weak compared to the circatidal rhythm. Together, our results suggest that the circatidal clock may be a conserved biological clock in animals, including osteichthyes.

Disclosures: T. Liang: None. **Y. Chiu:** None. **S. Chen:** None.

Poster

064. Biological Clocks and Rhythms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 064.13

Topic: F.07. Biological Rhythms and Sleep

Support: Dartmouth College Startup funds

Title: Can the rodent ventral striatum communicate through coherence?

Authors: *M. MOHAPATRA[1], J. E. CARMICHAEL[2], M. A. VAN DER MEER[3];
[1]Dartmouth Col. Psychological & Brain Sci., Hanover, NH; [2]McGill Univ., Westmount, QC, Canada; [3]Psychological & Brain Sci., Dartmouth Col., Hanover, NH

Abstract: The communication through coherence hypothesis (CTC) proposes that selective communication between brain areas can be achieved by synchrony of their oscillations (Fries 2015). This principle is especially powerful in scenarios where multiple upstream regions with independent local oscillations converge on a single downstream region. CTC has two main requirements: a) the phase of the downstream oscillation must affect the effectiveness (gain) of a given input; and b) the downstream region must be able to "tune in" to different inputs. The ventral striatum (vStr) receives convergent inputs from many oscillating upstream regions, including the hippocampus and medial prefrontal cortex, making it an excellent candidate for CTC, which we explored over two different experiments.
In the first experiment, we tested if inputs arriving at different phases of the vStr local field potential (LFP) are differentially effective at eliciting spikes in vStr neurons. Using optogenetic stimulation of ChR2-expressing fast spiking interneurons (FSI) in the vStr of awake, head-fixed PV-Cre x Ai-32 mice, the light stimulus was calibrated such that it evoked spikes in 20-80% of stimuli. Preliminary results indicate that half of the eligible neurons (12/23) show evidence of phase-dependent excitability, with more data collection currently ongoing.
The second experiment was motivated by Beatty et al. (2014), who showed that in vitro, striatal medium spiny neurons (MSNs) show spiking resonance at a frequency depending on their firing rate. If observed in vivo, we hypothesized that this would allow MSNs to phase lock to higher frequency LFP oscillations during higher firing rate epochs compared to lower firing rate epochs. We tested this prediction in vStr spiking and LFP data from 4 male Long-Evans rats as they performed a multiple T-maze task (van der Meer et al. 2009). While we found a small proportion of MSNs (11/220, 5%) matching our prediction, a similar number (15/220, 7%) showed the opposite effect. Thus we found no systematic support for the idea that MSN firing rate changes can implement an oscillatory switch.
In summary, we find some support for one key requirement for CTC (gain control through LFP phase) in vStr, but not for one possible implementation of the other key requirement (frequency switching). Future work should test alternative ways in which vStr local oscillations are controlled, such as via upstream inputs, to determine the extent to which CTC is relevant to the vStr.

Disclosures: **M. Mohapatra:** None. **J.E. Carmichael:** None. **M.A. van der Meer:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.01

Topic: F.07. Biological Rhythms and Sleep

Support: NINDS Grant NS110865

Title: Control of sleep by the preoptic area of the hypothalamus

Authors: *J. SMITH, A. HONIG-FRAND, H. ANTILA, F. WEBER, S. CHUNG;
Univ. of Pennsylvania, Philadelphia, PA

Abstract: The preoptic area (POA) is crucial for sleep regulation. Lesions in the POA lead to profound sleep impairment in humans and other mammalian species. The POA contains neurons that can promote sleep when activated and can be labeled with specific molecular markers. While these sleep-promoting neurons have been identified within the POA, it is not known how various subtypes of neurons change their activity throughout the sleep-wake cycle, and how they influence brain state transitions. Using fiber photometry combined with electroencephalogram (EEG) and electromyogram (EMG) recordings, we examined calcium activity in GABAergic and glutamatergic neurons within the POA throughout the sleep-wake cycle. Specifically, we found that GABAergic neurons are most active during REM sleep whereas glutamatergic neurons are least active during REMs while being most active during wakefulness. Furthermore, we used inhibitory optogenetic techniques to discover that inhibiting GABAergic neurons promotes wakefulness and suppresses NREM and REM sleep whereas inhibiting glutamatergic neurons decreases time spent in wakefulness. When performing closed loop inhibition of GABAergic neurons during REM, we found a significant decrease in the duration of REM episodes. We then conducted retrograde tracing experiments to determine if inputs of GABAergic and glutamatergic neurons in the POA originate from different regions throughout the brain. Our study will enhance our understanding of how POA neurons perform differing roles to control sleep.

Disclosures: J. Smith: None. **A. Honig-Frand:** None. **H. Antila:** None. **F. Weber:** None. **S. Chung:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 065.02

Topic: F.07. Biological Rhythms and Sleep

Support: MRC Grant MR/S01134X/1
BBSRC funded Oxford Interdisciplinary Bioscience DTP BB/M011224/1

Title: Local endoplasmic reticulum stress in mouse cortex increases electrophysiological markers of sleep pressure

Authors: *A. CHAKRABARTY, J. PRIUS-MENGUAL, H. ALFONSA, S. NEWEY, C. J. AKERMAN, V. V. VYAZOVSKIY;
Univ. of Oxford, Oxford, United Kingdom

Abstract: Sleep is a homeostatic process that is locally regulated in the mammalian cortex and has been shown to be activity-dependent. With prolonged periods of wakefulness or intense activity in a cortical area, subsequent sleep exhibits elevated levels of slow wave activity (SWA)

in the electroencephalogram, which then dissipate over the course of sleep. SWA power is considered a hallmark of "sleep pressure" and yet the cellular processes that mediate sleep homeostasis remain poorly understood. Prolonged wakefulness has been associated with a marked increase in endoplasmic reticulum (ER) stress response markers in mouse cortex, which is then restored with subsequent sleep. To test whether ER stress might mediate homeostatic sleep processes, we performed continuous recordings of sleep and wake in freely-moving adult C57BL/6J mice (n=8) following intracortical injections of Tunicamycin (TUN), which initiates an ER stress response. Results were compared to control mice that received vehicle (VEH) injections. There were no differences in sleep-wake architecture across 108h (4.5 days) following TUN compared to VEH injection. Spectral power density in the local field potential (LFP) was increased across frequencies between 0.5-30Hz following TUN compared to VEH injection (2-way ANOVA, treatment: $p=2x10^{-57}$, frequency: p=1.0, interaction: p=1.0; TUN n=8, VEH n=4). During the 72h period post-injection, TUN increased spectral power in the delta frequency range (0.5-4Hz) during NREM sleep, which is a marker of sleep intensity, compared to VEH (2-way ANOVA, treatment: $p=2x10^{-4}$, time: p=0.004, interaction: p=1.0), with the greatest increase at the 6-12 h timepoint. Similarly, TUN injection also increased low theta (4.5-6Hz) spectral power during wake, which is a marker of sleep pressure (2-way ANOVA, treatment: $p=2x10^{-5}$, time: p=0.569, interaction: p=0.987). The effect of TUN injection on delta and low theta power dissipated after 72h post-injection. The increase in NREM delta power was observed locally at the injection site, but not in the opposite hemisphere (2-way ANOVA, treatment: p=0.070, time: $p=6x10^{-7}$, interaction: p=0.998). Conversely, the increase in wake low theta power was present at the injection site as well as in the opposite hemisphere that did not receive an injection (2-way ANOVA, treatment: $p=3x10^{-7}$, time: p=0.039, interaction: p=0.911). Together, preliminary findings from this study show that Tunicamycin-induced ER stress in mouse cortex leads to broad changes in LFP activity in a sleep-wake history dependent manner. Specifically, ER stress may result in an increase in electrophysiological markers of homeostatic sleep pressure.

Disclosures: A. Chakrabarty: None. **J. Prius-Mengual:** None. **H. Alfonsa:** None. **S. Newey:** None. **C.J. Akerman:** None. **V.V. Vyazovskiy:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.03

Topic: F.07. Biological Rhythms and Sleep

Support: US Department of Veterans Affairs, Merit Award - BX005167 (M. N. Alam)

Title: Activation of the ventrolateral preoptic neurons projecting to the perifornical-lateral hypothalamic area promotes sleep: DREADD activation in wild type rats

Authors: A. KOSTIN[1], M. A. ALAM[2], A. SAEVSKIY[3], D. J. MCGINTY[4], *N. ALAM[1,5]; [2]Res. Service, [1]VA Greater Los Angeles Healthcare Syst., North Hills, CA; [3]5Scientific Res. and

Technol. Ctr. for Neurotechnology, Rostov-on-Don, Russian Federation; [4]VA Hosp 151A3, Sepulveda, CA; [5]Dept. of Medicine, UCLA, Los Angeles, CA

Abstract: The ventrolateral preoptic area (VLPO) predominantly contains sleep-active neurons and is involved in sleep regulation. The perifornical-lateral hypothalamic area (PF-LHA) is a wake-regulatory region and predominantly contains wake-active neurons. VLPO sleep-active GABAergic/galaninergic neurons project to the PF-LHA. The specific contribution of VLPO neurons projecting to the PF-HA (VLPO>PF-HA^{PRJ}) in sleep regulation in rats is not fully understood due to the lack of tools that could selectively target these neurons. We determined the contribution of VLPO>PF-HA^{PRJ} neurons in sleep regulation by selectively activating them using designer receptors exclusively activated by designer drugs (DREADDs) in wild-type Fischer-344 rats. We used a combination of two viral vectors to retrogradely deliver the Cre-recombinase gene specifically in VLPO>PF-HA^{PRJ} neurons and further express hM3Dq in those neurons to selectively activate them for delineating their specific contributions to sleep-wake functions. Compared to control, in DREADD rats, clozapine-N-oxide (CNO) significantly increased fos-expression, a marker of neuronal activation, in VLPO>PF-HA^{PRJ} neurons (2% vs. 20%, $p<0.01$) during the dark-phase. CNO treatment also increased nonREM sleep (27% vs. 40%, $p<0.01$) during the first 3h of the dark-phase when rats are typically awake and after exposure to the novel environment (55% vs. 65%; $p<0.01$), which induces acute arousal during the light-phase. These results support a hypothesis that VLPO>PF-HA^{PRJ} neurons constitute a critical component of the hypothalamic sleep-wake regulatory circuitry and promote sleep by suppressing wake-active PF-HA neurons.

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Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

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NIH Grant 1R15NS101692-02A1
Skidmore College Faculty Student Summer Research Award

Title: The effects of combined optogenetic activation of sleep- and wake-promoting neurons in Drosophila melanogaster

Authors: *C. R. KOOCHAGIAN, M. P. GREGA, C. G. VECSEY;
Skidmore Col., Saratoga Springs, NY

Abstract: Sleep is an essential behavior for nearly all organisms, and yet its underlying mechanisms remain largely unknown. The fruit fly *Drosophila melanogaster* has been a

prominent model organism for studying sleep to reveal the molecular and cellular pathways that control sleep and arousal. Within *D. melanogaster*, there are different types of neurotransmitters that play a role in the regulation of sleep, such as neuropeptides and biogenic amines. One neuropeptide, short neuropeptide F (sNPF), has been previously found to strongly promote sleep upon optogenetic activation of sNPF-releasing neurons. A second neuropeptide, pigment dispersing factor (PDF), conversely has been found to have a role in promoting arousal and wakefulness. Another signal that has been implicated in transmitting a wake promoting signal is the biogenic amine, octopamine (OA). The aim of the present study was to investigate how sNPF neurons' sleep-promoting signal is integrated with either PDF's or OA's wake promoting signal, and whether one signal could dominate over the others. In order to achieve this goal, sNPF-GAL4/UAS-Chrimson flies were crossed with PDF-GAL4 or TDC2-GAL4 (targeting octopamine/tyramine-producing neurons) flies so that both sleep-promoting and wake-promoting neuronal populations could be activated simultaneously by exposure to red light. We also examined if the prevailing signal depended on the time of day at which neuronal activation occurred. When sNPF and PDF neurons were co-activated during a period of general wakefulness during the subjective daytime, the flies exhibited an increase in sleep both during and after the red-light stimulation. Co-activation of sNPF and PDF neurons during the subjective nighttime, a time of increased sleep, caused flies to remain asleep. When sNPF and OA neurons were co-activated during the daytime, flies exhibited a decrease in sleep during the red-light exposure, followed by an increase in sleep once optogenetic activation ceased. Co-activation of sNPF and OA neurons during the nighttime resulted in flies exhibiting an increase in sleep during the red light, which was then followed by a decrease in sleep after the light turned off. Taken together, these data suggest that the sNPF sleep-promoting system likely interacts with the PDF and OA wake-promoting systems through different mechanisms to result in the differing sleep phenotypes observed with co-activation. Future experiments should be conducted to elucidate potential neuronal structures where these opposing signals are being integrated to build upon our current knowledge on the mechanisms and pathways that regulate sleep.

Disclosures: **C.R. Koochagian:** None. **M.P. Grega:** None. **C.G. Vecsey:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.05

Topic: F.07. Biological Rhythms and Sleep

Support: I01 BX001404
I01 BX004500
IK2 BX004905
R01 NS119227
R21 MH125242

Title: Gap-junction protein Cx36 of the thalamic reticular nucleus regulates cortical neuronal dynamics

Authors: *R. BASHEER, D. S. UYGUN, S. THANKACHAN, J. M. MCNALLY;
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Abstract: Sleep-wake-states are dynamic processes associated with distinct oscillatory activities that subserve various functions. Electrical synapses are particularly important for synchronization of neuronal activity in the brain which underlies the emergence of neuronal oscillations. Connexin36 (Cx36) is the predominant gap junction protein which forms the electrical synapses in neurons. Cx36 expression remains highly restricted to homogeneous neuronal subpopulations such as the parvalbumin expressing GABAergic (GABA-PV) neurons of brain areas including cortex and thalamic reticular nucleus (TRN). While the role of Cx36 containing gap junctions has been characterized, their role in TRN is less established. The TRN is unique in being almost exclusively dependent on gap junction coupling for local inter-neuronal communication between GABAergic neurons. TRN modulates thalamocortical oscillations that underlie various functions including sleep, arousal, attention and sensation. Here, using the CRISPR-gene editing method to locally knock-down (KD) electrical synapses we examine the role of the TRN-specific Cx36 in cortical neuronal dynamics during sleep and sensory perceptions. Sleep/wake recordings and cortical gamma activity associated with auditory and visual steady state responses (ASSR and VSSR) and during social interactions were examined in 1) global Cx36 knockout (KO) mice and compared with their wildtype (WT) littermates, and 2) before and after introducing the KD of Cx36 in a repeated measures design. To target TRN neurons, we bred parvalbumin expressing Cre recombinase (PV-cre mice) with lox-stop-lox-Cas9 mice to generate PV-Cas9 offspring. We then generated single-guide RNAs to target the Cx36 protein. When compared to WT mice, Cx36 KO mice (N=5) had significantly reduced sigma power (10-15Hz) during NREM to REM transitions ($p=0.0004$). Similarly, reduced sigma power was observed after TRN specific Cx36 KD (N=1). 40Hz ASSR and VSSR in EEG was significantly reduced ($p<0.05$) in Cx36 KO mice and after localized TRN Cx36 KD. Additionally, the gamma band response to social investigation was impaired after the TRN Cx36 KD. Our results, thus far, suggest that the electrical synapses within TRN play an important role in modulating sleep-wake and sensory task-associated cortical neuronal dynamics.

Disclosures: **R. Basheer:** None. **D.S. Uygun:** None. **S. Thankachan:** None. **J.M. McNally:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.06

Topic: F.07. Biological Rhythms and Sleep

Support: MRC AVR02190

Title: Physiologically relevant monocular light stimulation leads to local signatures of sleep pressure and differential expression of synaptic activity and ER stress-associated genes in the contralateral visual cortex in freely moving mice

Authors: *J. PRIUS MENGUAL[1], A. CHAKRABARTY[2], M. UNWIN[1], B. RUSSELL[1], L. TAYLOR[1], S. NEWEY[2], L. B. KRONE[1], C. AKERMAN[2], V. V. VYAZOVSKIY[1];
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Abstract: Aims: Extended wakefulness leads to a global increase in electroencephalogram(EEG) slow-wave activity (SWA: power density between 0.5-4Hz) during subsequentsleep. SWA is regulated at the local level, which led to the hypothesis that sleep is ause dependent process. We propose that sleep enables cellular maintenanceprocesses after prolonged wakefulness and the brain homeostasis can only berestored by a temporary reduction in synaptic activity manifested as SWA, allowingthe neurons to clear its backlog of biosynthetic work.
Methods: To induce sustained synaptic activity in the mice visual system, we positionlight-emitting diodes in front of the animal's eyes. EEG and/or 16-channel laminarelectrodes were used to record cortical activity. The mice were kept awake for 4 hoursstarting at light onset under monocular light stimulation (8 Hz, train duration 2 s every30 s, pulse duration 10 ms) and allowed to sleep undisturbed after the period of visualstimulation. In a cohort of animals, brains were collected after the visual stimulationfor qPCR analysis.
Results: During the first hour after sleep deprivation combined with visual stimulation,the occipital EEG spectral power density in part of the SWA frequency range (0.25-2Hz) was consistently higher in the stimulated visual cortex than the non-stimulatedhemisphere (mean ± SEM: 50±5.2% vs. 33±5.2%, n=8). A second stimulationgenerated a bigger difference (63±7% vs. 42±5%, n=8). Alongside, monocular lightstimulation resulted in the differential expression of synaptic activity- and ER stress-associated genes between the two visual cortices, suggesting that the local increasein synaptic activity due to the stimulation may impact synaptic plasticity and ER stress.
Conclusions: These preliminary results throw some light about the relationshipbetween homeostatic sleep need, synaptic plasticity, and ER stress. It helps tounderstand how sleep disturbances impact basic cellular physiology and how sleepallows efficient renormalization of brain function.

Disclosures: J. Prius Mengual: None. **A. Chakrabarty:** None. **M. Unwin:** None. **B. Russell:** None. **L. Taylor:** None. **S. Newey:** None. **L.B. Krone:** None. **C. Akerman:** None. **V.V. Vyazovskiy:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.07

Topic: F.07. Biological Rhythms and Sleep

Support: I01 BX001404
I01 BX004673
R01 NS119227
R21 NS079866
R21 MH125242

Title: Arousal mechanism of basal forebrain glutamatergic neurons

Authors: *C. YANG, E. L. HODGES, T. J. SPRATT, J. T. MCKENNA, R. E. BROWN, R. BASHEER;
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Abstract: The basal forebrain (BF) is an important subcortical region for sleep-wake control and sleep homeostasis regulation. Previous optogenetic studies described a role for BF glutamatergic neurons in both arousal and avoidance behavior. These neurons project locally to neighboring neurons, as well as to distant arousal and reward-associated areas. Here, using optodialysis and pharmacological intervention, we examined the role of local glutamatergic projections in modulating arousal. Male vGluT2-cre mice were unilaterally injected with AAV5-ChR2-EYFP viral vectors into the BF, implanted with the optodialysis probe targeting the BF for optostimulation and drug infusion, and with the electroencephalogram (EEG)/electromyography (EMG) headmount for sleep recording. The mice were subjected to the following recording paradigm: baseline day (no stimulation), optostimulation day (20Hz 5s/min optostimulation from ZT3-7), drug infusion day (ZT3-7 with the ionotropic glutamatergic receptor antagonists 200 µM DNQX and 500 µM D-AP5) and the optostimulation+drug infusion day (ZT3-7 with both 20Hz optostimulation and infusion of the glutamatergic receptor antagonists). Our data (N=7) show that consistent with previously reported findings, wakefulness was strongly and significantly increased with the optogenetic stimulation of BF vGluT2 neurons (wake% optostimulation vs baseline: 97.3±0.6% of total time vs 27.6±2.0%, $p<0.0001$, paired-t-test). The infusion of glutamatergic receptor antagonists during stimulation partially blocked the optostimulation-induced-arousal (optostimulation+drug infusion wake%: 85.9±4.3%, $p=0.0376$ compared to optostimulation). Drug infusion without optostimulation had no effect on spontaneous wakefulness (drug infusion wake%: 34.8±5.7%, $p=0.1978$ compared to baseline). Our findings here suggest that while local interactions are involved, extra-BF projections of vGluT2 neurons, perhaps those to the lateral hypothalamus, ventral tegmental area and lateral habenula, also play a central role in arousal promotion.

Disclosures: C. Yang: None. **E.L. Hodges:** None. **T.J. Spratt:** None. **J.T. McKenna:** A. Employment/Salary (full or part-time):; Merck MISP. 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.; Merck MISP. **R.E. Brown:** None. **R. Basheer:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.08

Topic: F.07. Biological Rhythms and Sleep

Support: DGA grant SAN-1-0413

Title: Neuropeptide S: a new target for sleep alterations caused by intense stress ?

Authors: *E. LARDANT[1,2], D. CLAVERIE[1], B. POLY THOMASSON[1], F. CHAUVEAU[1]; [1]I.R.B.A, Brétigny-sur-Orge, France; [2]Univ. Paris-Saclay, Ecole Doctorale BIOSIGNE, Le Kremlin-Bicêtre, France

Abstract: Sleep alterations such as insomnia, hyper-arousal and nightmares are the first post-traumatic stress disorder patients' complaints. However, no long-term effective treatments for traumatic-induced insomnia are available probably due to the lack of precise molecular and cellular knowledge. Among the potential therapeutic target, the Neuropeptide S (NPS), identified as a modulator of both stress and arousal (Xu, *et al.* 2004), could be of particular interest. In fact, while this brainstem-expressed peptide produces an anxiolytic-like effect on rodents in stressful context (Jungling, *et al.* 2008; Chauveau *et al*. 2012), as shown by the significantly elevated NPS mRNA in the Basolateral Amygdala of submissive mice during social aggression (Smith, *et al.* 2014), it also prevents slow-wave sleep (SWS) by the inhibition of the sleep-on galaninergic neurons in the preoptic area (Chauveau, *et al.* 2019). In order to investigate the role of the NPS in intense stress-induced sleep disturbances, a total of 30 SWISS male mice were used. Twenty animals were exposed to electrical foot shocks (1,6 mA; 2 sec) in an unique and specific context. Eight behavioral tests were conducted before and up to 28 days post-stress in order to assess the PTSD-like behaviors while their sleep was recorded during 24 hours sessions before, on the day of the intensive stress, and 7, and 14 days post-stress (D14). As a control group (CTL), 10 mice out of the 30 were exposed to the unique context without receiving any foot shock. Sleep analyses did not show an overall stress effect on sleep considering stressed *versus* non-stressed animals. However, some subtle differences in sleep amount appear when clustering stressed animals into two sub-groups according to their behavioral acute stress response (FRZ for freezers; ESC for escapers). More precisely, the percentage of time spent in SWS was significantly reduced and Rapid-eye movement sleep increased in FRZ mice during the light and dark periods at D14 whereas the ESC group showed an increase in wake percentage only in the light period. *In situ* hybridization data suggest that NPS mRNA is overexpressed in the preoptic area and amygdala of stressed animals, with differential effects between the two stressed subgroups of mice. To conclude, our data i) show the relevance to take into account the individual acute stress response to highlight delayed sleep alterations in stressed animals; ii) suggest a potential and essential role of NPS on these alterations.

Disclosures: E. Lardant: None. **D. Claverie:** None. **B. Poly thomasson:** None. **F. Chauveau:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.09

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01 GM121919
University of Michigan Department of Anesthesiology
Magnificent Michigan Summer Research Fellowship from the University of Michigan Neuroscience Innovators to Zuzanna Fracz

Title: Synergistic effect of combined dexmedetomidine and sevoflurane sedation on sleep homeostasis in rat

Authors: *T. GROENHOUT[1], Z. FRACZ[1], T. LIU[1], G. A. MASHOUR[1,2,3], D. PAL[1,2,3,4];
[1]Dept. of Anesthesiol., [2]Neurosci. Grad. Program, [3]Ctr. for Consciousness Sci., [4]Dept. of Mol. and Integrative Physiol., Univ. of Michigan, Ann Arbor, MI

Abstract: The effect of continuous long-term administration of sedative-hypnotics, as is routinely done in the intensive care units, on sleep homeostasis is not completely understood. Ongoing studies in our laboratory showed that sedation produced by continuous infusion (8 h) of dexmedetomidine (DEX, 0.5-1.0 μg/kg/min) increased wakefulness (WAKE), decreased slow-wave sleep (SWS), and reduced rapid eye movement (REM) sleep in the post-sedation period. In contrast, sedation with continuous (8 h) sevoflurane (SEVO, 1.5-2.1%) did not affect WAKE or SWS, but increased post-sedation REM sleep. To investigate the possibility of a synergistic effect of DEX and SEVO on sleep homeostasis, in the current study we sedated rats for 8 h by simultaneous administration of DEX and SEVO and quantified the effect on post-sedation sleep-wake states (48 h). Under isoflurane anesthesia, adult male and female Sprague Dawley rats (N=12, 6 male) were surgically prepared for sleep-wake recordings, and a catheter was chronically positioned in the jugular vein for DEX or lactated Ringer's (control) infusion. Each rat received 8 h of continuous (10:00 am - 6:00 pm) combined administration of subanesthetic SEVO (0.5-1.0%) and intravenous DEX (0.1 ug/kg/min). The rats also received continuous (8 h) intravenous lactated Ringer's on a different day, counterbalanced with SEVO+DEX administration, and with inter-experiment interval of at least 5-7 days. The SEVO and DEX concentrations were titrated to maintain loss of righting reflex and large-amplitude electroencephalographic waveforms, which are surrogates for loss of consciousness in rodents. Post-sedation sleep-wake data were recorded for 48 h and manually scored in 4 s epochs as WAKE, SWS, and REM sleep. A linear mixed model was used for statistical comparison of the time spent in sleep-wake states during the 48 h post-sedation and 48 h post-Ringer's period. The percent time spent in WAKE and SWS during the first 12 h post-sedation period was comparable to that observed after Ringer's infusion. In contrast, REM sleep was significantly reduced during the 12 h post-sedation period as compared to that observed after Ringer's infusion ($p<0.0001$). The time spent in WAKE, SWS, and REMS during the remaining 36 h of post-sedation period was comparable to that after Ringer's infusion. The effect of combined sedation with SEVO and DEX is distinct from the effects of either drug alone and provide evidence for minimal

interruption of sleep homeostatic processes leading to a sleep profile that more closely mirrors the natural sleep-wake states.

Disclosures: T. Groenhout: None. **Z. Fracz:** None. **T. Liu:** None. **G.A. Mashour:** None. **D. Pal:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.10

Topic: F.07. Biological Rhythms and Sleep

Support: NINDS Grant NS110865

Title: Neural circuit mechanisms underlying sleep disturbances in 16p11.2 deletion mouse model of autism

Authors: *A. CHOI, I. AN, H. ANTILA, A. SCHOTT, F. WEBER, S. CHUNG;
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Abstract: Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder, and its prevalence has markedly increased in the past 30 years. Many children with ASD suffer from insomnia, and sleep problems are positively correlated with the severity of ASD core symptoms such as communication deficits, withdrawal, and repetitive or stereotyped behaviors. In particular, the 16p11.2 hemideletion mouse model has been shown to sleep less and exhibit hyperactivity. However, neural circuit mechanisms underlying their sleep disturbances have not been well characterized. Using electroencephalogram (EEG) and electromyogram (EMG) recordings, we examined baseline sleep in 16p11.2 deletion and wild type mice and found that 16p11.2 deletion mice have increased microarousals resulting in fragmented sleep. To investigate the activity of the arousal promoting locus coeruleus (LC) noradrenergic neurons during sleep, we performed fiber photometry recordings and found that 16p11.2 deletion mice have an increased number of calcium peaks that largely overlap with microarousals during NREM sleep. Our study will contribute to understanding of circuit mechanisms underlying sleep fragmentation in 16p11.2 deletion mice, which may provide insight to neural mechanisms that promote disturbances in ASD.

Disclosures: A. Choi: None. **I. An:** None. **H. Antila:** None. **A. Schott:** None. **F. Weber:** None. **S. Chung:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.11

Topic: F.07. Biological Rhythms and Sleep

Support: Innovation Award from the Technology Development Group at UCLA.

Title: Non-invasive neuromodulation to stabilize blood pressure in obstructive sleep apnea

Authors: *V. LUBERA[1], M. ZEIDLER[4,2], D. N. SNODGRASS[3], E. K. SAUERLAND[5], R. K. HARPER[1], J. A. OGREN[2], R. J. STRETCH[4,5], R. M. HARPER[1];
[1]Neurobio., [2]Med., [3]Audiol. Clin., UCLA, Los Angeles, CA; [4]VA, West Los Angeles, CA; [5]Physiol. and Cell Biol., Univ. of Nevada, Reno, NV

Abstract: Obstructive sleep apnea (OSA) is the most prevalent sleep-related breathing disorder, with an estimated 1.36 billion adults aged 30-69 years affected globally. The repeated apneic events are often accompanied by increased blood pressure, with associated enhanced risks of stroke, heart failure, and other injuries. An intervention to assist control of blood pressure, preferably nonpharmacologic, would benefit protection against the exaggerated sympathetic tone underlying the development of hypertension. We earlier showed that vibratory stimulation to a set of cranial and cervical nerves (cranial nerves 5, 7, 8, 9, and 10; cervical nerves C2 and C3) with receptive fields in the auditory canal normalized blood pressure in hypertensive and hypotensive patients. The blood pressure/sympathetic nervous system outcomes likely arise from combined actions of all cranial nerves, and especially the 9th nerve with its projections to the carotid baroreceptors. The earlier studies were based on awake subjects undergoing interventions for migraine pain; whether comparable stimulation is effective for blood pressure control in sleeping patients with blood pressure changes that accompany obstructive sleep apnea is unknown. This study was a part of a larger study on overcoming breathing pauses in OSA, and both breathing and cardiovascular aspects were approved by the UCLA Institutional Review Board (#14000943). We recruited male and female subjects (18 to 78 years of age) who had been diagnosed with moderate to severe-obstructive sleep apnea, and who currently use the Continuous Positive Airway Pressure (CPAP) or another device as treatment. Baseline and experimental (neuromodulatory device) recordings were collected across two nights, respectively, and included airflow and chest wall breathing motion, heart rate, beat-by-beat blood pressure, EEG activity, eye movement, and chin muscle activity to assess sleep state, breathing, and cardiac patterns using Nihon-Khoden and SOMNOmedics^{TM} recorders. Seven subjects have been recorded to date, and beat-by-beat blood pressure values by state and intervention were assessed by conventional statistical means. The intervention introduced immediate declines in blood pressure, with systolic drops of as much as 26 mm Hg. The findings indicate that using neuromodulatory vibration may have the potential to provide a non-invasive, drug-free means to normalize blood pressure in a disease process that frequently leads to hypertension.

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Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.12

Topic: F.07. Biological Rhythms and Sleep

Support: JSPS KAKENHI Grant-in-Aid for Scientific Research (B) (JP 18H02595)
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JST CREST Grant Number JPMJCR1655 Japan

Title: A whole-brain input landscape of the GABA- and galaninergic neurons in the ventrolateral preoptic nucleus projecting to the lateral hypothalamus

Authors: *K. PROKOFEVA[1], Y. C. SAITO[1], Y. NIWA[2], S. MIZUNO[2], S. TAKAHASHI[2], A. HIRANO[1,2], T. SAKURAI[1,2,3];
[1]Intl. Inst. for Integrative Sleep Med. (WPI-IIIS), Univ. of Tsukuba, Tsukuba, Japan; [2]Fac. of Medicine, Univ. of Tsukuba, Tsukuba, Japan; [3]Life Sci. Ctr. for Tsukuba Advanced Res. Alliance (TARA), Univ. of Tsukuba, Tsukuba, Japan

Abstract: Deciphering of the anatomical and functional interactions between the preoptic area (POA) and lateral hypothalamus (LH) is crucial for comprehensive mechanistic understanding of sleep and arousal regulation. GABA- and galaninergic (GAL) neurons in the ventrolateral preoptic nucleus (VLPO) of the POA participate in sleep promotion, while orexinergic neurons in the LH play a vital role in consolidation of wakefulness. Our study aims to contribute to uncovering of the sleep/wake circuitry architecture through structural and functional examination of the VLPO→LH pathway and of its synaptic connectivity. Using monosynaptic retrograde rabies-mediated tracing in newly generated *orexin-iCre* knock-in mice (n=4) combined with fluorescence *in situ* hybridization, we found that vesicular GABA transporter (*Vgat*)- and galanin (*Gal*)-expressing neurons in the VLPO make monosynaptic inputs to orexin neurons. Over half (56.3±8%) of the VLPO inputs were also *Vgat*- and *Gal*-double-positive. Further, we examined presynaptic partners of the GABA and GAL VLPO neurons projecting to the LH via projection-specific rabies-mediated tracing using *Vgat-IRES-Cre* and *Gal-Cre* mice (n=5 for each mouse line), respectively. The largest number of input neurons for both populations was located within the subregions of the POA, such as the medial preoptic area and the VLPO, and in the LH, suggesting the presence of a highly developed intrapreoptic circuitry and of reciprocal connections between the VLPO and the LH. Among other input areas were the nucleus accumbens, bed nucleus of stria terminalis and wake-promoting regions, such as the tuberomammillary nucleus. Using multidimensional scaling, we determined that GABA and GAL VLPO neurons are clustered together according to their inputs ($p=0.1259$, stress=0.07), distribution of which was also positively correlated (Spearman correlation, $r=0.67$, $p<0.0001$). Therefore, our results suggest that subpopulations of the GABA and GAL VLPO neurons projecting to the LH compose a single neuronal population. We also optogenetically stimulated the $GABA^{VLPO}$→LH pathway using male *Vgat-IRES-Cre* mice and observed a nonsignificant trend towards sleep promotion in ChR2-(n=8) compared with GFP-delivered (n=7) mice. In conclusion, this study revealed a direct synaptic connection between the sleep-implicated VLPO and arousal-related LH neurons, as well as shed light on the connectivity of the GABA and GAL

VLPO→LH neurons. Despite observed modest influence of the $GABA^{VLPO}$→LH pathway on sleep, further examination of the pathway is necessary to disentangle its physiological role.

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Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.13

Topic: F.07. Biological Rhythms and Sleep

Support: VA CDA IK2 BX004905
I01 BX004673
I01 BX001404
NIH R01 NS119227

Title: Synaptic $GABA_A$ receptors of thalamocortical relay neurons sculpt sleep spindles

Authors: *D. S. UYGUN, R. BROWN, R. BASHEER;
VA Boston Healthcare Syst. & Harvard Med. Sch., West Roxbury, MA

Abstract: The thalamus is critical to the regulation of electroencephalographic (EEG) waveforms in both wakefulness and sleep. However, the circuitry underlying these oscillations is incompletely understood. Advances in *in vivo* CRISPR-Cas9 gene editing methods enable high-throughput and high precision gene targeting, within focused brain circuitry and neuronal subtypes. We used this technology to study the role of synaptic GABA inhibition onto the thalamocortical (TC) relay neurons in regulating sleep oscillations.
To target TC neurons, we bred vesicular glutamate transporter subtype 2 mice expressing Cre recombinase (vGlut2-cre mice) with lox-stop-lox-Cas9 mice to generate vGlut2-Cas9 offspring. The resulting mice express Cas9 in vGlut2+ cells, including the majority of TC relay neurons. We then generated single-guide RNAs to target the alpha1 subunit of $GABA_A$ receptors, which is the synapse-localizing $GABA_A$ receptor isoform in wild-type TC relay neurons. Sleep recordings were conducted before and after introducing the knockdown (KD) of alpha1 subunits in a repeated measures design.
Compared with baseline (BL), KD of alpha1 in TC relay neurons reduced 10-15 Hz (sigma; the frequency band of spindles) power in spindle enriched NREM sleep , and altered the morphology of the spindles, reducing their amplitude (BL: 2.48±0.17 μV vs alpha1KD: 2.16±0.12 μV), duration (BL: 1.97±0.02 s vs alpha1KD: 1.82±0.04 s) and characteristic shape (N=14; pending histologic validation). There was a trend-level reduction in delta power but no changes in other frequency bands during NREM sleep.
Sleep spindles have become a candidate target in disease because they are associated with learning and diminished by aberrant mental health. Our work suggests properly tuned spindles

require synaptic $GABA_A$ receptors on TC relay neurons, where thalamic reticular nucleus outputs are received. These receptors may therefore be a target for pharmaceutical manipulation of spindles.

Disclosures: D.S. Uygun: None. **R. Brown:** None. **R. Basheer:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.14

Topic: F.07. Biological Rhythms and Sleep

Support: GMU OSCAR URSP

Title: Mechanosensory stimulated sleep of Drosophila melanogaster as a treatment for Alzheimer's disease

Authors: *M. PEREZ[1], M. HAMMADI[2], R. GUERRIERO[2];
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Abstract: Alzheimer's Disease (AD) is a neurodegenerative affliction that erodes memory and exists as a looming threat over the aging population. The glymphatic flow disruption caused by fragmented sleep prevents the clearance of protein waste that progresses AD, which further fragments sleep. This degenerative cycle has been replicated in the fruit flies, *Drosophila melanogaster*. In a study from Dissel and colleagues, it was found that by enhancing the sleep of *Drosophila* using the drug THIP, the eroded memory of the flies began to recover and the clearance was partially restored (2017). Since the common side effects of sleeping drugs are dangerous for the elderly, this study will provide evidence that mechanosensory stimulation to enhance sleep can similarly treat AD models. Rhythmic lateral movements like rocking have already been shown to enhance the sleep of humans and mice and it has also been shown that reduced sleep often coincides with various neurodegenerative conditions. To link this information, AD model *Drosophila* will have their sleep enhanced throw rhythmic, lateral vibrations. By crossing stocks that possess pan-neuronal GAL4 drivers with stocks with UAS-APP:BACE and UAS-AB42, the flies will be put through short-term and long-term memory tests, while their sleep will be recorded using *Drosophila* Activity Monitors (DAM2). Aged flies, at 18 days old, will be moved to a plate shaker oscillating at 20 Hz during their sleeping hours. Western blots will be performed to measure the protein accumulation before and after sleep enhancement. Verifying that sleep enhancement via mechanosensory stimulation can combat the effects of Alzheimer's Disease in a model organism like *Drosophila*, there will be a sturdier foundation to carry out human studies.

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Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.15

Topic: F.07. Biological Rhythms and Sleep

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Title: Intracellular signaling components in homeostatic regulation of sleep

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Abstract: Sleep is ubiquitously observed across all species with the central nervous system, yet diverse in its manifestation as well as its qualitative traits.The switch between sleep and wake behavior is driven by both circadian rhythm and changes in the homeostatic sleep pressure. There has been growing emphasis on understanding the regulatory mechanism especially of the homeostatic drive at the molecular and cellular levels. However, the intracellular component of the mechanistic pathway remains largely unknown.
This study is based on our discovery of salt-inducible kinase 3 (SIK3) kinase as a key regulator of sleep homeostasisthrough the forward genetics study using mice. The mice with gain-of-function mutation of SIK3 showed marked increase in both daily non-REM sleep (NREMS) time and EEG delta power during NREMS. We also identified several SIK3 substrate molecules where SIK3-induced phosphorylation of these proteins localizes them to the cytoplasm and result in desuppression of target gene expression in the nucleus. EEG/EMG-based polysomnography analysis showed abnormal sleep and wake behavior in the loss-of-function mutants and phosphodeficient SIK3-substrate mice. The loss-of-function mutants showed increased NREMS time and EEG delta power during NREMS, consistent with the *Sik3-Sleepy* mutant phenotype. On the other hand, the phosphodeficient mutants showed decreased NREMS time and NREMS EEG delta power. These results suggest that the SIK3 cascade may constitute an important molecular pathway in the regulation of the sleep homeostasis.
To investigate the responsible neural population in modulating the sleep homeostasis by SIK3 signaling pathway, we employed both *Cre-loxP* recombination system and adeno-associated virus (AAV) mediated manipulation of the substrate proteins in a cell type- and region-specific manner. Several *Cre* driver mice were selected to mate with the floxed mice to induce deletion of targeted genes in a *Cre*-dependent manner. Our results revealed that SIK3 signaling in the

excitatory neurons is critical for the regulation of NREMS EEG delta power, whereas the NREMS amount control requires intact signaling in the hypothalamic neurons. In addition, we adopted the blood-brain barrier-permeable AAV that allows effective delivery of the targe gene via retro-orbital injection. Here we show that, consistent with the systemic mutant mice, brain-specific alteration of the target gene expression induces changes in the animals' sleep/wake behavior. Our results suggest the common intracellular signaling components and the link between the cellular and circuitry mechanism that control the quality and quantity of NREMS.

Disclosures: S.J. Kim: None. **S. Nakata:** None. **N. Hotta-Hirashima:** None. **N. Asama:** None. **T. Tsukamoto:** None. **M. Kakizaki:** None. **S. Mizuno:** None. **S. Takahashi:** None. **C. Miyoshi:** None. **H. Funato:** None. **M. Yanagisawa:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH Grant DC-016293
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Title: Time and sex-dependent modulatory functions of serotonin cells within the Drosophila olfactory system

Authors: O. M. COOK, J. JONAITIS, K. E. COATES, A. M. DACKS;
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Abstract: Sleep is essential for survival across the animal kingdom. In *Drosophila*, sleep regulation is largely managed by two distinct mechanisms: the circadian clock network, which signals the timing of sleep, and the sleep homeostat, which signals sleep drive. Sleep regulation can also be modulated by additional physiological drives, such as feeding status, dietary needs or sex-specific factors. Altogether, this complexity has led to an "integrator neuron" model of sleep/wake transitions. Here, we propose that serotonin cells within the *Drosophila* olfactory system, known as the CSDns, are one example of an integrator neuron contributing to sleep regulation. First, we established sleep phenotypes attributed to the CSDns neuronal activity via constitutive silencing or activation. The observed phenotypes were both sex and time-specific, with male sleep affected in evenings and female sleep in the mornings. Sleep is regulated in part by various neuropeptide signals, so we therefore screened various neuropeptide receptor reporter lines to assess which pathways could regulate the CSDns. We observed that the CSDns express the receptor for short neuropeptide F (sNPF), which has been shown to play a sleep-promoting role. Using two-photon calcium imaging, we found that the CSDns are activated upon exogenous sNPF application. Furthermore, knocking down expression of the sNPF receptor in the CSDns increased evening sleep in males, phenocopying our activation experiments. Finally, we used a

retrograde synapse tracing technique to determine the sNPFergic cells upstream to the CSDns and that in both males and females, the CSDns receive synaptic input from sNPFergic αβ and γ-lobe Kenyon cells of the mushroom bodies, which have been shown to play a role in sleep regulation. Together, these results suggest that the CSDns integrate neuropeptide signals to affect behavioral outputs such as sleep in a sex-specific manner, and in the case of sNPF, the synaptic connections may be localized to distinct neuropil.

Disclosures: O.M. Cook: None. **J. Jonaitis:** None. **K.E. Coates:** None. **A.M. Dacks:** None.

Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 065.17

Topic: F.07. Biological Rhythms and Sleep

Title: Sleep/wake behavior of mice lacking PKA phosphorylation site in SIK3

Authors: *M. PARK[1], T. HONDA[2], K. IWASAKI[1], C. MIYOSHI[1], T. FUJIYAMA[1], A. IKKYU[1], S. MIZUNO[1], S. TAKAHASHI[1], M. YANAGISAWA[1,3], H. FUNATO[1,4]; [1]Univ. of Tsukuba, Tsukuba, Japan; [2]MIT, Cambridge, MA; [3]Univ. of Texas, Texas, TX; [4]Toho Univ. Sch. of Med., Tokyo, Japan

Abstract: We previously identified a kinase, SIK3, as an important sleep regulator through a screening of randomly mutagenized mice. Mice that express mutant SIK3 lacking 52 amino acids encoded by exon 13 showed a decrease in wake time and an increase in NREM sleep time. SIK3 is an AMPK-family protein kinase containing a well-conserved protein kinase A (PKA)-phosphorylation site, serine 551. The skipping of exon 13 results in a deletion of 52 amino acids including S551. Also, *Sik3 S551A* knock-in mice showed reduced total wake time and increased sleep need. These results suggest that the existence of S551, a PKA recognition site, is crucial for the normal sleep/wake regulation and maintenance of daily sleep need.In addition to S551, there are two PKA recognition sites, threonine 469 and serine 674. To examine whether the phosphorylation of T469 and S674 of SIK3 is required for proper sleep/wake behavior, we generated mutant mice in which SIK3 T469 and SIK3 S674 were substituted by alanine through the CRISPR/Cas9 method. *Sik3 T469A* mice showed increased NREM sleep time and NREM sleep delta power, an index for sleep need. However, *Sik3 S674A* mice showed no changes in NREM sleep time and NREM sleep delta power. These findings indicate the PKA recognition sites of SIK3, especially T469 and S551 are required for the regulation of sleep/wake behavior.Furthermore, we generated Flag-tagged *Sik3* mice with PKA-phosphorylation site mutations. Using these mouse brains, we are working to identify proteins that bind to SIK3 in a phosphorylation-dependent manner and that are involved in the regulation of sleep/wake behavior.

Disclosures: **M. Park:** None. **T. Honda:** None. **K. Iwasaki:** None. **C. Miyoshi:** None. **T. Fujiyama:** None. **A. Ikkyu:** None. **S. Mizuno:** None. **S. Takahashi:** None. **M. Yanagisawa:** None. **H. Funato:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01NS103529

Title: Wakefulness Induced by TAAR1 Partial Agonism is Mediated Through Dopaminergic Neurotransmission

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Abstract: The partial TAAR1 agonist RO5253397 promotes wakefulness and suppresses REM sleep in mice, rats, and non-human primates. TAAR1 negatively regulates dopaminergic (DA) neuronal activity in the ventral tegmental area (VTA) and VTA DA neurons are primarily wake- and REM-active. Accordingly, we sought to determine whether pretreatment with DA D1- and D2-receptor antagonists affects the wake-promoting activity of TAAR1 partial agonism. Male C57BL6/J mice (n=8), maintained on a 12:12 light/dark cycle with food and water ad lib, were implanted with F20-EET telemetry transmitters (DSI, Inc., St. Paul, MN) for measurement of EEG, EMG, body temperature and activity. Three weeks after surgery, mice were acclimated to handling and oral gavage dosing. Mice received 8 combinations of compounds in a repeated measures design: D1 receptor antagonist SCH23390 (0.25 mg/kg, i.p.), D2 antagonist Eticlopride (1 mg/kg, i.p.), a combination of D1+D2, or saline was administered at ZT5.5. The TAAR1 agonist RO3397 (1 mg/kg, p.o.) or vehicle (10% DMSO) was then administered 30 min later at ZT6. EEG/EMG was recorded from ZT6-12 and scored for wakefulness, NREM, and REM sleep using Neuroscore (DSI, Inc). Data were analyzed by repeated measures analysis of variance (RM-ANOVA) followed by post hoc tests. Since most of the effects were evident only for the first few hours after dosing, data were analyzed in 3-h bins from ZT6-8 and ZT9-11. RO3397 increased cumulative wakefulness and the latency to NREM and REM sleep as shown previously. Compared to Sal+Veh, pretreatment with D1 or D2 antagonists had no effect on wake time. D1, D2, and D1+D2 pretreatment tended to reduce RO3397-induced wake time during the first hour after dosing, but only the D1+D2 combination attenuated the wake-promoting effect of RO3397 from ZT6-8. Although D1+D2 blocked the wake-promoting effect of RO3397, only D1+RO3397 and D2+RO3397 but not D1+D2+RO3397 reduced NREM and REM latencies compared to RO3397. While the half-lives of the D1 and D2 antagonists differ somewhat, these results indicate that the wake-promoting effects of RO3397 are mediated in part

by dopaminergic neurotransmission. In contrast to the clear effects on wakefulness, the interaction between DA and TAAR1 on sleep appears to be more complex.

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Poster

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Program #/Poster #: 065.19

Topic: F.07. Biological Rhythms and Sleep

Support: GMU OSCAR URSP

Title: Molecular Processes of Novel Sleep-Related Genes in Fruit Flies, Drosophila melanogaster

Authors: *M. HAMMADI, M. PEREZ, R. GUERRIERO;
George Mason Univ., George Mason Univ., Fairfax, VA

Abstract: Sleep is a vital part of human life known for rejuvenation and memory consolidation, but the molecular processes of this state are poorly understood. *Drosophila melanogaster* has been shown to exhibit this similar behavior under circadian and homeostatic control. Thus, to perform genetic manipulations that will enhance or degrade sleep, this model organism will be utilized to present a better understanding of the molecular aspect of sleep in humans. The three novel sleep-related genes analyzed within this study include *Ptx1*, *stj* (straightjacket), and *Spn* (Spinophilin). A UAS-Gal4 system will be utilized to carry either an RNAi or knockdown mutation of the gene. The Gal4 targets known sleep-regulating areas in the *Drosophila* brain, including the mushroom body, fan-shaped body, and ellipsoid-shaped body. Drosophila Activity Monitors (DAM2) will be used to record their locomotor activity over time. Sleep is determined if the infrared beam has not been broken in 5 minutes. Preliminary data has shown that *stj* in mice (*Cacna2d3*) codes for a calcium channel protein that interacts with various circadian genes that control the internal clock (Joshi et al., 2019). The *Cacna2d3* knockout mice were shown to have reduced sleep during their rest phase. Thus, since fruit flies are diurnal, this dip in sleep is expected during the dark phase. Pilot data has shown in *Ptx1* RNAi flies there was an increase in sleep time in males and females. *Spn*'s pilot data has shown increased total sleep times, more specifically aroundZT12 and ZT21 in both sexes. The importance of validating these novel sleep-related genes is to provide scientists with an understanding of the molecular processes of the circadian rhythm and even the homeostatic responses to sleep. This molecular understanding will allow scientists to better develop medicines or therapies that can counteract sleep-related diseases, includinginsomnia and restless leg syndrome (RLS).

Disclosures: M. Hammadi: None. **M. Perez:** None. **R. Guerriero:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Program #/Poster #: 065.20

Topic: F.07. Biological Rhythms and Sleep

Support: NIH R01NS108713
NIH T32NS095775

Title: Rhythmic gene expression in the substantia nigra and ventral tegmental area dopaminergic neurons and implications for the molecular clock in dopaminergic neurophysiology and function.

Authors: *A. SWAROOP[1], J. R. PAUL[2], L. J. MCMEEKIN[3], R. M. COWELL[3], K. L. GAMBLE[4];
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Abstract: Rhythmicity of physiological and behavioral processes is driven by 24-hr changes in gene expression and neuronal activity. Though these have been extensively studied in the circadian pacemaker, the suprachiasmatic nucleus (SCN), circadian regulation in other brain regions such as the substantia nigra (SN) is poorly characterized. Death of dopaminergic (DAergic) neurons of the SN occur in Parkinson's disease (PD) whereas DAergic neurons in the ventral tegmental area (VTA) are relatively spared. Given that circadian disruption is a common non-motor symptom of PD and the molecular clock is key to timing several homeostatic cellular processes, the goal of this study was to characterize rhythms in DAergic neurons and determine impact of clock deletion to understand mechanisms that regulate DAergic functions and outputs. To establish presence of a cell intrinsic DAergic clock, midbrain slices were collected from wild-type mice housed in constant darkness every 4 hrs over the 24-hr cycle and assayed for clock and clock-controlled gene expression over time and between sexes. Tyrosine hydroxylase expressing neurons showed significant 24-hr rhythms in clock and clock-controlled mRNA that were similar in phase and amplitude in both SN and VTA (n=4 male & female mice/time point, 3-6 mo. age; cosinor analysis). To explore a larger number of DAergic clock-controlled genes, we sub dissected SN and VTA of BAC-TRAP L10 $^{+/+}$; Dat^{Cre} mice in the day and night (n=2-3 samples/group, sample=10 pooled male & female mice). In comparing SN vs VTA we found several differentially expressed genes through RNA seq across time of day.
Ablation of the DAergic clock (via TH-Cre AAV injection in SN of 3-4 mo. Bmal1$^{Fl/Fl}$ mice) resulted in no group differences in wheel-running locomotor behavior and pole assay (n=6 male mice/group, 6-7 mo.; rm-ANOVA, t-test). Using a complementary model that ablates the molecular clock in all DAT-expressing neurons (DAT^{Cre}; Bmal1$^{Fl/Fl}$) we found that spike rate activity of SN DAergic neurons in control males significantly varied across 24 hours (6 mice/group; cosinor analysis). However, this significant firing rhythm in DAergic neurons was lost in DAT^{Cre}; Bmal1$^{Fl/Fl}$ mice suggesting that firing rhythms in the SN DAergic neurons are

dependent upon the molecular clock. Taken together, these experiments further our understanding of clock regulation of DAergic neurons, which may reveal therapeutic targets to abate neurodegeneration in PD.

Disclosures: A. Swaroop: None. **J.R. Paul:** None. **L.J. McMeekin:** None. **R.M. Cowell:** None. **K.L. Gamble:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: T32NS063391
1F30HL145901
5RO1HL129138

Title: The Effects of Estradiol on A_1R and $A_{2A}R$ Signaling and Sleep in the Median Preoptic Nucleus

Authors: *K. D. KRUK[1], P. C. SMITH[2], S. VIECHWEG[3], J. A. MONG[3];
[1]Univ. of Maryland Sch. of Med. Program In Neurosci., Baltimore, MD; [2]Washington Univ. Sch. of Med. Dept. of Anesthesiol., St. Louis, MO; [3]Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Studies have shown that women report more sleep difficulties and are more likely to be diagnosed with insomnia compared to men. Sleep disturbances are more likely to occur in women during times of hormonal fluctuations, including pregnancy and menopause, thus indicating that sex hormones play a role in the sleep-wake cycle. Understanding more about how sex hormones act to influence sleep can help us develop targeted treatments for women who suffer from sleep disorders. There are 2 major sleep centers in the brain, both located in the hypothalamus - the median preoptic nucleus (MnPO) and the ventrolateral preoptic nucleus (VLPO). There are estrogen receptors located in the MnPO, but not in the VLPO, suggesting that estrogen acts via the MnPO to regulate sleep-wake states. The MnPO is thought to promote sleep by inhibiting wake-promoting neurons in the brain. It has been found that estradiol (E2) injection increases wake and decreases sleep in ovariectomized female rats, however the mechanism by which sleep is disrupted by estradiol is largely unknown. During wakefulness, adenosine accumulates in the brain and increases sleep pressure, causing tiredness. The A_1 and A_2A receptors (A_1R and $A_{2A}R$) are expressed in the MnPO and play an important role in regulating the effects of adenosine in the brain. Infusion of an A_1R agonist into the MnPO has been found to increase wake and decrease sleep in rats, while infusion of an $A_{2A}R$ agonist has been found to increase sleep and decrease wake. E2 has been hypothesized to influence the inhibitory/excitatory adenosinergic balance in the MnPO. One potential target of E2 could be G protein-coupled receptor 37 (GPR37), as it has been shown to inhibit $A_{2A}R$ surface expression

and function in the striatum. To examine the effects of E2 on $A_{2A}R$ signaling, ovariectomized female rats with EEG and EMG leads implanted were treated with E2 or oil and then infused with an $A_{2A}R$ agonist via cannulation. Their sleep-wake states were measured prior to E2 or oil injection, after E2 or oil injection, and after drug injection. To examine GPR37 expression, ovariectomized female rats were treated with E2 or oil for 2 days and sacrificed on the third day; male rats were treated with oil and not castrated. The brains were fixed and sectioned, and a standard RNA scope protocol was utilized to detect GPR37 mRNA. We found that E2 blocks $A_{2A}R$ signaling in the MnPO while increasing GPR37 expression. Overall, estradiol appears to have an effect on the sleep/wake phenotype through adenosinergic signaling and/or expression, potentially through GPR37.

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Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: 5 T32 HL 110952-9

Title: Temporal dynamics of synaptic and intrinsic ion currents underlying homeostatic sleep pressure of hypocretin neurons

Authors: *O. C. GONZALEZ, R. COULSON, G. WANG, L. DE LECEA;
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Abstract: Sleep is a well conserved behavioral state observed across all known animals. Its role in learning and memory has been recently established, and disrupted sleep has been associated with various neurological and psychiatric disorders. Sleep has been the focus of intense research for several decades. As the amount of time spent in the awake state increases, there is a homeostatic increase in sleep pressure. Following sleep deprivation, this buildup of sleep pressure leads to homeostatic sleep rebound. However, how the amount of time spent in the awake state impacts the brain, thereby driving it towards sleep, remains to be fully understood. It is likely that state dependent changes in wake promoting hypocretin neurons of the lateral hypothalamus may be involved in accumulated sleep pressures developed across the awake state. We hypothesize that accumulated changes in synaptic and intrinsic currents on hypocretin neurons may regulate their relative excitability and underly homeostatic sleep pressure. In this new study, we use super-resolution array tomography to explore the temporal dynamics of changes in synaptic and intrinsic ion channel densities on hypocretin neurons of the lateral hypothalamus. We compare relative densities of excitatory and inhibitory synapses on mouse hypocretin neurons across dark/light cycles to establish general trends in synapse densities as impacted by periods of wake/sleep. Additionally, we explore state dependent changes of intrinsic

potassium channel expression on hypocretin neurons. Together, these data provide novel insights into the mechanisms underlying local circuit sleep pressure in wake-promoting hypocretin neurons of the lateral hypothalamus, which may drive wake/sleep transitions.

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Poster

065. Sleep Behaviors and Mechanisms

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JSPS KAKENHI 22H04918

Title: Kinase activity of SIK3 has a crucial role in sleep homeostatic regulation

Authors: *S. NAKATA[1], H. ABE-KOMIYA[1], T. FUJIYAMA[1], C. MIYOSHI[1], A. IKKYU[1], F. ASANO[1], K. IWASAKI[1], S. MIZUNO[2], F. SUGIYAMA[2], S. TAKAHASHI[2], H. FUNATO[1,3], M. YANAGISAWA[1];
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Abstract: Sleep time within a day is tightly regulated by the homeostatic sleep need accumulating during wakefulness and dissipating during sleep. Although neural circuits responsible for switching vigilance states have been increasingly elucidated, intracellular signaling pathways which connect the sleep need with the state-switching remain unknown. We recently identified *Sik3* as a sleep-promoting gene functioning in neurons. The gain-of-function mutation named *Sleepy* in *Sik3*, which leads to the expression of exon13-deleted SIK3 proteins (Slp), induces increased sleep need and non-REM (NREM) sleep time in mice. On the other hand, sleep deprivation, which increases sleep need, specifically enhances the phosphorylation level at Thr221 (T221) of SIK3 in wild-type mice. Phosphorylation at T221 in the SIK3 kinase domain modulates the kinase activity, suggesting that SIK3 kinase activity increases in mice with a higher sleep need. However, how the SIK3 kinase activity is involved in the sleep homeostatic regulation has not been elucidated. Here, to examine the role of SIK3 kinase activity in sleep/wake regulation, we performed EEG/EMG-based sleep/wake analysis for mice carrying a T221A or T221E mutation in *Sik3*. *Sik3*$^{T221A/+}$ heterozygous mice showed decreased delta density (1-4Hz) during NREM sleep, which is a reliable marker for sleep need, with no changes in sleep

time. On the other hand, *Sik3*$^{T221E/T221E}$ homozygous mice also showed a tendency to decrease delta density during NREM sleep, which is consistent with our results that SIK3(T221E) had a partial constitutive kinase activity at about a half of wild-type activity. Additionally, we examined the dynamics of sleep need in *Sik3*$^{T221A/+}$ and *Sik3*$^{T221E/T221E}$ mice by simulating NREM sleep delta power under the baseline and during and after sleep deprivation. While *Sik3*$^{T221A/+}$ heterozygous mice showed a faster dissipation rate of sleep need, *Sik3*$^{T221E/T221E}$ homozygous mice exhibited normal sleep homeostatic response. These results suggest that SIK3 kinase acts to delay the resolution of sleep need. Moreover, to examine whether the kinase activity of SLP mutant SIK3 is required for hypersomnia in *Sik3*^{Slp} mice, we generated and analyzed *Sik3*$^{T221A\text{-}Slp}$ mice and *Sik3*$^{T221E\text{-}Slp}$ mice. *Sik3*$^{T221A\text{-}Slp/+}$ heterozygous mice showed decreased sleep need and NREM sleep time compared with *Sik3*$^{Slp/+}$ heterozygous mice. In contrast, *Sik3*$^{T221E\text{-}Slp/T221E\text{-}Slp}$ homozygous mice showed increased sleep time compared with *Sik3* $^{T221E/T221E}$ homozygous mice. In conclusion, our current results indicate that SIK3 kinase activity has a critical role in sleep-need regulation.

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Poster

065. Sleep Behaviors and Mechanisms

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Topic: F.07. Biological Rhythms and Sleep

Support: NIH R35 NS122172
NIH F31 NS105487

Title: Genetic and neuronal substrates of melatonin signaling in zebrafish sleep

Authors: *A. HILL, D. PROBER;
Caltech, Pasadena, CA

Abstract: Sleep appears to be conserved across the animal kingdom, and adequate sleep is essential for human health. However, despite its ubiquity and importance, surprisingly little is known about the genetic, molecular, and neuronal mechanisms that govern sleep. Exogenous melatonin (MT) is recognized as a somnogen in humans, but endogenous MT, whose synthesis is controlled by the circadian clock, has only recently been shown to be required for normal sleep levels. Using the diurnal vertebrate zebrafish, our lab demonstrated that loss of an enzyme essential for MT synthesis causes a dramatic decrease in sleep at night. We also found that loss of MT has no effect on circadian rhythms but abolishes circadian regulation of sleep, indicating that MT acts downstream of the clock to promote sleep. We are now investigating the mechanisms by which MT promotes sleep. The human genome encodes two MT receptors, MT1

and MT2, whose effects on sleep are not well-understood. The zebrafish genome encodes six MT receptors: 3 that are orthologous to mammalian MT1, 2 that are orthologous to MT2, and an additional receptor not present in mammals. We created zebrafish lines harboring mutations in each of the six receptors, as well as double and triple mutant combinations. We found that zebrafish larvae lacking all three MT1-type receptor paralogs sleep less at night, similar to MT-deficient animals, and are nearly totally resistant to sleep induced by exogenous MT. Next, using *in situ* hybridization chain reaction (HCR), we found that the MT1 receptors are primarily expressed in the optic tectum, a structure homologous to the superior colliculus of mammals, and in a discrete cluster of cells in the hindbrain. We are currently optimizing HCR to better ascertain the location of these receptors, which we anticipate will point to critical sleep-regulating parts of the brain. Last, we are using 2-photon selective plane illumination microscopy (2P-SPIM) to image the entire brain of larval zebrafish expressing the calcium sensor GCaMP7f pan-neuronally. We hypothesize that animals treated with MT will exhibit specific changes in neuronal activity, that these changes represent a subset of neuronal changes observed during normal sleep, and that these changes are not observed in MT1 receptor mutants.

Disclosures: **A. Hill:** None. **D. Prober:** None.

Poster

065. Sleep Behaviors and Mechanisms

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Title: The Role of Pumilio on Intestinal Stem Cells

Authors: J. A. RODRIGUEZ-CORDERO, ***C. I. MALDONADO-VALEDON**, Y. ORTIZ, A. GHEZZI, I. A. RODRIGUEZ-FERNANDEZ, J. L. AGOSTO RIVERA;
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Abstract: In the past few years, accumulating evidence indicates that the gut and its microbial communities play a major role in the regulation of a wide range of behaviors. However, the cellular and molecular mechanisms underlying this regulation remain to be elucidated. We have previously shown that an RNA binding protein called Pumilio (pum) plays a role in sleep regulation, in Drosophila melanogaster. While performing a behavioral screen to identify the anatomical loci of pum effects on sleep regulation, we found that pum silencing in the enterocytes of the gut increases sleep and increases overall intestinal bacterial populations. Since pum is known to play a key role in stem cell differentiation and self-renewal, we explored the effects of pum silencing exclusively on intestinal stem cells (ISCs) on sleep. Moreover, to make sure that any potential effect is associated with physiological regulation rather than a

developmental effect, we restricted pum silencing to the adult phase using the GAL80ts system. Interestingly, we found that pum knockdown during adulthood specifically decreases sleep latency in a reversible manner, in males. Furthermore, the knockdown of pum on ISCs produced an increase in the amount of ISCs, as quantified via immunohistochemistry. Based on these findings, we hypothesize that pum silencing affects sleep by altering the expression of genes involved in gut-brain signaling, either directly in ISCs, or indirectly through one of the differentiated cells. To test this hypothesis, we will use ribosome profiling to quantify translational activities specifically in ISCs between control and pum knockdown flies. Our work represents the first study linking ISCs with sleep regulation. Moreover, our findings will shine light into the mechanisms of behavioral regulation by gut-brain communication.

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Poster

065. Sleep Behaviors and Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 065.26

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

Support: Heritage Medical Research Institute
Vallee Foundation
the Center for Molecular and Cellular Neuroscience in the Tianqiao and Chrissy Chen Institute for Neuroscience at Caltech
Caltech Biology and Biological Engineering division postdoctoral fellowship
Moore Distinguished Scholar at Caltech

Title: The role of suprachiasmatic VIP neurons in circadian rhythm regulation of the estrous cycle in female mice

Authors: *A. KAHAN[1], G. M. COUGHLIN[1], M. BORSOS[1], J. E. ROBINSON[1,2], B. W. BRUNTON[3], V. GRADINARU[1];
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Abstract: Ovulation is fundamental for female fertility and assessing health. In humans, disruptions to the regular light-dark (LD) cycle, such as jetlag or shift work, affect the menstrual cycle and decrease ovulation. To elucidate the underlying mechanism, we examined the effect of light conditions on estrous cycle regularity in mice. Under 12:12 LD conditions, female mice ovulate every 4-5 days, whereas, under near-complete dark-dark (DD) conditions, ovulation was reduced to every 7-8 days. The effect was reversed by one hour of light in the late afternoon at Zeitgeber (ZT)10. This temporal specificity led us to suspect the involvement of the circadian

rhythm pacemaker, the suprachiasmatic nucleus (SCN). A key neuronal population in the SCN expressing vasoactive intestinal peptide (VIP) exhibits immediate responses to light, as well as direct and indirect connectivity to gonadotropin-releasing hormone (GnRH) neurons which control the release of reproductive hormones such as luteinizing hormone (LH). Although signaling from SCN^{VIP} to GnRH neurons has been hypothesized to control estrous cycle timing, it is unknown if and how SCN^{VIP} neuronal activity stimulates or depends on the estrous state and whether GnRH neuronal sensitivity to VIP originates from SCN^{VIP} neurons. To address these questions, we first ablated SCN^{VIP} neurons with Caspase-3 and observed a significant reduction in ovulation. Next, we recorded GCaMP6s signal via fiber photometry (FP) from SCN^{VIP} neurons *in vivo* in male and female mice for ten minutes every hour over 30 days. In a separate paradigm, we recorded the activity from ZT10 to 13, corresponding to the LH surge. Analysis of the FP data showed that SCN^{VIP} activity depends on time-of-day and not estrous-cycle. A machine learning classifier could distinguish between sex but not between estrous cycle days. Our recordings revealed that SCN^{VIP} neurons' activity in ZT10-13 is estrous-cycle dependent; rates at ZT11, just before ovulation, are two-fold lower than on the day before or after. This suggests that SCN^{VIP} neurons act on GnRH neurons mainly to inform light status and have hormonal control at a specific time window. Finally, we used opto- and chemogenetics to test whether, under DD conditions, estrous cycle regularity can be rescued by SCN^{VIP} neuron activation at ZT10. No significant rescue was found, suggesting that other activation patterns or cell populations are involved in this process. Together, these experiments provide direct evidence for SCN^{VIP} neurons being the source of VIP peptide in the circadian regulation of ovulation and suggest that the time-of-day-dependent activity of SCN^{VIP} neurons is essential for estrous cycle regularity.

Disclosures: **A. Kahan:** None. **G.M. Coughlin:** None. **M. Borsos:** None. **J.E. Robinson:** None. **B.W. Brunton:** None. **V. Gradinaru:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Novo Nordisk AG
Eccellenza Grant from the Swiss National Science Foundation (grant number 181094)

Title: Does GLP-1 receptor agonist alter food-related sensory pleasure? Arandomised controlled trial in patients with obesity

Authors: *G. COPPIN[1,3,4], D. MUNOZ TORD[4,2,3], E. R. POOL[4,3], L. LOCATELLI[5], A. ACHAIBOU[2,4], A. ERDEMLI[4,3], L. LEON PEREZ[4,3], L. WUENSCH[4,3], D. CEREGHETTI[6], A. GOLAY[4], D. SANDER[4,3], Z. PATAKY[4];

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Abstract: GLP-1 receptor agonist (liraglutide) has been demonstrated to successfully promote weight loss in patients suffering from obesity. Yet, it is unclear whether the observed weight loss is driven by an alteration of food reward processing. Here we investigated the effects of liraglutide on cerebral correlates of food-related sensory pleasure in obesity. We conducted a randomized, single-centre, double-blind, placebo-controlled, parallel group, prospective clinical trial. 73 patients with obesity and without diabetes were randomly assigned to receive liraglutide 3.0 mg (37.40±11.18 years old, Body Mass Index = 35.89±3.01) or placebo (40.04±14.10 years old, Body Mass Index = 34.88±2.87) subcutaneously once daily, for 16 weeks. We investigated sensory pleasure during food consumption (liking). Participants reported their trial-by-trial hedonic experience while consuming a high-calorie food (milkshake) and a tasteless solution. The solutions were administered inside the scanner with a Magnetic Resonance Imaging-compatible gustometer to assess neural responses during consumption. The same procedure was repeated for pre- and post-intervention sessions. The liraglutide group lost more weight (8.50 kg ±0.70) compared to the placebo group (2.12 kg ±0.63). The sensory pleasure during food reward consumption was associated with the activation of the ventromedial prefrontal cortex and the amygdala. We did not find any statistically significant difference in the liraglutide group between the pre and post sessions, neither at a subjective level nor at a neural level in response to the milkshake. In summary, we did not find evidence supporting a reduction of food-related sensory pleasure in patients suffering from obesity concomitant to the weight loss induced by liraglutide.

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Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.02

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Conditioned approach behavior of SHR and SD rats during Pavlovian conditioning

Authors: *B. SILIC[1], M. AGGARWAL[3], K. LIYANAGAMA[1], G. TRIPP[2], J. WICKENS[1]; [1]Neurobio. Res. Unit, [2]Human Developmental Neurobio. Unit, Okinawa Inst. of Sci. and Technol., Onna, Japan; [3]RIKEN Ctr. for Brain Sci., Wako City, Japan

Abstract: The spontaneously hypertensive rat (SHR), an animal model with ADHD-like behavior, has a greater sensitivity to delay of reward than comparison strains such as Sprague-

Dawley (SD) rats. However, little evidence is available on the impact of reward cues on the behavior of SHRs compared to SD rats. We used Pavlovian conditioned approach (PCA) to investigate the acquisition of a conditioned response and tendency to attribute incentive salience to reward cues in SHR (n=19) and SD rats (n=20). Across 11 days of Pavlovian training, a lever-cue was paired with the food reward. During the 8 s of cue presentation, some rats approached and interacted with the cue, while others approached and entered the reward location. We recorded the number of lever contacts and presses as a measure of cue-directed conditioned responding. To measure the reward location-directed conditioned response, we recorded a number of magazine entries during the cue presentation. We found that SHR and SD rats had a similar number of lever contacts across 11 days of training. However, when we measured the vigor of lever interaction using lever presses, SD rats had more lever presses than SHR rats across the training. In contrast, SHR rats had strikingly more magazine entries than SD rats during the cue presentation. Both strains had fewer magazine entries 8 s before the cue presentation than during the cue presentation. Moreover, SHR rats had fewer magazine entries than SD rats before the cue presentation. Additionally, we used a Pavlovian conditioned approach (PCA) index to classify rats as sign trackers (rats that are predominately approaching the cue), intermediate responders (rats that are most of the time approach both the lever and the reward location), or goal trackers (rats that are mostly approaching reward location during the cue presentation). The majority of SHR rats (89%) and SD rats (50%) were classified as goal trackers. 11% of SHR and 40% of SD rats are classified as intermediate responders, while no SHR and 10% of SD rats were classified as sign trackers. These results suggest that SHR rats have a greater tendency toward goal tracking than SD rats, possibly because the cue develops less incentive salience in the SHRs, which might explain their sensitivity to delayed reward.

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Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NRF-2020M3E5D9080734

Title: Lateral habenula(lhb) mediates the association of CS with non-reward in Pavlovian appetitive conditioning

Authors: *I.-B. JIN, D.-H. KIM, Y.-J. JEON, J.-S. HAN;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: The presentations of an unconditioned stimulus (US) contingent upon the occurrence of a conditioned stimulus (CS) establishes excitatory conditioning or conditioned excitation (e.g.,

approaching a food-cup area). On the other hand, a CS's association with a US's nonoccurrence generates inhibitory conditioning or conditioned inhibition (e.g., not approaching a food-cup area). Research on conditioned excitation has long dominated and revealed the neural substrate of the conditioned excitation, but the neural substrate of conditioned inhibition is poorly studied. Therefore, we performed a retardation-of-acquisition task of unpaired learning following paired learning to prove a CS's association with a US's nonoccurrence. Animals with unpaired learning were slower to acquire conditioned excitatory properties in the subsequent paired learning than comparison animals that received the paired alone. In the previous study, c-Fos levels were higher in the lateral habenula (LHb) of rats with unpaired learning compared with rats with paired learning or the other controls. Therefore, the present experiment used a retardation-of-acquisition task with LHb lesions to investigate whether LHb mediated the association of CS with the nonoccurrence of a US. Sham-operated rats showed a slow acquisition of the paired learning followed by the unpaired learning, which was not observed in rats with LHb lesions. These results indicate that neurotoxic LHb lesions interfere with acquiring a CS's association with a US's nonoccurrence in the unpaired training. Next, chemogenetic inhibitory effects of the LHb on the association of a CS with the nonoccurrence of a US were evaluated in the retardation-of-acquisition test. The retarded acquisition of subsequent excitatory learning following unpaired learning was not observed in animals with chemogenetic LHb inhibition throughout the unpaired training but in animals with chemogenetic LHb inhibition throughout the paired training. These findings suggest that LHb mediates the association of CS with the nonoccurrence of US.

Disclosures: I. Jin: None. **D. Kim:** None. **Y. Jeon:** None. **J. Han:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

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Program #/Poster #: 066.04

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA Grant P50 DA037844
NIAA Grant T32 AA007583

Title: Genome-wide association study in outbred heterogeneous stock rats identifies convergent loci for the attribution of incentive salience to reward cues.

Authors: *C. P. KING[1], A. S. CHITRE[2], O. POLESSKAYA[2], B. M. THOMPSON[1], S. B. FLAGEL[3], T. E. ROBINSON[4], L. C. SOLBERG WOODS[5], H. CHEN[6], A. A. PALMER[2], P. J. MEYER[1];
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Medicine, Mol. Medicine, Ctr. on Diabetes, Obesity and Metabolism, Wake Forest Sch. of Med., Winston-Salem, NC; [6]Dept Pharmacol, Univ. Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Addiction vulnerability is associated with non-drug traits such as the tendency to attribute incentive salience to reward cues and is influenced by environmental and genetic factors. To characterize the genomic regions associated with these behaviors, we performed a genome-wide association study (GWAS) in a cohort of 1,645 phenotypically and genetically diverse N/NIH Heterogeneous Stock (HS) rats. We first tested HS rats in a Pavlovian Conditioned Approach task, in which we characterized the individual responses to food-associated stimuli ("cues"), and report two general categories of Pavlovian conditioned responses: cue-directed "sign-tracking" behavior, and food-cup directed "goal-tracking" behavior. We then used the conditioned reinforcement procedure to examine whether rats would perform a novel operant response for presentations of the food cue alone. We demonstrate that sign-tracking was associated with heightened cue-directed responding, and identified genetic loci associated with multiple measures across both tasks in HS rats at each institution.
We found high genetic correlations for terminal measures of Pavlovian conditioned approach and conditioned reinforcement. GWAS yielded 18 unique quantitative trait loci (QTL) across these two tasks, many on chromosome 1 that were associated with sign-tracking measures with the largest heritability values (h^2 = .189-.215). Interval sizes of loci varied, although some of the strongest associations for sign-tracking contained few genes (*e.g. Tenm4, Mir708*). Expression-QTL in mesocorticolimbic regions of the central nervous system revealed additional candidate genes (*e.g. Wnt11, Capn5)*. We demonstrate that HS rats are useful for investigating the genetic variants underlying complex behavior and may be useful for identifying candidate genes for future testing.

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Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Pritzker Neuropsychiatric Disorders Research Consortium
NIDA R21 DA052594
T32DA07268

Title: Investigating the effect of corticosterone on the acquisition of sign-tracking behavior in female rats from different vendors

Authors: *A. E. TURFE[1], S. R. WESTBROOK[2], S. A. LOPEZ[4], V. SANCHEZ FRANCO[5], J. BHATTI[3], S. B. FLAGEL[2];
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Abstract: Cues in the environment become predictors of biologically relevant stimuli through associative learning. These cues can also become imbued with incentive value, leading to maladaptive behaviors characteristic of numerous psychopathologies. We can model incentive learning in animals using Pavlovian conditioned approach (PavCA) training, where a discrete lever cue (conditioned stimulus, CS) is followed by the delivery of a food reward (unconditioned stimulus, US). Rats that undergo this training will develop a conditioned response directed towards the lever-CS (i.e., sign-trackers), the food cup (i.e., goal-trackers), or vacillate between the two (i.e., intermediate responders). We have previously reported that after a single PavCA session, plasma corticosterone (CORT) levels are higher in male rats that become sign-trackers relative to those that become goal-trackers. We have also found that CORT administration increases the propensity to sign-track in male rats, but appears to do so selectively in rats from Charles River, and not those from Taconic. Here, we assessed the effect of CORT administration on the propensity to sign-track in female rats, and included vendor as a variable of analysis. Adult female rats from either Charles River or Taconic underwent 5 sessions of PavCA training with 3 mg/kg CORT or vehicle injections (i.p.) administered 30 minutes prior to each session. The effect of CORT administration on sign-tracking and goal-tracking behaviors were assessed using several outcome measures, including the number of lever-CS vs. food cup contacts, the probability to approach the lever-CS vs. food cup, and the latency to approach the lever-CS vs. the food cup. In contrast to our prior findings with male rats, CORT administration did not significantly affect sign-tracking behavior in female rats from either vendor. It is important to note, however, that female rats from Charles River displayed significantly greater sign-tracking behavior compared to those from Taconic. These findings support the notion that individual variation and the distribution of behavioral phenotypes differs between vendors. Blood samples were collected and will be analyzed to determine whether differences in baseline CORT levels between rats from different vendors underlie the reported behavioral effects. The effect of estrous cycle is also being examined. These results underscore the importance of reporting vendor in scientific communications and including it as a critical variable of analysis.

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Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.06

Title: WITHDRAWN

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.07

Topic: G.02. Reward and Appetitive Learning and Memory

Support: 1F32DA054767-01A1
5R01DA035943-09

Title: Midbrain dopamine is sensitive to Pavlovian information loss

Authors: *E. GARR[1], Y. CHENG[1], A. BAL[2], S. BROOKE[1], L. CASTELL[1], P. H. JANAK[1]; [2]Psychology, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: There has long been evidence that Pavlovian learning is a function of the mutual information between cue and reward—the degree to which a cue signals a change in reward rate. We investigated whether midbrain dopamine dynamics are sensitive to Pavlovian information loss. We used fiber photometry to record from dopamine neurons in the ventral tegmental area (VTA) during cue-reward conditioning followed by contingency degradation in TH-Cre+ rats (*n* = 13). Contingency degradation attenuated the rate, timing, and latency of conditioned port entries. Calcium transients at the time of reward were sensitive to local reward history in a manner consistent with prediction error encoding, but this sensitivity disappeared with contingency degradation. Calcium transients at the time of cue onset did not depend on local reward history regardless of cue-reward contingency, opposing the prediction error hypothesis. We further show that sensitivity to contingency degradation is predicted by how much larger the dopamine response is to non-contingent rewards. In a separate experiment, we asked whether degrading the contingency between a cue and optogenetic VTA dopamine neuron stimulation would attenuate conditioned locomotion. During acquisition, conditioned locomotion increased in TH-Cre+ rats (*n* = 19), but not in TH-Cre- rats (*n* = 9) or TH-Cre+ rats that experienced contingency degradation (*n* = 6). Rats that acquired the conditioned response were split into groups that were either maintained on the conditioning protocol or underwent contingency degradation. Conditioned locomotion was attenuated only in the latter group. Together, these results suggest that the mutual information between cues and VTA dopamine transients inform animals' propensity to respond to motivationally salient cues.

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Poster

066. Reward and Appetitive Learning

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Topic: G.02. Reward and Appetitive Learning and Memory

Support: JSPS Grant 20J00921
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JSPS Grant 19H01775

Title: Transition between habits and goal-directed actions in the renewal effect

Authors: *S. FUJIMAKI, Y. KOSAKI;
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Abstract: Two experiments with rats explored whether previously extinguished goal-directed and habitual responding recover with the same status using an ABA renewal preparation. In Experiments 1a and 1b, a lever-press response was minimally (4 sessions) or extensively (16 sessions) trained in one context (Context A) and extinguished in another context (Context B). Then, outcome devaluation took place in either Context A or Context B in which a food pellet reinforcing the response was paired with lithium chloride (LiCl). Finally, renewal of the extinguished response was tested in both Contexts A and B. We confirmed that both minimally and extensively trained responses renewed as goal-directed action regardless of the context in which devaluation took place. This finding was replicated in Experiment 2 even after more extended acquisition training (32 sessions). However, another group that received outcome devaluation before but not after extinction training showed habitual performance during extinction training as well as in a subsequent renewal test. This result extends previous findings suggesting that actions and habits renew with the same status by returning to the original context after extinction. Overall, the present results revealed the differential effects of pre- and post-extinction devaluation on expression of habitual behavior; extinction prior to devaluation may convert from a habitual performance to a goal-directed action.

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Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.09

Title: WITHDRAWN

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

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Topic: G.02. Reward and Appetitive Learning and Memory

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Title: Accessibility over time to palatable food determined binge like intake during adolescence

Authors: *M. CHAVEZ-HERNANDEZ[1], L. RODRIGUEZ-SERRANO[2], S. MUÑOZ-JUAREZ[3], R. ESCARTIN-PEREZ[4], D. DIAZ-URBINA[5], A. MONTENEGRO-PEREZ[3], J. RODRÍGUEZ-AGUILAR[6], M. H. BUENROSTRO-JAUREGUI[7];
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Abstract: A growing number of studies suggest that palatable food can lead to chronic overconsumption and, subsequently, obesity. Furthermore, it has been shown that food intake palatable can lead to synaptic plasticity alteration in the mesolimbic system. Additionally, it has been shown that there are two systems involved in feeding: homeostatic, necessary for basic metabolic processes and survival, and hedonic, which is driven by sensory perception or pleasure (Rossi & Stuber, 2017). It has been shown that ad libitum and continuous restricted access to palatable food (PF) have differential effects, with restricted access inducing binge eating behavior to PF in adult rats (Muñoz-Escobar et al., 2019). Nevertheless, it is not known if an increased intake of PF is regarding its access, particularly in adolescent rats. Therefore, we hypothesized that intake of PF is a risk factor that can lead to chronic overconsumption in conditions of non-homeostatic feeding, exacerbating its effects if they occur at an early age. The present study aims to evaluate which PF access protocol is the most sensitive to induce increased intake of PF during adolescence. We used thirty male Wistar rats (30 days postnatal); all animals were housed individually and had ad libitum access to a standard diet (SD) and water; animal weight and SD food intake were manually recorded every 24h. Rats were assigned to one of three groups: a) Continuous, with daily access to PF; b) Intermittent A, with one-day access, one-day no-access; or c) Intermittent B (weekend), with 3 days-access/4 days no-access. All groups had 1h access to PF (Oreo® Cookies Nabisco®) according to diet protocol; SD and water were removed during the PF access, and PF was weighed before and after the 1h access to register consumption. PF and SD caloric intake, and binge eating criterion (defined as consuming ≥20% of total caloric intake per day during the 1h access to PF) were analyzed. Results show that there are no significant differences between groups in binge eating criterion and PF caloric intake

during the first 2 weeks. Nevertheless, on week 3, the continuous and the intermittent A groups significantly increase PF caloric intake when compared to the Intermittent B group, and the continuous group presents significantly more binge eating criteria when compared to the intermittent B group. Overall, these results indicate that an increased intake of PF during adolescence is in regard to its access, and that it can lead to chronic overconsumption in conditions of non-homeostatic feeding.

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Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.11

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CONACyT grant FOINS 474

Title: Glutamatergic afferents from the anterior insular cortex to the ventral tegmental area play a critical role in reward memory formation

Authors: *E. HERNÁNDEZ-ORTIZ[1], J. LUIS-ISLAS[2], R. GUTIÉRREZ[2], F. TECUAPETLA[1], F. BERMÚDEZ-RATTONI[1];
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Abstract: The insular cortex's (IC) activity has been implicated in processing interoceptive and exteroceptive signals associated with the maintenance of addictive behavior. Previous reports have shown differential functions of anterior (aIC) or posterior (pIC) IC in the maintenance of drug addiction. Nevertheless, the insular activity and neuronal outputs implicated in reward memory formation have not been explored. Here, we test the hypothesis that the aIC, but not the pIC, can induced a contextual reward-memory through communication whit the ventral tegmental area (VTA). Our results show that the aIC, but not the pIC insular cortex, sends a robust axonal projection to VTA, the primary source of forebrain dopamine, which has a vital role in reward-seeking and drug abuse. We show that photoactivation of axonal projection of aIC neurons expressing CamKII (aIC^{CamKII}), but not pIC^{CamKII}, in VTA induced the formation and maintenance of a reward-memory in a real-time conditioning place preference (rtCPP). Consistently, electrophysiological recordings show that the photoactivation of aIC^{CamKII} to VTA circuit, but not pIC^{CamKII} to VTA, can modulate the VTA activity and elicit a liberation of glutamate and local dopamine release in VTA through in vivo microdialysis. Moreover, employing a transsynaptic anterograde spread whit AAV1, we show that aIC establishes synaptic contacts, preferentially, whit VTA dopaminergic neurons. Finally, we show that the

photoactivation of this subpopulation elicited a dopamine release in VTA, which was associated with the formation and maintenance of reward-memory. Our findings show, for the first time, that the aIC can modulate the electrical activity of VTA and suggest a new functional circuit implicated in the formation and maintenance of different addictive behaviors.

Disclosures: E. Hernández-Ortiz: None. **J. Luis-Islas:** None. **R. Gutiérrez:** None. **F. Tecuapetla:** None. **F. Bermúdez-Rattoni:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.12

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA R01 DA044960

Title: Effects of chemogenetic manipulation of the ventral hippocampus to nucleus accumbens pathway on sign- and goal-tracking behaviors

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Abstract: Cues in the environment can gain motivational value when paired with rewarding stimuli. This relationship becomes maladaptive when cues are associated with addictive substances. Pavlovian conditioned approach (PCA) training is used to assess individual variation in the attribution of incentive value to reward cues, which is linked to a proclivity towards addiction and relapse. When the presentation of a lever precedes a food reward, ‘goal-tracking’ rats (GT) direct their behavior away from the cue and towards the food reward, suggesting that the lever is solely a predictor. Meanwhile, ‘sign-tracking’ rats (ST) approach the lever, indicating that they are attributing incentive salience to the cue itself, which makes them prone to addiction-like behaviors. Despite the apparent relevance to addiction and other psychopathologies, the underlying neurocircuitry remains poorly understood. Glutamatergic transmission to the nucleus accumbens (NAc) is important for sign-tracking behaviors in rats. Additionally, our previous work has found that lesions of the ventral hippocampus (vHPC), which densely innervates the NAc, lead to decreases in sign-tracking. We therefor hypothesized that the vHPC-NAc glutamatergic projection may influence sign- and goal-tracking. To test the effects of this pathway, we used an in-vivo dual vector approach to inject Cre recombinase into the NAc and either an inhibitory Gi-coupled or excitatory Gq-coupled DREADD into the vHPC. Rats then received injections of CNO or vehicle before PCA training for six days to analyze effects of pathway manipulation on behavior acquisition. On the 7th day, rats received a crossover injection with the other treatment (CNO or vehicle) to examine effects on behavior expression. Our results show that chronic inhibition of the pathway does not seem to affect sign- and goal-tracking behaviors, while ceasing chronic inhibition, through crossover treatment, leads to

decreased sign-tracking behaviors in sign-trackers. Chronic excitation revealed a sex difference in sign-tracking, with male rats showing decreased sign-tracking. These results indicate that the vHPC-NAc projection may modulate the acquisition and expression of sign-tracking behaviors in a sex-dependent manner.

Disclosures: F. Czesak: None. **C.E. Maria Rios:** None. **J. Staffeld:** None. **J.D. Morrow:** None.

Poster

066. Reward and Appetitive Learning

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Program #/Poster #: 066.13

Topic: G.02. Reward and Appetitive Learning and Memory

Support: European Research Council (ERC-2015-CoG-682422)
Deutsche Forschungsgemeinschaft (FOR 2143, SFB1315)
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Title: Primary somatosensory cortex is required for the consolidation of a one-session thermal detection

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Abstract: Fear conditioning tasks have established that the 2 hours following training is a critical time window for memory consolidation, however the neural mechanisms are unclear. In part this is because monitoring and manipulating cellular activity during and immediately after training is technically challenging. Sensory detection tasks are amenable to cellular resolution recordings in head-fixed mice, but training is typically incremental, taking multiple sessions, thereby preventing access to the early consolidation phase. To address this, we developed a single session go/no-go thermal detection task for head-fixed mice with tight control of the stimuli and rewards. Mice were trained to report a 10°C cooling stimulus delivered to their forepaw by licking for a water reward. Remarkably, mice learnt the task within ~50 trials (n = 65 mice) without any prior exposure to the stimulus or reward. Rather than a gradual improvement in reporting throughout the training session, our data show that mice performed a switch in their behavior from poor to expert performance. To examine the impact of forepaw primary somatosensory cortex (fS1) on consolidation, we went on to manipulate fS1 activity after the end of the training session. Inhibition of fS1 by local pharmacological silencing or optogenetic stimulation of GABA-ergic inhibitory interneurons <2 hours following the end of training impaired memory consolidation as measured by reduced task performance on the subsequent

day. Consolidation remained unaffected if fS1 manipulations were performed >2 hours following the end of training session. Furthermore, acoustic training and optogenetic manipulation in cortical regions outside of fS1 confirmed that these effects were specific to fS1. Moreover, optogenetic manipulation a day before the first training session or in expert mice did not affect task performance, indicating that manipulation did not alter the ability of mice to perceive thermal stimuli. Together, these data indicate that fS1 is involved in the early consolidation of thermal learning. We provide a new, rapidly learnt behavioral task to examine the cellular and circuit mechanisms of learning and sensory memory consolidation.

Disclosures: A. Udhayachandran: None. **C. Whitmire:** None. **R. Paricio-Montesinos:** None. **Z. Pauly:** None. **C. Memler:** None. **J.F. Poulet:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

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Program #/Poster #: 066.14

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R01DC018650
R00DC015014

Title: Sudden transitions from goal-directed to habitual behavior during sensorimotor learning in mice

Authors: *S. MOORE[1], Z. WANG[1], A. LEE[2], K. KUCHIBHOTLA[3];
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Abstract: Animals use different decision processes to efficiently adapt to complex environments. When operating in a goal-directed mode, animals deliberate amongst alternatives in a slow and cognitively demanding process. As the statistics of the environment become predictable, animals can shift to a faster and more automatic habit mode. To what extent are transitions between goal-directed to habitual behavior sudden or slow? The nature and timescale of these transitions remains poorly understood. Standard methods to determine whether a behavior is 'goal-directed' or 'habitual' include outcome devaluation and contingency degradation. They, however, interfere with ongoing behavior and must be implemented at discrete time points, making it difficult to identify the precise moment when a transition occurs. We hypothesized that by shifting an animals' motivation from a *need* (for survival) to a *preference* (for palatability), we could use action rate variability as a behavioral indicator of goal-directed performance (highly variable) versus habit-like performance (stably high) without impacting accuracy. We leveraged a recent protocol in which mice get *ad libitum* access to water with citric acid (CA), a less-palatable substance that fulfills the hydration needs. We compared CA mice with mice under standard water restriction (WR) protocols. Mice were trained in an

auditory go/no-go task in which they learned to lick in response to a tone for a water reward and withhold licking in response to another tone to avoid a timeout. Our data shows that mice acquired task contingencies at similar rates in all groups. Interestingly, throughout training most CA mice initially showed high action rate variability, regularly shifting from epochs of high engagement to low engagement. Surprisingly, CA mice exhibited an abrupt reduction in action rate variability, typically at the beginning of a new session, suggesting a sudden shift to habit-like performance. This shift was not evident in WR mice. Detailed analysis of behavioral microstructures demonstrated a sudden increase in consummatory licks in CA mice post transition, a signature of 'automaticity' commonly related to habits. Ongoing work aims to isolate pupil-based biomarkers of goal-directed and habitual behavior during learning. This approach allows us to identify naturalistic transitions between goal-directed and habitual behavior and provides an opportunity to uncover new insights into the neural basis of habit formation. Our data suggests that the transition from goal-directed to habit-like performance during learning is sudden, concomitant with automaticity, and may result from a winner-take-all decision process.

Disclosures: S. Moore: None. **Z. Wang:** None. **A. Lee:** None. **K. Kuchibhotla:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.15

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Mesolimbic dopamine adapts the rate of learning from action

Authors: *L. T. CODDINGTON[1], J. T. DUDMAN[2];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Janelia Res. Campus, HHMI, Ashburn, VA

Abstract: Recent success in training artificial agents and robots derives from a combination of direct learning of behavioral policies and indirect learning via value functions. Policy learning and value learning employ distinct algorithms that optimize behavioral performance and reward prediction, respectively. In animals, behavioral learning and the role of mesolimbic dopamine signaling have been extensively evaluated with respect to reward prediction; however, to date there has been little consideration of how direct policy learning might inform our understanding. Here we used a comprehensive dataset of orofacial and body movements to understand how behavioral policies evolve as naive, head-restrained mice learned a trace conditioning paradigm. Individual differences in initial dopaminergic reward responses correlated with the emergence of learned behavioral policy, but not the emergence of putative value encoding for a predictive cue. Likewise, physiologically-calibrated manipulations of mesolimbic dopamine produced multiple effects inconsistent with value learning but predicted by a neural network-based model that used dopamine signals to set an adaptive rate, not an error signal, for behavioral policy learning. This work provides strong evidence that phasic dopamine activity can regulate direct learning of

behavioral policies, expanding the explanatory power of reinforcement learning models for animal learning.

Disclosures: L.T. Coddington: None. **J.T. Dudman:** None.

Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.16

Topic: G.02. Reward and Appetitive Learning and Memory

Support: JSPS KAKENHI (#18K03182)

Title: Effects of reward delays on both sign-tracking and goal-tracking behaviors: What kind of mice can acquire more significant rewards by lever-pressing in unpredictable delayed reward trials?

Authors: *T. SATO[1], T. YAMAKUNI[2,3];
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Abstract: Sometimes the presentation of an anticipated reward may be unpredictably delayed in an instrumental learning situation. Certain behavioral patterns might facilitate or hinder reward acquisition in experimental sessions with delayed reward presentation. This study examined what behavioral parameters related more to the number of reward pellets acquired by using a small sample of well-trained C57BL/6N mice in a Skinner box. We focused on their preference between the right or left lever and their lever-pressing and nose-poke responses to a pellet under five different time lengths from lever-pressing to the delayed food pellet presentation (in seconds): 0 (no delay), 10, 20, 30, and 40. Animals could press the levers in the predefined delay duration but not acquire food pellets. Response latency to the lever presentation after the predetermined delay was also measured for each trial. The differences observed in the number of responses between the trials at 10 and 20 seconds, 20 and 30 seconds, and 30 and 40 seconds were used to estimate the frequency of lever-pressing and nose-poke responses at the second, third, and fourth 10-second intervals, respectively, within the five reward delay trials. A Spearman correlation coefficient was used to assess the relationship between the number of reward pellets acquired and other behavioral parameters, including the estimated number of the lever-pressing and the nose-poke response at each 10-second interval. In the results, the number of pellets acquired was moderately correlated with the estimated number of the lever-pressing responses at the first and second 10-second intervals. Contrastingly, it was negatively correlated with the nose-poke responses at the second 10-second interval, thus demonstrating that the animals which showed reward-less lever-pressing responses more frequently with shorter delay tended to acquire a more significant amount of food pellets. In comparison, those with reward-less nose-poke responses more regularly with more temporary delay obtained a smaller amount

of the food pellets. The number of pellets acquired was moderately correlated with the estimated number of the nose-poke responses at the fourth 10-second intervals. Those that showed the nose-poke responses more frequently with longer delay acquired a more significant amount of food pellets. Furthermore, the amount of the pellets acquired moderately positively correlated with the high preference for pressing either of the two levers. Therefore, the side preference of either lever could have some advantage in collecting more pellets.

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Poster

066. Reward and Appetitive Learning

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Support: NIH Grant DA042111
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Title: Schedule-dependent production of stereotyped sequences of actions

Authors: *E. G. FOLLMAN[1,2], M. CHEVÉE[2], C. J. KIM[2], A. R. JOHNSON[2], J. TAT[2], E. S. CALIPARI[2,3,4,5,6];
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Abstract: Automatic routines sacrifice flexibility for efficiency, a tradeoff which can become pathological in disease states such as obsessive-compulsive disorder (OCD) and substance use disorder (SUD). Understanding maladaptive behavior in the context of disease states requires an understanding of the parameters that control how actions become more stereotypical. Here, we investigated the ability of different operant conditioning contingencies to promote reproducible sequences of lever presses. We trained four groups of mice on distinct Fixed Ratio 5 (FR5) lever pressing procedures. In one procedure, lever presses only counted toward a target of five if they were executed after the previous reinforcer had been collected (*w/ MustCollect*, n=6). In a second procedure, a light cue signaled reinforcer delivery (*w/ LightCue*, n=8). A third group of mice trained with both conditions (*w/ LightCue&MustCollect*, n=9). For the fourth cohort, the variance of the inter response intervals (IRI) in a sequence of five presses needed to be below a dynamic target variance to trigger reinforcer delivery (*LowVariance*, n=14). Firstly, we found that presenting a light cue to signal reinforcer delivery was critical for behavioral performance. Even though all groups learned the task, the absence of this cue diminished learning rates and

impaired the ability to cluster lever presses into bouts. We also found that reinforcing low variance sequences was not more effective than a traditional FR5 schedule in promoting reproducible behavior. While the mean IRI variance within reinforced sequences decreased during training for both the *w/ LightCue&MustCollect* and *LowVariance* cohorts, there was no significant difference between the changes in these two groups. Further, we found that the *LowVariance* cohort successfully generated clustered lever presses but failed to produce sequences of five presses. Lastly, we investigated whether individual lever press movements become more reproducible with training. By tracking lever displacement, we found that the pairwise correlation coefficient between presses increased from midway to late in training and that mice trained on *w/ LightCue&MustCollect* had more reproducible presses than mice trained on the *LowVariance* paradigm. Nonetheless, the *LowVariance* mice continued to respond despite reward devaluation, which makes this cohort a useful comparison to schedules known to induce habitual behavior, such as random interval training. Together, our findings provide insights into the parameters of behavioral training that promote reproducible sequences and serve as a roadmap to investigating the neural substrates of automatic behaviors.

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Poster

066. Reward and Appetitive Learning

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 066.18

Topic: G.02. Reward and Appetitive Learning and Memory

Title: Differential hippocampal Arc protein synthesis in social memory tasks Differential hippocampal Arc protein synthesis in social memory tasks

Authors: *V. DIAZ[1], P. GARCIA- DE LA TORRE[2], L. MENDOZA-VIVEROS[3], M. ALCÁNTARA-GRESS[4], K. R. GUZMAN[1];
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Abstract: Differential hippocampal Arc protein synthesis in social memory tasks.Vanessa Díaz-Medina[1], Paola García de la Torre[2], Lucia Mendoza Viveros[3], Margarita Alcántara Gress[1], Kioko R. Guzmán Ramos[11]Health Sciences Department, Universidad Autónoma Metropolitana, Unidad Lerma CP 52005, [2] Unidad de Investigación en Enfermedades Neurológicas, Centro Médico SXXI, Mexico City, Mexico.
Social memory reflects different cognitive and behavioral processes, such as the ability to recognize a relative or a conspecific, and is essential for group survival, social hierarchy, territorial defense, interspecies recognition, and group maintenance. The molecular mechanisms that underly consolidation of social recognition memory are poorly understood. The activity-

regulated cytoskeleton-associated protein (Arc, also known as Arg3.1) participates in the plastic changes linked to the long-term stabilization of memory traces and has also been implicated in familiarization processes. In the present study, we used two social tasks in male mice: The social recognition test and social transmission of food preference to assess the synthesis patterns of Arc protein within the dorsal hippocampus during the socialization phase using immunohistochemical techniques. We found Arc+ cells differentially activated within the hippocampus, being more abundant within CA1 during social transmission of food preference and within CA3 during the socialization phase of the social recognition task. These results indicate that these hippocampal regions are involved in social memory under distinct conditions suggesting that increased social experience, such as the involved in the social transmission of food preference requires CA1 plasticity, whereas CA3 plasticity appears to be crucial for rapid social information encoding. Keywords: Social Memory, Social Recognition, Protein Arc, ConsolidationThe authors declare no conflict of interest.

Disclosures: V. Diaz: None. **P. Garcia- De la Torre:** None. **L. Mendoza-Viveros:** None. **M. Alcántara-Gress:** None. **K.R. Guzman:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.01

Topic: G.05. Mood Disorders

Support: NIGMS Grant SC3GM130467
NIGMS Grant SC2GM109811

Title: Effects of juvenile ketamine and/or psychological stress on spatial memory in mice

Authors: *I. GARCIA CARACHURE, O. LIRA, S. IÑIGUEZ;
Psychology, The Univ. of Texas At El Paso, El Paso, TX

Abstract: Effects of juvenile ketamine and/or psychological stress on spatial memory in adult mice Garcia-Carachure, Israel; Lira, Omar, and Iñiguez, Sergio D Department of Psychology, The University of Texas at El Paso, El Paso, TX Ketamine is currently being used for the management of treatment resistant depression in adolescent patients. However, the possible long-term effects of ketamine exposure during adolescence have not been assessed. Thus, we examined whether repeated exposure to concomitant ketamine and/or psychological stress, during the adolescent stage of development, results in long-lasting spatial memory alterations in male and female C57BL/6 mice (N=36; 9 per group, N=44; 11 per group, respectively). Specifically, male and female postnatal day (PD)-35 mice underwent 10 days of vicarious defeat stress (VDS; a form of psychological stress) with or without ketamine exposure (20 mg/kg; PD35-44). Once mice reached adulthood (PD70) separate groups were assessed for spatial memory performance adopting a water maze task. Our results suggest that singular pre-

exposure to ketamine or VDS increased the latency (sec) to locate the escape platform in adult male, but not female, mice – revealing that ketamine, like psychological stress, induces an enduring spatial memory impairment in males only. However, history of concomitant ketamine and VDS prevented spatial memory impairment in adulthood. Together, our findings suggest that ketamine, as a prophylactic treatment for adolescent psychological stress-induced illnesses, does not lead to long-term changes in spatial memory. However, juvenile recreational ketamine-use, like psychological stress history, results in an enduring spatial memory deficit in a male-specific manner.

Disclosures: I. Garcia Carachure: None. **O. Lira:** None. **S. Iñiguez:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

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Program #/Poster #: 067.02

Topic: G.05. Mood Disorders

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CSULB/CLA
CSUPERB

Title: Liraglutide Attenuates Methamphetamine Preference in Female Adolescent Rats

Authors: *F. S. OMERJEE[1], G. C. VERDUZCO[2], H. C. PONCE[3], A. R. ZAVALA[4]; [1]California State University, Long Beach, Artesia, CA; [2]Psychology, California State University, Long Beach, Mira Loma, CA; [3]Psychology, California State University, Long Beach, Torrance, CA; [4]Psychology, California State University, Long Beach, Long Beach, CA

Abstract: Methamphetamine (METH) abuse is a serious public health concern. In the last decade, deaths attributed to METH have increased dramatically in the United States. METH use is especially damaging during adolescence, as early initiation leads to even poorer treatment outcomes and a decreased ability for patients to stay sober. Interestingly, activation of Glucagon-Like Peptide-1 Receptors (GLP-1R) reduces the rewarding and reinforcing effects of psychostimulant drugs, like cocaine, suggesting a potential therapeutic target for psychostimulant abuse. Liraglutide is a Glucagon-Like Peptide-1 Receptor (GLP-1) agonist drug currently approved by the FDA to treat Type II diabetes. However, Liraglutide's effect on the rewarding effects of METH is unknown. Thus, using the Conditioned Place Preference (CPP) paradigm, a validated animal model of reward, we examined the hypothesis that Liraglutide would reduce the rewarding effects of METH in adolescent female Sprague Dawley rats, evident as a decrease in the acquisition of METH-induced CPP. On days 1 and 10 of the 10-day CPP procedure, rats were tested in 15-minute sessions to examine their place preference. On days 2 to 9, rats were

conditioned with either METH (0.0, 0.3, or 1.0 mg/kg, intraperitoneally) one day and saline on another day for 30 minutes a day. This two-day procedure was repeated over the next six days. During the METH conditioning sessions, rats were pretreated with Liraglutide (0.0 or 0.1 mg/kg) or saline 60 minutes before being conditioned with METH. The results of our study indicate that Liraglutide reduces the rewarding properties of METH in female adolescent rats. These findings provide valuable insights into the ability of Liraglutide to reduce the rewarding effects of METH. Future studies should focus on investigating the Liraglutide's effect on the self-administration of methamphetamine in rats to further delineate its potential viability as a pharmacological treatment for METH addiction.

Disclosures: F.S. Omerjee: None. **G.C. Verduzco:** None. **H.C. Ponce:** None. **A.R. Zavala:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Topic: G.05. Mood Disorders

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CSULB/CLA
CSUPERB

Title: Activation of 5-HT1B receptors attenuates the acquisition of nicotine reward in adolescent male rats

Authors: *T. A. GONZALEZ-GUTIERREZ, A. K. GARCIA, A. R. ZAVALA;
California State University, Long Beach, Long Beach, CA

Abstract: Activation of serotonin (5-HT)1B receptors decreases the rewarding and reinforcing effects of stimulant drugs, such as cocaine and methamphetamine. In the present study, we examined the hypothesis that administration of CP 94,253, a 5-HT1B receptor agonist, would reduce nicotine preference in adolescent male rats using a 10-day Conditioned Place Preference (CPP) procedure, a well-established animal model of drug reward. On postnatal day (PD) 28, baseline preference for a two-sided apparatus was assessed during a 15 min session. In two-day cycles, rats received an injection of CP 94,253 (0 or 5.6 mg/kg) 15 min before the administration of nicotine (0, 0.2, or 0.6 mg/kg) on one day and saline administration on the other day before being confined to one side of the two-chamber apparatus for 15 min. This two-day cycle was repeated over the next 6 days. On day 10, the preference for the nicotine-paired chamber was assessed for 15 min. Rats exhibited nicotine-induced CPP when conditioned with either 0.2 or 0.6 mg/kg of nicotine. Administration of CP 94,253 (5.6 mg/kg) before nicotine (0.2 or 0.6

mg/kg) resulted in a decreased preference for the nicotine-paired compartment. The present findings demonstrate that activation of 5-HT1B receptors with CP 94,253 attenuated the acquisition of nicotine-induced CPP in male adolescent rats. Overall, these findings further add to a growing body of literature that points to the 5-HT1B receptor as a pharmacological target for treating psychostimulant addiction.

Disclosures: T.A. Gonzalez-Gutierrez: None. **A.K. Garcia:** None. **A.R. Zavala:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.04

Topic: G.05. Mood Disorders

Support: NIGMS Grant SC2GM109811
NIGMS Grant SC3GM130467

Title: Effects of ketamine and/or psychological stress during adolescence on hippocampal AKT-mTOR protein expression in adulthood

Authors: *A. THEMANN, S. D. INIGUEZ;
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Abstract: Depression is a pervasive psychiatric illness that negatively affects the adolescent population. Currently, ketamine, a general anesthetic, is being used off-label to manage juvenile treatment-resistant depression, given its recent classification as a fast-acting antidepressant. However, the potential long-term molecular changes of early-life ketamine exposure have not been thoroughly accessed. This is surprising, given that preclinical work indicates that early-life exposure to ketamine alters responses to stress and reward-related stimuli in adulthood. To address this gap in the literature, we examined if juvenile exposure to vicarious defeat stress (VDS; a form of psychological stress) and/or ketamine can lead to alterations of the AKT-mTOR (protein kinase b-mammalian target of rapamycin) signaling pathway in the hippocampus of adult C57BL/6 mice. Specifically, separate groups of male adolescent mice [postnatal day (PD)-35] were exposed to VDS, ketamine (20 mg/kg), or both, for 10 consecutive days (PD35-44). Once mice reached adulthood (PD70) hippocampal tissue was dissected, and subsequent immunoblot analyses were adopted to evaluate protein expression of AKT-mTOR-related proteins. Our results indicate that total levels of AKT, mTOR, and Raptor, were significantly decreased in the hippocampus of adult mice with history of singular exposure to psychological stress (VDS) or ketamine. Interestingly, no differences in any of the proteins evaluated were noted between non-stressed controls and mice exposed to concomitant ketamine and VDS. Collectively, these data indicate that juvenile ketamine exposure, like psychological stress, decreases hippocampal AKT-mTOR-related signaling in adulthood. As such, this work provides

awareness of the potential long-term molecular consequences associated with juvenile ketamine exposure.

Disclosures: **A. Themann:** None. **S.D. Iniguez:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

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Program #/Poster #: 067.05

Topic: G.05. Mood Disorders

Title: Establishment of a clinical setting-associated behavioral model of ECT and investigation of the mechanism of action of ECT with focus on astrocytes

Authors: *K. MIYAKO, Y. KOGA, N. KAJITANI, S. BOKU, M. TAKEBAYASHI;
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Abstract: Background: Electroconvulsive therapy (ECT) has superior efficacy and rapid response compared with pharmacotherapy and is often used for treatment-refractory depression. However, the mechanism of action of ECT remains unclear. Moreover, a clinical setting-associated behavioral model of ECT, in which depression-like behaviors showed recovery by electroconvulsive stimulation (ECS), has not yet been established. Therefore, the present study first established a clinical setting-associated behavioral model of ECT with corticosterone (CORT)-administrated mice, a common mouse model of depression, and ECS. Recent studies have suggested the potential role of astrocytes in the pathophysiology of depression. Therefore, as the next step, we isolated astrocytes from the hippocampus of our ECT mouse model and performed RNA-seq with isolated astrocytes-derived RNA to identify molecules and signaling pathways involved in the mechanism of action of ECT in astrocytes.
Methods: 35 µg/mL aqueous solution of CORT was administrated to 8-week-old male C57BL/6J mice by ad libitum feeding for 6 weeks. During the last 2 weeks of corticosterone administration, ECS was performed under anesthesia at 30 mA, 1000 Hz, 1 s via an ear clip. According to clinical practice, ECS was administrated once a day and three times a week. After the course of ECS, sucrose preference test (SPT) and novelty suppressed feeding test (NSFT) were performed to evaluate depression-like behaviors. After these behavioral tests, the hippocampus was dissected from the mice brain. The hippocampal astrocytes were isolated using FACS -based method. Random displacement amplification sequencing (RamDA-seq) (Hayashi et al., 2018) was performed to comprehensively analyze gene expression of astrocytes at a small-scale (100 cells).
Results: CORT-administrated mice presented with depression-like behavioral changes in both SPT and NFST, which were significantly recovered by ECS. The isolated astrocytes expressed astrocyte markers, such as Atp1b2 and Aqr4, but not the markers of neurons, oligodendrocytes, and microglial cells. The in silico analyses based on the data of RamDA-seq showed that the genes and signaling pathways related to cytoskeleton, angiogenesis, and cytokines may be

associated with the recovery effects of ECT on depression-like behaviors.
Conclusion: Herein, we established a clinical setting-associated mice model of ECT. Using this model, the genes and signaling pathways associated with the antidepressive mechanisms of ECT were identified in hippocampal astrocytes. The findings of the present study are expected to contribute to the elucidation of the mechanism of action of ECT.

Disclosures: K. miyako: None. **Y. koga:** None. **N. kajitani:** None. **S. boku:** None. **M. Takebayashi:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.06

Topic: G.05. Mood Disorders

Title: Comprehensive analysis of gene expression in hippocampal astrocytes in a model of depression

Authors: *Y. KOGA, K. MIYAKO, N. KAJITANI, S. BOKU, M. TAKEBAYASHI;
Dept. of Neuropsychiatry, Fac. of Life Sci., Kumamoto Univ., Kumamoto, Japan

Abstract: Background: Hippocampal astrocytes have been implicated in the pathophysiology of depression. Previous in vitro studies have reported that amitriptyline (AMI), a prototypic antidepressant, induces neurogenesis via neurotrophic factors produced by direct action on astrocytes (Kajitani et al., 2012; Boku et al., 2013). However, the molecular mechanisms in hippocampal astrocytes that are involved in mediating antidepressant behaviors are not well understood. In this study, we comprehensively analyzed gene expression patterns in hippocampal astrocytes from corticosterone (CORT)-treated mice, a mouse model of depression.
Methods: Eight-week-old male C57BL/6J mice were treated with CORT (35 mg/L in bottles, available ad libitum in the drinking water) for 7 weeks, with or without AMI (10 mg/kg, intraperitoneally) for the final 3 weeks of the treatment period. A novelty-suppressed feeding test was performed to evaluate depression-like behavior. After behavioral testing, hippocampal astrocytes were isolated using fluorescence-activated cell sorting. Random displacement amplification sequencing (Hayashi et al., 2018) was performed to comprehensively analyze gene expression patterns in a small number of astrocytes (100 cells). Differentially expressed genes (DEGs) between groups were determined using DESeq2.
Results: CORT-treated mice showed depression-like behavior, which was reversed by AMI treatment. There were 553 DEGs between control and CORT-treated mice and 284 DEGs between CORT and AMI-treated mice. Upstream regulators of the DEGs using Ingenuity Pathway Analysis predicted that TCF7L2 was inhibited in CORT-treated mice and activated in AMI-treated mice.
Conclusion: Herein, we identified hippocampal astrocytic genes and upstream regulators

(TCF7L2) associated with antidepressant functions. Our findings help elucidate the mechanisms of antidepressant behavior targeting astrocytes.

Disclosures: Y. Koga: None. **K. Miyako:** None. **N. Kajitani:** None. **S. Boku:** None. **M. Takebayashi:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.07

Topic: G.05. Mood Disorders

Support: JSPS KAKENHI 15K09805
JSPS KAKENHI 18K07556

Title: Neonatal maternal separation-induced alternation of miRNAs andtheir possibility as biomarkers of major depressive disorder

Authors: *S. BOKU[1], H. TODA[2], M. KOGA[2], N. KAJITANI[1], M. TAKEBAYASHI[1]; [1]Kumamoto Univ. Fac. of Life Sci., Kumamoto Univ. Fac. of Life Sci., Kumamoto, Japan; [2]Natl. Def. Med. Col., Natl. Def. Med. Col., Saitama, Japan

Abstract: As major depressive disorder (MDD) impairs a patient's life over a long period, early detection of MDD is essential for minimization of loss and suffering due to MDD. However, early detection of MDD remains difficult because of the lack of clinically useful biomarkers of MDD. A lot of studies have shown that early-life stress (ELS) is involved in the vulnerability and treatment-resistance of major depressive disorder in adults. In addition, recent studies have reported that miRNAs may be involved in the biological effects of ELS. These suggest that ELS-associated miRNA may be a potential biomarker of MDD. With neonatal maternal separation (NMS) in rats, a common animal model of ELS, we performed the microarray analysis of miRNAs derived from peripheral blood of rats and identified four miRNAs which were significantly altered in NMS rats and expressed in both rats and human. As a next step, receiver operating characteristic (ROC) curve analyses were performed to estimate the possibility of each miRNA and combinations of the identified four miRNAs as biomarkers of MDD with peripheral blood of MDD patients (N=64) and healthy controls (N=75). The area under the curve (AUC) of each miRNA was under 0.7000, which means that each miRNA has only limited diagnostic power of MDD. However, AUC of the combination of these four miRNAs was 0.9444 with sensitivity of 0.8750 and specificity of 0.9200, which means that the combination of these four miRNAs has the high diagnostic power of MDD. These results suggest that the combination of the four ELS-associated miRNAs is expected as a clinically useful biomarker of MDD.

Disclosures: S. Boku: None. **H. Toda:** None. **M. Koga:** None. **N. Kajitani:** None. **M. Takebayashi:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.08

Topic: G.05. Mood Disorders

Support: NIH Grant R01NS116594
McKnight Memory and Cognitive Disorders Award

Title: Prefrontal parvalbumin interneurons support neural encoding during behavioral adaptation in mice

Authors: *C. JOHNSON-CRUZ, V. SOHAL;
Univ. of California San Francisco, San Francisco, CA

Abstract: Rodents, primates, and humans use their medial prefrontal cortex (mPFC) to adapt behavior to changing environments. Inhibitory cells in the mPFC expressing parvalbumin, or parvalbumin-positive interneuron (PVIs), are important when adopting new behavioral strategies in cognitive tasks. Their specific role in shaping the activity of mPFC neurons during cognitive flexibility is little understood. To study this, we used a transgenic mouse line with impaired cognitive flexibility due to disrupted PVI development. Using one-photon micro-endoscopic calcium imaging, we record single-cell neural activity in the mPFC of these mice before and after they receive a benzodiazepine improving their performance on a task requiring cognitive flexibility. We compared these mutant mice to wild type mice. We found that this behavioral rescue accompanied changes in the single-cell and population-level neural encoding. Treatment increased the proportion of mPFC neurons encoding trial phases above chance when mice were learning a new task rule, while the per-neuron encoding strength remained unchanged. The similarity of population activity during similar types of trials increased in mutant mice after treatment. Finally, treatment altered the performance of neural networks trained to predict task features from mPFC neural activity during the task. Understanding the neural locus of cognitive impairments associated with interneuron deficits could help better target treatments for psychiatric disorders like schizophrenia, that affect parvalbumin-positive interneurons.

Disclosures: C. Johnson-Cruz: None. **V. Sohal:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.09

Topic: G.05. Mood Disorders

Support: SFARI
UCSF Dolby Family Center for Mood Disorders

Title: Encoding of behavioral states in the medial prefrontal cortex is disrupted in a Tbr1-KO mouse model of autism

Authors: *M. TURNER, V. SOHAL;
Univ. of California, San Francisco, San Francisco, CA

Abstract: Recently, much work has been done to identify neural activity patterns underlying behaviors. How activity is disrupted in neuropsychiatric conditions is less well understood, and could shed light on the features of neural activity that are critical for driving normal behavior. One way to explore this is to record activity specifically from neuron sub-types that have been implicated in human disorders. Exome sequencing in humans with autism spectrum disorder (ASD) has revealed a set of high-confidence risk genes which are enriched in deep cortical layers, particularly in prefrontal cortex (PFC). One such gene is *Tbr1*, which encodes a transcription factor critical for cortical neuron development and function. Here, a mouse model of ASD was generated by crossing *Tbr1*-floxed mice with *Rbp4-cre* mice, which leads to deletion of *Tbr1* specifically in layer 5 (L5) pyramidal neurons. These conditional knockout mice (*Rbp4-cre*$^{+/-}$::*Tbr1*$^{f/f}$ *or* cKO) displayed abnormal social behavior, measured by time spent exploring a novel juvenile mouse, and abnormal anxiety-related avoidance behavior, measured by time spent exploring the open arms of the elevated plus maze (EPM), as compared to wild-type littermates (*Rbp4-cre*$^{+/-}$::*Tbr1*$^{+/+}$). To explore how this genetic manipulation altered behaviorally associated neural activity, microendoscopic calcium imaging was employed. Using a *Cre*-dependent, genetically encoded calcium indicator (AAV9-*hSyn*-DIO-*GCaMP7f*), activity of L5 pyramidal neurons in the mPFC was recorded in WT (n = 6 mice, 324 neurons) and cKO (n = 4 mice, 205 neurons) mice during a social interaction assay and in the EPM. Generally, the activity of cKO neurons was found to be significantly lower in both behavioral assays. To identify neuronal ensembles associated with behavior, a neural network classifier was utilized. For the social assay, an ensemble associated with the first exposure to a juvenile mouse was identified. In WT mice, these social ensembles were reactivated during a second exposure to the juvenile, while in cKO mice social ensemble activity decreased significantly. For the EPM, ensembles associated with exploration of the open arms and the closed arms were identified. In WT mice, Open-arm ensembles predicted subsequent open arm exploration, i.e., they increased activity immediately prior to center entries during closed to open arm transitions. This pre-transition activity was not seen in the Open ensembles of *Tbr1*^{L5}KO mice. Altogether, these data indicate that L5 neurons of the mPFC encode behavioral states related to socialization and anxiety, and that *Tbr1* is critical for the appropriate recruitment of these neuronal ensembles during behavior.

Disclosures: M. Turner: None. **V. Sohal:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.10

Topic: G.05. Mood Disorders

Support: UCSF Dolby Family Center for Mood Disorders

Title: Circuit dissection of a cross-species biomarker for emotional state

Authors: *A. D. JACKSON, V. S. SOHAL;
Dept. of Psychiatry, UCSF, San Francisco, CA

Abstract: Under normal conditions, anxiety acts as an instructive signal that promotes adaptive avoidance behaviors which assist individuals navigating through an uncertain world. However, these signals can also become maladaptive, producing excessive and inappropriate levels of avoidance characteristic of many anxiety disorders. Collectively, anxiety disorders comprise the most common psychiatric disorder in the world and result in a high burden of disease for those affected. The identification of robust and valid biomarkers of psychiatric disorders is a crucial tool for improvements in their prevention, diagnosis and treatment. Emotional responses arise from limbic circuits including the hippocampus and amygdala. In the human brain, beta-frequency (13-30Hz) communication between these structures correlates with self-reported mood and anxiety (Kirkby et al., 2018. ***Cell***. 175:1688-1700). However, both the physiological underpinnings and the functional significance of this biomarker as a readout vs. driver of emotional state remain unknown. Here we show that beta-frequency communication between the ventral hippocampus and basolateral amygdala also predicts anxiety-related behavior in mice on both long timescales (~30 min) and immediately preceding behavioral choices (~seconds). Using genetically encoded voltage indicators, we identified that this biomarker reflects synchronization between somatostatin interneurons across both structures. Synchrony between these neuronal populations dynamically predicts approach vs. avoidance, and optogenetically inducing or disrupting this synchrony is sufficient to bidirectionally modulate risk assessment behaviors. Therefore, via back-translation we show that a human biomarker is not only a predictor but also a causal determinant of emotional state and identify its underlying circuit mechanisms which may provide future therapeutic targets for anxiety disorders.

Disclosures: A.D. Jackson: None. **V.S. Sohal:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.11

Topic: G.05. Mood Disorders

Title: Investigating the role of CCR5 in mood and behavior

Authors: *K. HUMMEL[1], G. A. GRECO[3], K. CONANT[2];
[1]Georgetown Univ. Med. Ctr., Washington, DC; [2]neuroscience, Georgetown Univ. Med. Ctr., washington, DC; [3]Georgetown Univ., Washington, DC

Abstract: C-C motif chemokine receptor 5 (CCR5) is a G-protein coupled receptor that is widely expressed in the central nervous system. CCR5 is believed to be critical for learning and temporal control of memory. Activation of CCR5 is thought to close the window for memory linking. Knockdown of CCR5 is associated with better long-term potentiation (LTP), enhanced MAPk/CREB signaling, and improved plasticity. While there is strong data to support the role of CCR5 in memory and cognition, its role in mood and behavior unclear. In this study, we use battery of behavioral assays to examine the role of CCR5 in anxiety and mood related behavioral endpoints, as well as locomotion, and contextual memory. In a comparison of normally housed, age-matched wild-type (WT) controls (n=8 males), we found that CCR5 knockout (KO) mice (n=8 males) show improved mood related behavioral endpoints. In the Elevated Plus Maze, we saw both decreased anxiety and increased exploratory behavior in CCR5 KO mice as compared to controls. Additionally, we found that in a Novelty Suppressed Feeding paradigm, CCR5 knockout mice showed greater food motivation and decreased anxiety. In a contextual Fear Conditioning test, CCR5 KO mice showed significantly improved recall compared to WT controls. Locomotor assays did not show significant differences between groups, suggesting that results are not due to differences in activity levels. Additionally, we conducted Western blot analyses of glycogen synthase kinase-3 beta (GSK-3β), which, in its phosphorylated form, is positively correlated with mood stability. We found that when compared to WT controls, CCR5 KO mice show increased levels of phospho-GSK-3β. These results support the possibility that CCR5 expression may be an important regulator of mood and behavior. Ongoing studies include those related to plasticity-associated structural and functional changes in the hippocampus and cortex, as well as measure of neuronal oscillations linked to improved mood. Future studies will be conducted on female CCR5 KO and WT mice as well, in order to assess any sex-related differences.

Disclosures: K. Hummel: None. **G.A. Greco:** None. **K. Conant:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.12

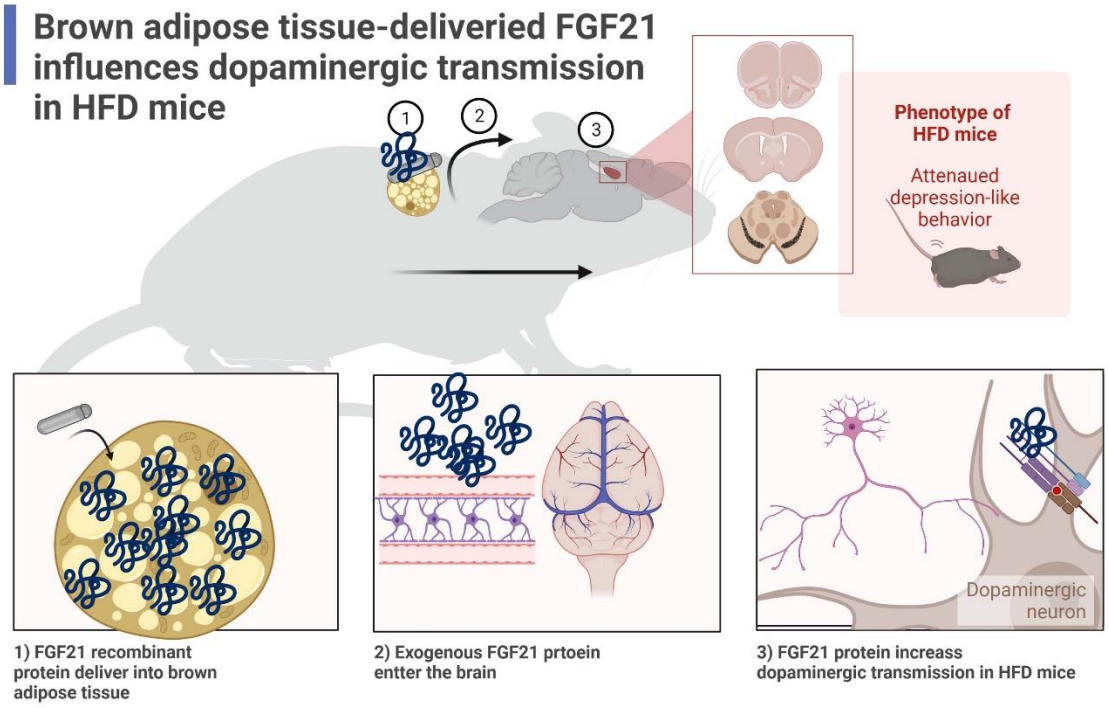
Topic: G.05. Mood Disorders

Support: MOST107-2320-B-006-014
MOST108-2320-B-006-0002
MOST 109-2320-B-006-018-MY3

Title: Correct dimerization of FGF21 receptors in the VTA dopaminergic neurons attenuates depression-like symptoms via activation of brown adipocyte-K_{ATP} channels

Authors: *P.-C. CHEN, **Y.-Y. KUO**;
Natl. Cheng-Kung Univ., Natl. Cheng Kung Univ. Col. of Med., Tainan, Taiwan

Abstract: Cumulating epidemiological evidence has indicated the correlation between obesity and depression. Our previous publication demonstrated that ATP-sensitive potassium (K_{ATP}) channels are functionally expressed in the brown adipose tissue (BAT). Inhibition of BAT-K_{ATP} channels improves metabolic syndromes and reduces depressive symptoms. BAT act as an endocrine organ that releases a variety of factors that have been indicated to have beneficial effects on neurons, such as fibroblast growth factor 21 (FGF21) and brain-derived neurotrophic factor (BDNF), and nerve growth factor (NGF). Therefore, we investigated the anti-depressant effect of FGF21. High fat diet-fed mice were implanted with a miniosmotic pump containing FGF21 protein into the interscapular BAT for two weeks, followed by FGF21 (3 mg/kg) intraperitoneal administration for four consecutive days. FGF21 treatment reduced metabolic disorder symptoms, improved depressive-like behaviors, and restored mesolimbic dopamine projection. FGF21 treatment recovered the dysregulation of FGF21 receptors, comprising FGFR1 and a co-receptor β-klotho, in the ventral tegmental area. Next, we investigated if the closure of K_{ATP} channels by K_{ATP} channels blocker (glibenclamide, GB) administration increases FGF21 protein. GB treatment increased FGF21 mRNA level and promoted FGF21 releasing from the BAT. GB corrected dimerization of FGF21 receptors in the VTA dopaminergic neurons significantly attenuated depression-like symptoms.



Disclosures: **P. Chen:** None. **Y. Kuo:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.13

Topic: G.05. Mood Disorders

Title: Effects of chronic caffeine administration on depressive behaviors and neuron morphology in long evans rats

Authors: *J. J. CORTRIGHT, L. LANDAETA, D. ROEVER, C. PETERSON, S. PECK, T. ST. VINCENT, J. ARNT, D. G. EHLINGER;
Univ. of Wisconsin River Falls, Univ. of Wisconsin River Falls, River Falls, WI

Abstract: Caffeine, the most consumed psychoactive drug, appeals to populations with psychiatric histories due to its readily available nature and alertness exerting effects. Given the prevalence of psychiatric conditions such as major depressive disorder (MDD), in which symptoms such as lethargy are commonplace, a potential relationship between caffeine administration and attenuation of MDD behaviors has been suggested. In particular, in groups exposed repeatedly to stress, a frequent trigger of MDD, caffeine intake surges. This has led to an observed inverse correlation between caffeine intake and MDD behaviors. Clinically, the current state regarding caffeine administration as part of MDD treatment remains limited, but established. When administered in the presence of selective serotonin reuptake inhibitors (SSRIs), caffeine has shown to augment the therapeutic effects in human and animal models. However, the mechanism of action by which caffeine specifically exerts these effects remains unknown. The purpose of our study was to expand upon previous literature and to elucidate these mechanisms by combining behavioral and morphological analysis. Subjects were exposed to 28 days of randomized stressors during early adulthood including forced swim, cage tilt, wet bedding, mild restraint and restriction of food and water. Control animals were housed in pairs, while animals exposed to randomized stress were housed in isolation. Following stress exposure, a subset of control and stress-exposed animals were orally administered caffeine (15 - 20 mg) twice daily for five weeks. Subjects were then tested for latency in a forced swim test and hot plate test, for motivation in a radial arm maze, for lethargy in an open field test, and for anhedonia using sugar pellets. Following testing, rats were deeply anesthetized, transcardially perfused with saline, and their brains were placed in a Golgi cox solution for subsequent dendritic spine density analysis in the medial prefrontal cortex (mPFC), nucleus accumbens (NAcc), hippocampus and basolateral amygdala (BAL). To date, an attenuation in latency in the forced swim test and hot plate test has been found in stress-exposed animals which had receive caffeine treatment compared to controls. Currently, the effects on depressive behaviors in other tests and dendritic spine density are being analyzed. Collectively, these findings hold significance in that they build on recent research that has aimed to link caffeine intake with the attenuation of depressive behaviors.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.14

Topic: G.05. Mood Disorders

Title: Behavioral effects of the monoamine stabilizer (-)-OSU6162 in the forced swim and context conditioned fear tests

Authors: *D. ATANASOVSKI, S. M. HAGSÄTER, L. SANDGREN, M. MYREHAG, E. ERIKSSON;
Dept. of Pharmacol., Univ. of Gothenburg, Gothenburg, Sweden

Abstract: Background: Augmentation-therapy with atypical antipsychotics is a common and effective treatment approach for patients suffering from major depressive disorder but not responding to first-line treatment. While these pharmaceuticals may be excellent at managing symptoms against their primary indication of psychosis, the side effects accompanying their use can be plentiful and are often cause for discontinuation. The monoamine stabilizer (-)-OSU6162 displays certain pharmacological similarities to atypical antipsychotics but a clinical profile with less adverse events and better compliance, which warrants exploration of its therapeutic properties against anxiety and depressive disorders. **Aims:** To explore the efficacy of the monoamine stabilizer (-)-OSU6162 in preclinical animal models of anxiolytic and antidepressant effect; the context conditioned fear and forced swim tests respectively. **Method:** Anxiolytic-like effects of (-)-OSU6162 were assessed in 10-week-old male Sprague Dawley rats (n = 9-10) as expression of context-conditioned freezing one week following exposure to a conditioning chamber in which they had received mild electrical foot shocks. Antidepressant-like effects were assessed in 9-week-old male Wistar rats (n = 6-12) as reversal of immobility in the forced swim test following induction of anhedonia-like behavior as measured by the sucrose preference test following seven weeks of chronic mild stress. Comparisons between groups were performed with ANOVA or Kruskal-Wallis tests followed by Fisher's least significant difference or Uncorrected Dunn's test respectively. **Results:** (-)-OSU6162 administered to rats in doses of 10 mg/kg ($p=0.023$) and 30 mg/kg ($p=0.019$) reduced context conditioned freezing. (-)-OSU6162 also decreased duration of immobility in the forced swim test in doses of 10 mg/kg ($p=0.029$) and 30 mg/kg ($p=0.005$) in rats subjected to chronic mild stress. **Discussion & Conclusion:** (-)-OSU6162 showed anxiolytic- and antidepressant-like efficacy in the context-conditioned freezing and forced swim tests. Atypical antipsychotics are prescribed as mono- or adjunctive therapy for difficult-to-treat depressive and anxiety disorders, but lack of tolerability is cause for discontinuation. The pharmacological similarity between these compounds and the better tolerated monoaminergic stabilizer (-)-OSU6162, in combination with the results described above, imply a therapeutic prospect for use of (-)-OSU6162 against mood disorders.

Disclosures: D. Atanasovski: None. **S.M. Hagsäter:** None. **L. Sandgren:** None. **M. Myrehag:** None. **E. Eriksson:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.15

Topic: G.05. Mood Disorders

Support: CAPES
FAPESP

Title: Maternal separation model for induction of postpartum depression in lactating rats cause few behavioral and neurochemical alterations in the offspring

Authors: *L. D. PANTALEON[1], G. ABREU[1], J. ZACCARELLI MAGALHÃES[1], B. RIBEIRO[2], C. MUNHOZ[2], M. MANES[1], M. LIMA[3], J. MIGLIOLI[3], J. FLÓRIO[1], E. RICCI[1], A. FUKUSHIMA[1], H. SPINOSA[1];
[1]Univ. de Sao Paulo, Univ. de São Paulo, São Paulo, Brazil; [2]Univ. Presbiteriana Mackenzie, São Paulo, Brazil; [3]Ctr. Universitário das Américas, São Paulo, Brazil

Abstract: Postpartum depression is a serious mentally disabling illness that affects 1 in 7 women worldwide. Several studies show that this disease in the first six months after birth can lead to behavioral and cognitive alterations in the child development in the first infancy through adolescence. These changes also occur in animal models, such as rodents, since the dam-offspring bond is crucial for the offspring development and maternal behavior alterations can lead to permanent effects in the progeny. Thus, the aim of this study was to evaluate the behavior and neurochemistry of offspring of dams induced to postpartum depression through the maternal separation model in the infancy and adulthood. Sixteen lactating rats were subdivided into 2 groups, control and MS (n=8 animals/group). MS dams were separated from their litter for 3h daily from postnatal day (PND) 2 to 12. The behavioral evaluation was performed in the infancy (open field and light/dark box tests on PND 21) and in adulthood (open field, light/dark box and social interaction tests on PND 60 to 62). On PNDs 21 and 62 animals were euthanized by decapitation for collection of hippocampus and prefrontal cortex for monoamines analyses. The results showed that: 1) MS male offspring had higher number of crossing in the light/dark box test in the infancy compared to control (t=0.0025) but not in the adulthood; 2) MS female offspring had lower social interaction time than control (t=0.0169); and 3) MS male offspring had lower DOPAC/DA rate compared to control (t=0.0083) in adulthood. The higher number of crossing in the light/dark box can be explained by the higher sensibility to stress caused by the maternal separation model, as well as a higher probability for these animals to develop depression and anxiety. The social interaction results corroborate other studies that show that the maternal separation model lead to low sociability only in female offspring. Regarding the neurochemical evaluation, our results show only a lower turnover rate in the dopaminergic system in male offspring in adulthood. These results combined showed that the maternal separation model leads to few neurophysiology alterations in the offspring, some of which are

not permanent, since they are only found in the infancy, and some take longer to stablish, as they just appear in the adulthood.**Acknowledgements**: CAPES and FAPESP.

Disclosures: L.D. Pantaleon: None. **G. Abreu:** 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.; CAPES. **J. Zaccarelli Magalhães:** 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.; CAPES. **B. Ribeiro:** None. **C. Munhoz:** None. **M. Manes:** None. **M. Lima:** None. **J. Miglioli:** None. **J. Flório:** None. **E. Ricci:** None. **A. Fukushima:** None. **H. Spinosa:** 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.; FAPESP.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.16

Topic: G.05. Mood Disorders

Support: CIHR CGS-M

Title: Parallel fast-acting effects of Reelin and Ketamine in an animal model of chronic stress

Authors: *K. K. SCHEIL, J. N. JOHNSTON, C. LIRIA SANCHEZ-LAFUENTE, B. S. REIVE, L. E. KALYNCHUK, H. J. CARUNCHO;
Univ. of Victoria, Victoria, BC, Canada

Abstract: Depression is the leading cause of disability worldwide, disproportionately affecting females. As 30-40% of those diagnosed do not respond adequately to first line treatment, there is a pressing need for novel therapeutics. Ketamine, an N-methyl-d-aspartate receptor (NMDAR) antagonist, has rapid antidepressant effects following a single dosage. We have previously shown that independently, both Reelin, an endogenous glycoprotein, and ketamine have behavioural and neurochemical antidepressant effects 24h post-treatment. In this study, we used repeated injections of corticosterone (CORT) (40mg/kg, subcutaneously for 21 days) to induce a chronic stress phenotype in female Long Evans rats (N = 58). A single acute dose of Reelin (3µg, intravenously) and/or ketamine (10mg/kg, intraperitoneally) was administered on the 21st day. Behavioural changes were assessed at 1h-, 6h-, 12h-, and 24h- post injection. Compared with controls, CORT injections were found to significantly increase the time spent immobile in the forced-swim test (FST) ($p < 0.001$), a test commonly used in preclinical studies of putative antidepressant drugs. We showed that ketamine had a significant effect on immobility at 1h [$F(2,9) = 13.00$, $p = 0.002$], 6h [$F(2,9) = 11.70$, $p = 0.003$], and 12h [$F(2,9) = 8.94$, $p = 0.007$].

We further showed that Reelin had a significant effect on immobility at 1h [$F(2,9) = 9.65$, $p = 0.006$], 6h [$F(2,9) = 12.11$, $p = 0.003$], and 12h [$F(2,9) = 8.94$, $p = 0.007$]. These results demonstrate that Reelin has an effect similar to ketamine in the FST that parallels the time-course of action. We also demonstrated that Reelin and ketamine have a synergic effect [$F(4,13) = 7.40$, $p = 0.003$] on immobility. To examine the duration of the antidepressant effect, we included a cohort who received CORT injections for one-week following the therapeutic treatment. We found that a single injection of Reelin [$F(2,9) = 14.30$, $p = 0.002$], or ketamine [$F(2,9) = 37.64$, $p = 0.003$], was able to maintain a lasting antidepressant effect on immobility. Overall, these findings indicate that the Reelin has a fast and lasting effect in the FST that parallels that of ketamine, and that their effects can be synergic. The findings of this study could contribute towards the development of Reelin-based therapeutics with putative fast-acting antidepressant actions.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.17

Topic: G.05. Mood Disorders

Support: Fundamental Research Grant Scheme, Ministry of Higher Education, Malaysia (FRGS/1/2021/WAB13/UCSI/02/1)
UCSI University (REIG-FPS-2020/065)
Faculty of Pharmaceutical Sciences, UCSI University (BP491)

Title: Alpha-tocopherol alleviates interferon-induced mood disorders in mice.

Authors: *M.-T. LEE[1], J.-Y. LAI[1], J.-X. HO[1], A. KOW[1], C.-L. THAM[2];
[1]Fac. of Pharmaceut. Sci., UCSI Univ., Kuala Lumpur, Malaysia; [2]Dept. of Biomed. Sci., Universiti Putra Malaysia, Serdang, Malaysia

Abstract: Interferon-alpha (IFN-α) is widely used against severe illnesses, including hepatitis C, cancers, multiple sclerosis, etc. However, its clinical use is associated with severe neuropsychiatric side-effects such as depression, anxiety, and in severe cases, suicidal tendencies. These incidences may have dampened the clinical benefits of the therapy. Elevated IFN- levels are known to cause oxidative stress, which causes monoamine depletion, hyperactivation of the HPA axis, and cytokine network disruption, all of which are associated with depression and anxiety. Alpha-tocopherol (α-tocopherol), the most common isomer of the Vitamin E family, was reported to possess strong pharmacological activity as an antioxidant and an anti-inflammatory agent. The present study aimed to investigate the potential ameliorative effect of α-tocopherol on IFN-induced mood disorder in mice. Male ICR mice received 10 daily

doses of IFN-α (36 μg/kg) or saline via intraperitoneal injection (i.p.). In separate roups of mice, α-tocopherol (50 or 100 mg/kg, p.o.) or its vehicle were co-administered with i.p. IFN-α for the 10 daily doses. At the end of the final dose, each mouse was subjected open field test, social interaction test and tail suspension test. These behaviors were recorded and analyzed with ANY-MAZE software. In the open field test, mice treated with IFN- travelled significantly less distance and spent less time in the middle, compared to the saline control group. In the social interaction test, mice treated with IFN- had a shorter social interaction time than the saline-treated group. Immobility time was significantly higher in IFN-α-treated group in the tail suspension test. Interestingly, co-administration of α-tocopherol reversed the depressive- and anxiety-like behaviors induced by IFN-α. Our findings show that α-tocopherol, possibly through its antioxidative and anti-neuroinflammatory effects, can prevent the emergence of anxiety- and depressive-like responses in mice induced by repeated IFN- treatment.

Disclosures: **M. Lee:** None. **J. Lai:** None. **J. Ho:** None. **A. Kow:** None. **C. Tham:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

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Program #/Poster #: 067.18

Topic: G.05. Mood Disorders

Support: CAPES
FAPESP

Title: Maternal separation model for induction of postpartum depression cause disturbance on maternal care in lactating rats

Authors: *J. Z. MAGALHÃES[1], G. R. ABREU[1], L. P. PANTALEON[1], B. B. RIBEIRO[2], C. MUNHOZ[2], M. MANES[1], M. A. LIMA[3], J. MIGLIOLI[3], J. C. FLÓRIO[1], A. R. FUKUSHIMA[1], E. L. RICCI[1], H. S. SPINOSA[1];
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Abstract: Postpartum depression is a complex disease with multifactorial etiology that affects women worldwide. Despite the high prevalence, this disease is still poorly studied and underdiagnosed. One of its main characteristics is the poor maternal care that these women express towards the child. It is well documented in the literature that lack of maternal care can lead to severe consequences on child behavioral and cognitive development. Thus, the aim of the present study is to evaluate the maternal care sphere of dams induced to postpartum depression through the maternal separation model. Sixteen lactating rats were subdivided into 2 groups, control and MS (n=8 animals/group). MS dams were separated from their litter for 3h daily from lactating day (LD) 2 to 12, while the control dams were handled only for the behavioral tests. The behavioral evaluation was performed on LD5 and 6 through the maternal behavior and the

maternal aggressive behavior tests. On LD21, right after weaning, all dams were euthanized by decapitation for collection of hippocampus and prefrontal cortex for dopaminergic system analysis and blood for prolactin and oxytocin dosage. The results showed dysfunction on the maternal care sphere of the MS dams evidenced by alterations in several behavioral parameters [1st pup retrieval (t=0.0422); 2nd pup retrieval (t=0.0366); 3rd pup retrieval (t=0.0375); 4th pup retrieval (t=0.0429), self-grooming (t=0.0331); latency for nursing (t=0.0290); total time of nursing (t=0.0389); latency to start the 1st fight (t<0.0001); number of fights (t=0.0406); and number of boxing (t=0.0024)] and in the dopaminergic system analysis [HVA (t=0.0440) and HVA/DA rate (t=0.0341) in the hippocampus; and DA (t=0.0311), DOPAC (t=0.0045) and DOPAC/DA rate (t=0.0339) in the prefrontal cortex]. These results indicate that the maternal separation model mimics the maternal care dysfunction expressed by woman with postpartum depression, suggesting that this model can be use to induced postpartum depression in female rats.**Acknowledgements**: CAPES and FAPESP.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.19

Topic: G.05. Mood Disorders

Support: 456306

Title: Combined low doses of mirtazapine and venlafaxine produce a similar antidepressant-like effect in male and female rats in the forced swim test

Authors: *L. ALVAREZ-SILVA[1], A. FERNÁNDEZ-GUASTI[2];
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Abstract: Depression is a psychiatric disorder that affects nearly 2.8 million people. Despite the plenty of pharmacological treatments, almost half of the patients have a poor response to a single antidepressant therapy, and the persistence of depressive symptoms negatively affects their

quality of life. For treating resistant depression, some strategies have been explored, like the use of two antidepressants of different classes. An example is the “California Rocket Fuel”, a combination of mirtazapine plus venlafaxine. This combination seems to be effective to treat depression in humans; however, data are scarce, and there are no studies of the optimal doses that should be used for an adequate antidepressant response with less adverse effects in humans. The objective of this study was to evaluate if the chronic administration of low doses of mirtazapine and venlafaxine combined show an antidepressant-like effect in male and female rats using the forced swim test (FST). For this purpose, young adult male and female Wistar rats were used. The animals were separated by sex and submitted to a forced swim pretest in a cylinder with water 30 cm deep for 15 minutes, and then divided into 2 groups: vehicle and treated (mirtazapine and venlafaxine 2.5/3.75 and 5/7.5 mg/kg) for 14-16 days. After this time, rats were evaluated in the FST (same conditions described above, for 5 minutes), and the tests were videotaped for scoring immobility (depressive-like behavior), swimming, and climbing (active behaviors). The estrous cycle was determined from 2 weeks before the pretest until the end of the study, the FST was made when the rats were in proestrus/estrus. The results showed that, in both male and female rats, the combination of mirtazapine and venlafaxine 5/7.5 mg/kg was equally effective in reducing the depressive-like behavior, while 2.5/3.75 mg/kg lacked an antidepressant-like effect. In male rats, the effective combination produced a significant increase of both active behaviors, while in females, only swimming behavior was enhanced. The regularity of the estrous cycle was unmodified by the treatments. These results show that the combination of low doses of mirtazapine plus venlafaxine in a chronic schedule has an antidepressant-like effect in rats of both sexes and might represent a therapeutic option for treating patients with resistant depression.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Topic: G.05. Mood Disorders

Support: VA grant I21 BX002085
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NSF grant IOS-1656626
NIH grant F31AG066501

Title: Differential effects of high fat diet on endocrine, metabolic and depressive-like behaviors in male and female rats

Authors: *J. L. WOODRUFF[1], M. K. BYKALO[2], F. Z. LOYO-ROSADO[2], E. S. MAISSY[2], A. T. SADEK[2], M. HERSEY[2], K. E. AYALA[1], N. D. MAXWELL[2], C. A. GRILLO[2], L. P. REAGAN[1];

[1]Columbia VA Hlth. Care Syst., Columbia, SC; [2]Univ. of South Carolina Sch. of Med., Columbia, SC

Abstract: Current data estimate that greater than ~ 64% of the adult US population may be categorized as either overweight or obese. The neurological complications of obesity extend beyond the periphery to the central nervous system (CNS) and include an increased risk of developing neuropsychiatric co-morbidities like depressive illness. This concept is supported by preclinical studies, including studies that have examined the effects of high-fat diet (HFD) on depressive-like behaviors. Previously, most studies have focused on the effects of HFD in male rats even though women are approximately two-fold more likely to develop depressive illness compared to men. Accordingly, the goal of this study was to examine depressive-like behaviors in male and female rats provided access to a HFD. Approximately 50% of male and female rats fed a HFD developed an obesity phenotype (i.e., diet-induced obesity; DIO) as assessed by increases in body weight, body composition and plasma leptin levels; the remaining rats did not exhibit these metabolic and endocrine changes and are referred to as diet resistant (DR). Upon confirmation of the obesity phenotype, behavioral measures were performed in control chow rats, DR rats and DIO rats. In the sucrose preference test,
male DIO rats exhibited significant decreases in sucrose consumption (i.e., anhedonia) compared to male DR and male control rats. In the forced swim test (FST), male DR rats exhibited increases in immobility and decreases in active behaviors. Female rats exhibited no differences in sucrose preference test, but female DIO rats exhibited increases in immobility and decreases in active behaviors in the FST compared to DR and control-chow female rats. Interestingly, while the estrous cycle did not affect immobility or active behaviors, metestrus/diestrous DR rats exhibited significant increases in latency to float compared to control-chow or DIO females. Collectively, these studies demonstrate that male and female rats exhibit different behavioral responses following access to HFD, including a diet-estrous cycle interaction in the FST in DR female rats.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.21

Topic: G.05. Mood Disorders

Support: Fundação Araucária/CNPq - PRONEX 02/2016, protocol 46843

Title: Specialized pro-resolving mediator Protectin DX induces antidepressant-like and anxiolytic-like effects in an animal model of type 1 diabetes mellitus

Authors: *A. P. F. WALTRICK[1], W. A. VERRI, Jr[2], J. M. DA CUNHA[1], J. M. ZANOVELI[1]; [1]Pharmacol., Univ. Federal do Paraná, Curitiba, Brazil; [2]Univ. Estadual De Londrina, Londrina, Brazil

Abstract: Type 1 diabetes mellitus (T1DM) is a chronic, inflammatory, and metabolic disease that affects the central nervous system, leading to psychiatric comorbidities such as depression and anxiety. It is well established that the treatment of these comorbidities is a great challenge, since the number of diabetic patients resistant and refractory to the currently treatments used is high. Considering chronic inflammation as an important characteristic of T1DM and that pro-resolving lipid mediators (PRLMs) can exert protective actions on this process, this study aimed to evaluate the therapeutic potential of PRLM protectin DX (PDX) in behaviors such as depressive-like and anxious-like in an animal model of T1DM1. The experimental protocol started with the induction of T1DM (streptozotocin; 60 mg/kg, i.p), considered day 0. Male *Wistar* rats received 6 injections of PDX (0, 1, 3 and 10 ng/animal i.p, 200 µl) performed on days 14, 15, 18, 21, 24 and 27 after experimental induction of T1DM. On day 26 of the protocol, the animals were submitted to the elevated plus maze test (EPM) and, 24 hours later, they were submitted to a pre-test session of the modified forced swimming test (mFST). On day 28 of the protocol, the open field test (OFT) was performed followed by mFST. All protocols were approved by the UFPR Ethics Committee for the Use of Animals (CEUA/UFPR; #1108). Treatment with PDX improved dysregulated parameters related to the diabetic condition of weight gain reduction and also hyperglycemia. In addition, PDX increased open-arm time and entries, as well as the frequency of head dipping in the EPM test, indicative of an anxiolytic-like effect. In the same direction, in OFT, treatment with PDX increased the time spent in the center of the apparatus, reinforcing this anxiolytic-like effect, without altering locomotor activity. Finally, in mFST, PDX induced an antidepressant-like action, by decreasing immobility and increasing swimming frequencies. Our findings indicate that the PRLM PDX presents a therapeutic potential in relieving anxiety and depression associated with T1DM. In addition, PDX treatment also improved diabetic parameters of hyperglycemia and reduced weight gain.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.22

Topic: G.05. Mood Disorders

Support: Caltech Innovation Initiative
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HHMI

Title: A Family of Genetically Encoded Biosensors for Rapidly Acting Antidepressants (RAADs) inside Neurons

Authors: *K. BERA[1], Z. BLUMENFELD[1], E. LIN[1], E. J. FINE[1], H. M. AMBROSINO[1], A. ANDREEV[1], C. H. KIM[1], A. V. SHIVANGE[1], B. N. COHEN[1], J. S. MARVIN[2], L. L. LOOGER[3], D. PROBER[1], H. A. LESTER[1];
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Abstract: Rapidly acting antidepressants (RAADs) partially relieve depression in < 1 day and have sustained antidepressant effects for several days. This rapid onset is 10- to 100-fold faster than selective serotonin and norepinephrine reuptake inhibitors (SSRIs and SNRIs). The mechanism(s) of RAAD action are largely unknown at levels ranging from the molecular target, the signaling pathway, the cell types they target, and other circuits they may influence. Thus, it is important to detect and quantify RAADs inside subcellular regions of neurons. Therefore, we developed the "**i**ntensity-based **RAAD**-**S**e**n**sing **F**luorescent **R**eporter" [iRAADSnFR] genetically encoded biosensor family for RAADs. This biosensor family structurally resembles a previously published nicotine biosensor: a fusion between a mutated periplasmic binding protein OpuBC, interdomain linkers, and circularly permuted green fluorescent protein (GFP). Biosensor mutations are created by site-saturated mutagenesis and are tested with computational docking models, high-throughput fluorescence screens, and fluorescence measurements with their purified protein form. We have mutated residues at the binding site to account for unique sensitivity to various RAADs in the nanomolar (nM) range.
We incorporate additional sequences to the iRAADSnFR constructs to target the sensors to the plasma membrane (PM), cytoplasm, peroxisome, mitochondria, endoplasmic reticulum (ER), nucleus, nucleolus, F-Actin, Golgi, lysosome, and synaptic vesicles. Our research will allow us to measure the accumulation of RAADs in various intracellular compartments, the time course of RAAD metabolism, RAAD-induced synapse development, discover new receptor(s), and the potential physiological mechanism of RAAD action. Fluorescence dose-response experiments to test the efficacy of various iRAADSnFRs in live mammalian cells have progressed. Fluorescence microscopy shows that, at the nM concentrations relevant to the antidepressant action, RAADs enter and leave the various organelles within a few seconds, attaining extracellular levels. We have created six transgenic zebrafish lines (for various iRAADSnFRs) to determine the neural concentrations of various RAADs in the amelioration of depression-like behavior in zebrafish. Finally, we will utilize AAV capsids to facilitate efficient and noninvasive iRAADSnFR gene transfer to the central and peripheral nervous systems which may help us unravel RAADs' mechanism of action in mice. This information will, in turn, help researchers develop safer and more effective rapidly acting antidepressants.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

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Program #/Poster #: 067.23

Topic: G.05. Mood Disorders

Support: R15MH-114026-02

Title: Role of ovarian hormones in rapid exercise-induced stress resilience in females

Authors: *N. JAMIL[1], M. K. TANNER[1], A. HOHORST[1], E. C. LOETZ[2], B. N. GREENWOOD[3];
[1]Integrative Biol., [3]Psychology, [2]Univ. of Colorado Denver, Denver, CO

Abstract: Stress-related disorders affect females more than males. Physical activity enables stress resilience in both sexes, but little research has characterized exercise-induced stress resilience in females. We observed that female rats are more responsive to the stress-buffering effects of exercise than males. In females, three weeks of voluntary wheel running prevents anxiety-like behavioral effects of inescapable stress (IS), while males require six weeks of running. Although the expression of exercise-induced stress resilience occurs regardless of estrous phase at the time of IS or behavioral testing, the role of ovarian hormones during the three weeks of exercise in mediating resilience is unknown. The goal of this study is to determine the role of ovarian hormones in accelerated exercise-induced stress resilience in females. Ovariectomized and SHAM rats had access to locked wheels or running wheels for three weeks. After three weeks, rats were exposed to IS and anxiety-like behaviors were assessed twenty-four hours later. Since females run more than males, we limited the distance run in another group of females to 3 h per day, in order to determine the degree to which running distance contributes to rapid stress resilience and the potential effect of ovariectomy. Results demonstrate three weeks of running prevents IS-induced behavior sequalae independent of the presence of ovarian hormones. Additionally, even though limited access to running wheels reduced the running distance to less than that of males, three weeks of limited running prevented anxiety-like behavior in females. These data suggest that sex differences in the acquisition of exercise-induced stress resilience is independent of ovarian hormones and running distance.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.24

Topic: G.05. Mood Disorders

Support: T32MH087004
R01NS107512

Title: Early and sustained vulnerability to variable stress in mice carrying a Parkinson's disease-linked Lrrk2^{G2019S} mutation

Authors: *C. A. GUEVARA, K. ALLOO, R. THOMAS, K. BLAKE, N. MEMAN, A. TIELEMANS, D. L. BENSON, G. W. HUNTLEY;
Nash Family Dept. of Neurosci., The Friedman Brain Institute, Grad. Sch. of Biomed. Sciences, Icahn Sch. of Med. at Mount Sinai, New York, NY

Abstract: Parkinson's disease (PD) is associated with psychiatric non-motor symptoms such as depression and anxiety that emerge early, appear to be independent of dopamine neuron loss, and are poorly understood. Risk for both PD and depression is increased by behavioral stress, and previous work in the lab showed that male mice carrying a knockin G2019S mutation in the *Lrrk2* gene, which in humans greatly increases risk for PD, show altered behavioral and electrophysiological responses to social stress. In order to determine whether social stress-effects are generalized to other forms and durations of behavioral stress, we subjected young adult male wildtype (WT) and G2019S knockin (GS) mice to a standard, daily variable stress (VS) protocol for either 6 days (6d-VS) or 28 days (28d-VS). Stressed and unstressed mice then underwent a battery of assays (open field test, social interaction test, and novelty suppressed feeding) to probe for stress-induced behavioral changes. As expected from prior work, we found no changes in WT mice from 6d-VS, but significant stress effects in each of the post-stress behavioral assays which emerged at 28d-VS. In contrast, GS mice already displayed significant stress susceptibility from 6d-VS which persisted at 28d-VS. These differences are driven by the stressful experience, as no behavioral or locomotor differences were observed between genotypes in unstressed conditions. Together, these data suggest that the G2019S mutation lowers the threshold for stress susceptibility, and drives a temporally evolving set of neural adaptations that differ from WT. Such differential vulnerabilities may impact the onset of psychiatric symptoms in human PD patients. Future studies are probing the impact of sex as a variable, as there is a lower prevalence of PD overall in females, but a higher risk for developing stress-induced depression in females. Understanding these interactions will provide insight into the differential adaptations between individuals harboring the G2019S mutation, revealing novel targets for ameliorating mood-related symptoms associated with PD.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.25

Topic: G.05. Mood Disorders

Title: The interferon-alpha rat model of neuroinflammation as a back-translational model of depression to investigate the efficacy of antidepressant and anti-inflammatory drugs

Authors: *J. KEALY, C. DE PASQUALE, A. FREEBURN, M. GLASS, C. MCGURK, D. DISHA, K. MURRAY, C. CALLAGHAN, M. BIANCHI;
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Abstract: Interferon (IFN)-alpha is an innate inflammatory cytokine clinically used for treating various cancers, and some forms of hepatitis. Patients undergoing IFN-alpha treatment often report CNS side effects including depression-like symptomatology, with up to 80% being referred for psychiatric assessment. The IFN-alpha-induced depression is rescued by chronic treatment with SSRIs.We have previously shown that administration of IFN-alpha (170,000IU/kg) in Wistar rats (three times per week for 3-4 weeks) induces a depression and anxiety-like behavioural phenotype paralleled by increased peripheral IL-6 and decreased BDNF, similar to what is observed in patients.Here we expand on our previous observation with results showing that behavioural and molecular IFN-alpha-induced phenotypes can be significantly reversed by pharmacological challenge with clinical and experimental antidepressant drugs.Thus, since June 2019 we ran eight studies with the IFN-alpha model, with forced swim test (FST) behaviour (passive coping behaviour), IL-6 (central and peripheral; pro-inflammatory cytokine), BDNF (central and peripheral; neurotrophin) and hippocampal neurogenesis being the most studied endpoints. The experiments were conducted using IFN-alpha/saline treatment in separate cohorts of animals, which were then challenged with fluoxetine (SSRI, chronic (once per day for 14 days) or acute; 10mg/kg, PO), ketamine (NMDA antagonist, acute; 5mg/kg, SC), SB216763 (GSK-3beta inhibitor, chronic [once per day for 14 days; 2-10 mg/kg, IP), and rolipram (acute, PDE-4 inhibitor; 0.3 mg/kg, IP). IFN-alpha increases FST immobility behaviour, indicative of depressive-like behaviour, with a significant replication rate of 95% between studies. This behavioural phenotype was rescued by all drug treatments excluding acute fluoxetine administration, which is in line with clinical studies. Moreover, increased central IL-6 expression induced by IFN-alpha was replicated in 90% (medial prefrontal cortex (mPFC)) and 100% (hippocampus (HIPP)) of studies, while increased plasma IL-6 expression was replicated with a 60% reliability. IFN-alpha-induced decreases in central (mPFC, HIPP) and plasma BDNF were replicated with 60% reliability and hippocampal neurogenesis was replicated in 100% of studies. Pharmacological challenge rescued these molecular alterations with different patterns depending on the mechanism of action and length of treatment.In conclusion, a back translational model of neuroinflammation and depression was developed displaying both central and peripheral molecular sensitivity to clinical and experimental antidepressant drugs.

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Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

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Program #/Poster #: 067.26

Topic: G.05. Mood Disorders

Support: Knut and Alice Wallenberg Foundation
Swedish Research Council

Title: Enkephalinergic neurons in the lateral septum bi-directionally control affective responding

Authors: *A. M. KLAWONN[1], P. LLORACH[2], J. S. SALGADO[2], B. D. HEIFETS[2], M. HEILIG[3], R. C. MALENKA[2];
[1]Univ. of Copenhagen, Univ. of Copenhagen, Copenhagen, Denmark; [2]Stanford Univ., Stanford, CA; [3]Linkoeping Univ., Linkoeping, Sweden

Abstract: The role of the Lateral septum (LS) in affective and motivational regulation has remained largely unexplored since the 1960's, when Robert G. Heath performed a series of deep brain stimulations (DBS) revealing the LS as a potential site that evokes pleasure. Subsequently, a handful of human DBS studies have further linked the LS to positive affective subjective experiences. However, the specific roles of LS neuron subpopulations in regulating affective states have not been fully elucidated. Here we first investigated the effects of manipulating the activity of LS neurons non-specifically in a variety of assays of affective experiences in mice. Optogenetic stimulation of LS neurons with ChR2 generated increased hedonic responding (orofacial reactivity) and increased social interaction (opposite gender), without influencing real time place preference, locomotion in the open field or exploration of a novel object. The LS in rats was recently found to project to NAc hedonic 'hotspots', in which enkephalinergic signaling reinforces hedonic responding. Therefore, we went on to explore the consequences of selective manipulations of LS enkephalinergic neurons. Surprisingly, selective activation of LS enkephalinergic neurons by expressing Cre-dependent ChR2 in the Penk-Cre driver line lead to behavioral effects that were essentially opposite to those elicited by non-selective LS neuron activation: decreased hedonic responding and social interactions as well as a real time place aversion. Conversely, inhibition of LS enkephalinergic neurons with a DREADD increased hedonic responding, social interaction and self-care. Neither activation nor inhibition of LS enkephalinergic neuron affected locomotion in the open field or exploration of a novel object. Collectively these results suggest that the LS influences affective behaviors in a complex manner that depends on the specific subpopulations of LS neurons involved.

Disclosures: A.M. Klawonn: None. **P. Llorach:** None. **J.S. Salgado:** None. **B.D. Heifets:** None. **M. Heilig:** None. **R.C. Malenka:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.27

Topic: G.05. Mood Disorders

Support: NRF-2019R1A6A3A01095232

Title: Sociality regulation by cortico-habenula pathway in an animal model of depression

Authors: *H. PARK, C. CHUNG;
Konkuk Univ., Seoul, Korea, Republic of

Abstract: Impaired sociality is one of the behavioral symptoms oftenaccompanied with depression. The medial prefrontal cortex (mPFC) is known to beinvolved in social behaviors as well as physiological responses to stress andprojects to the lateral habenula (LHb). Given abnormal hyperactivity of the LHbin depressed patients and animal models, it has been suggested that themPFC-LHb pathway modulates depression-like behaviors. Previous studies mostlyfocused on animal models of depression caused by social defeat stress, however,the underlying mechanisms of sociality impairment in depression animal modelsinduced by non-social stress remain largely uninvestigated. In the presentstudy, we employed acute learned helplessness (aLH) mice and investigated anystructural and functional alterations in mPFC neurons projecting to the LHb byemploying electrophysiology, optogenetics and in vivo fiber-photometry. Usingretrograde tracing, we observed mPFC neurons project to the LHb, and theseneurons also project to the laterodorsal thalamus (LP) and paraventricularthalamus (PVT). We found that LHb-projecting mPFC neurons exhibited increasedneuronal excitability in aLH mice. Moreover, specific synaptic transmissiononto LHb neurons was potentiated. We also tested functional alteration of themPFC-LHb pathway during social interaction behaviors. The Ca2+ transient signalfrom soma and axon terminal of LHb-projecting mPFC neurons were facilitated inaLH mice. Finally, optogenetic suppression of the mPFC-LHb pathway restoresimpaired sociality of aLH mice. Our observations provide a highly valuableneural circuit to investigate mechanisms underlying the impaired sociality indepressive disorders.

Disclosures: H. Park: None. **C. Chung:** None.

Poster

067. Animal Models of Relevance to Psychiatric Illness and Treatment I

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 067.28

Topic: G.05. Mood Disorders

Support: UWRF USE Grant

Title: The effect of adolescent isotretinoin exposure on the hippocampus, stress coping behavior, and recognition memory

Authors: *C. PETERSON[1], K. LAROCCO[2], H. KOST[1], D. EHLINGER[1];
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Abstract: 13-cis-retinoic acid (Isotretinoin) is a commonly prescribed medication for the treatment of persistent cystic acne but has also been shown to cause depressive behaviors and suicidal thoughts in patients. The hippocampus is a brain area involved in regulation of the stress response as well as cognitive functions such as novel memory formation, and alterations to this area may be important to the development of symptoms following adolescent isotretinoin exposure. In the present study, we assessed the effects of adolescent isotretinoin exposure on stress-coping behavior and novel object recognition, followed by measurement of hippocampal dopamine D2 receptor expression and dendritic spine density. From approximately postnatal day (P)35 through P49, C57BL/6J mice were injected with 1 mg/kg of isotretinoin intraperitoneally for 10 total injection days. From approximately P50 through P52, mice underwent novel object recognition testing, followed by a 6-minute forced swim test on approximately P53. Brains were extracted for western blotting of dopamine D2 receptor density in the hippocampus and striatum, and Golgi-Cox tissue staining for analysis of dendritic spine density. To date, our results indicate an increase in immobility in the forced swim test, but no effect on novel object recognition, in isotretinoin exposed mice compared to controls. These results suggest that adolescent isotretinoin exposure is associated with depressive-like behavior in mice but does not influence hippocampal dependent memory formation. Ongoing research aims to correlate hippocampal dopamine D2 receptor expression and dendritic spine density with our behavioral results.**Acknowledgements**: Funding Source (UWRF undergraduate stipends and expenses grant), we would also like to acknowledge Sid Peck, Dan Roever, James Cortright for animal care and assistance in data collection.

Disclosures: C. Peterson: None. **K. LaRocco:** None. **H. Kost:** None. **D. Ehlinger:** None.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.01

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics

Title: Pharmacological and Neurophysiological Effects of Psilocybin and Related Novel Tryptamines

Authors: *M. MCMURRAY, J. JONES, R. J. RAKOCZY;
Miami Univ., Oxford, OH

Abstract: Demand for more efficacious antidepressants, particularly those with a rapid onset of action, has resulted in a reevaluation of psychedelic drugs such as psilocybin for their therapeutic potential. Recently several tryptamines found in ‘magic’ mushrooms have been characterized including psilocybin, baeocystin, norbaeocystin, and aeruginascin. Preliminary evidence suggests these other tryptamines may rapidly alleviate symptoms of depression, but the mechanisms of

action and physiological targets remain unknown. The overall purpose of this work is to better understand the pharmacokinetics and neural effects of these tryptamines. As these novel compounds share structural similarity with psilocybin, it was hypothesized they are metabolized by the same enzymes, serve as ligands for similar, centrally-located receptors, and modulate activity of neurons in similar brain regions. In vitro enzyme kinetics, receptor binding, and blood brain barrier permeability assays provide supporting evidence for some of these hypotheses. Specifically, preliminary experiments suggest similar rates of dephosphorylation via alkaline phosphatase, demonstrate permeability across a blood brain barrier mimetic, and identify unique receptor binding profiles for each of these compounds. Furthermore, in vivo electrophysiology recordings in rodents suggest tryptamine administration evokes changes in activity in brain regions known to correlate with positive therapeutic effects. Specifically, local field potential and single unit recordings from medial prefrontal cortex implanted multiwire electrode arrays suggest regional decreased activity. Future work will isolate antidepressant effects to specific neuronal populations, continue to expand the knowledge of the mechanism of action of psychedelic drugs, and further identify and characterize potential therapeutic targets for these novel tryptamines. Funding for this project was provided by PsyBio Therapeutics, Inc.

Disclosures: **M. McMurray:** A. Employment/Salary (full or part-time):; Miami University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsyBio Therapeutics. **J. Jones:** A. Employment/Salary (full or part-time):; Miami University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsyBio Therapeutics. **R.J. Rakoczy:** A. Employment/Salary (full or part-time):; Miami University. B. Contracted Research/Research Grant (principal investigator for a drug study, collaborator or consultant and pending and current grants). If you are a PI for a drug study, report that research relationship even if those funds come to an institution.; PsyBio Therapeutics.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.02

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics
Miami University Undergraduate Research Award
Miami University Summer Scholars Award

Title: The Effects of Adolescent Psilocybin Exposure on Behavior in Adulthood

Authors: *B. F. ROBERTS, A. L. ZYLKO, J. A. JONES, M. S. MCMURRAY;
Miami Univ., Oxford, OH

Abstract: The prevalence of adolescent depression is at an all-time high. Pharmacological treatments are similar to adults but elicit different—and oftentimes adverse—consequences in adolescents, including anxiety and suicidal ideations. Thus, significant demand exists for improved therapeutic interventions for this age group. Psilocybin, a serotonergic agonist, has demonstrated potential for therapeutic efficacy in adulthood; however, its effects have not been evaluated in adolescents. Therefore, the purpose of this study is to investigate age-related differences in psilocybin efficacy. Additionally, we sought to determine whether adolescent treatment of psilocybin has long-term effects on anxiety-associated behaviors and decision-making. In separate groups of rats (8-10 per group), head twitch responses were quantified following administration of 1 mg/kg psilocybin or vehicle via gavage in early adolescence (35 days old) or late adolescence (45 days old) and in adulthood (110+ days old). In adulthood, adolescent-treated subjects completed an elevated zero maze and a novel object test to measure long-term effects of psilocybin on anxiety-associated behaviors. Lastly, animals underwent a reversal learning paradigm to assess effects on behavioral flexibility. Preliminary results demonstrate weaker acute responses to psilocybin in adolescents than adults (fewer head twitches), and adolescent treatment with psilocybin at either age increases adult behavioral flexibility in males. Although significant sex differences in adult anxiety-like behaviors were also detected, these were not affected by adolescent psilocybin treatment. This pattern of effects suggests age-related differences in 5-HT2A/2C levels, which will be examined in future studies. Combined, these results imply that age-related differences in drug efficacy should be considered when evaluating the therapeutic efficacy of psilocybin or other psychedelic drugs, and that lasting changes in behavior may occur following adolescent treatment. Funding for this project was provided by PsyBio Therapeutics, Inc.

Disclosures: B.F. Roberts: None. **A.L. Zylko:** None. **J.A. Jones:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics. **M.S. McMurray:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.03

Topic: G.05. Mood Disorders

Support: PsyBio Therapeutics
Miami University Undergraduate Research Award
Miami University Undergraduate Summer Scholars

Title: Psilocybin and PsyBio-11040 improve depressive-like behaviors in rats after chronic early life stress

Authors: *J. SCIORTINO, B. ROBERTS, M. MCMURRAY;
Miami Univ., Oxford, OH

Abstract: Recent research has focused on psilocybin as a potential rapid-onset antidepressant, but its status as a Schedule 1 substance makes it difficult to study. Preliminary studies of the non-hallucinogenic, psilocybin-related tryptamine, PsyBio-11040, suggest it may have similar antidepressant effects. Evaluating antidepressant efficacy in animals relies upon utilization of chronic and/or early life stress paradigms, such as the limited nesting/bedding model. Dams in the limited nesting/bedding conditions often display hallmark behaviors indicative of childhood neglect, and pups raised in this environment have been shown to display robust depressive behaviors in adulthood. Therefore, to evaluate the antidepressant efficacy of these drugs, we treated 128 male and female Sprague Dawley rats (8 per group per sex per rearing condition) with psilocybin (1 mg/kg), PsyBio-11040 (1 mg/kg), fluoxetine (15 mg/kg), or vehicle intragastrically via gavage. Early life stress subjects experienced the limited nesting/bedding model during postnatal days 2-11 and control animals were raised under normal nesting conditions. Depression-associated phenotypes were assessed in adulthood, including measures of anhedonia (sucrose preference), exploration and anxiety (open field test), and despair (forced swim test). Preliminary results indicate that psilocybin, PsyBio-11040, and fluoxetine all increased sucrose preference in limited nesting/bedding animals and decreased immobility in the forced swim test, while having no significant effect on open field behavior in either sex. These results indicate that psilocybin, PsyBio-11040, and fluoxetine may alleviate symptoms of anhedonia and despair, while not significantly affecting anxiety-like and exploratory behaviors. Future studies will examine whether these treatments alter neural markers of antidepressant efficacy, such as BDNF expression. The results provide support for the antidepressant efficacy of psilocybin and PsyBio-11040 in an established rodent model of depression. This may demonstrate a potential to treat depression or other conditions in humans. Likewise, with preliminary results indicating no significant sex differences, they suggest that these compounds may be effective in both sexes. Funding for this research was provided by PsyBio Therapeutics, Inc.

Disclosures: **J. Sciortino:** None. **B. Roberts:** None. **M. McMurray:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.04

Topic: G.05. Mood Disorders

Support: Miami University ORU
Miami University Family Fund
PsyBio

Title: Effectiveness of psilocybin in females depends on estrous stage

Authors: *M. WILSON, Á. POWELL, M. HEITKAMP, D. FEIST, A. PAGE, B. ROBERTS, M. MCMURRAY;
Miami Univ., Oxford, OH

Abstract: Effectiveness of Psilocybin in Females Depends on Estrous Stage
AuthorsM. Wilson, Á. Powell, M. Heitkamp, D. Feist, A. Page, B.F. Roberts, M.S. McMurray; Department of Psychology, Miami University, Oxford, OH
AbstractThe tryptamine psilocybin exerts its hallucinogenic effects primarily through agonism of 5-HT2A receptors. 5-HT2A receptor expression fluctuates across the estrous cycle, driven by variation in estradiol level. Therefore, it is likely that the hallucinogenic effects of the drug may also vary across the estrous cycle. The head twitch response, also called wet dog shake, can be used to assess 5-HT2A receptor activation in rodents. Therefore, we studied the ability of psilocybin to induce head twitch responses in females at various stages of their estrous cycle. Psilocybin (1mg/kg) was administered intragastrically to 12 naturally cycling Long Evans females in proestrus or diestrus, with head twitch responses quantified for 30 minutes immediately after. Preliminary results showed that psilocybin administered in the proestrus phase failed to increase head twitch responses, while administration during diestrus produced the expected increase in head twitch responses. Additionally, separate animals placed in an anestrous state (n=12) by subcutaneous injection of 3.5mg medroxyprogesterone acetate 24 hours prior to testing also showed the expected increase in head twitch responses. To determine a potential mechanism for these effects, we conducted a Western Blot analysis of 5-HT2A and 5-HT2C receptor levels in the medial prefrontal cortex during proestrus, diestrus, and in anestrous animals. Activation of 5-HT2C receptors has been previously shown to inhibit the action of 5-HT2A agonists. We found that the balance between these receptors shifts across the estrous cycle, potentially explaining the observed variation in head twitch responses. Recent studies utilizing psilocybin point towards potential therapeutic benefits for serotonin-linked mood disorders common in women, such as Major Depressive Disorder and Premenstrual Dysphoric Disorder. Funding for this research was provided by PsyBio Therapeutics, Inc.

Disclosures: M. Wilson: None. **Á. Powell:** None. **M. Heitkamp:** None. **D. Feist:** None. **A. Page:** None. **B. Roberts:** None. **M. McMurray:** 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.; PsyBio Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); PsyBio Therapeutics. F. Consulting Fees (e.g., advisory boards); PsyBio Therapeutics.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.05

Topic: G.05. Mood Disorders

Support: PsyBio Grant

Title: Drug Discrimination of Psilocybin and Novel Tryptamines

Authors: *O. SANDOVAL, B. ROBERTS, Z. PLATOW, J. SCIORTINO, J. JONES, M. MCMURRAY;
Miami Univ., Oxford, OH

Abstract: Drug Discrimination of Psilocybin and Novel Tryptamines
AuthorsO. Sandoval[1], B.F. Roberts[1], Z.L. Platow[1], J.H. Sciortino[1], J.A. Jones[2], M.S. McMurray[1]; Departments of [1] Psychology and [2] Chemical, Paper, and Biomedical Engineering, Miami University, Oxford, OH;
Abstract Clinical trials utilizing psilocybin to treat depression are promising, but they are still in preliminary stages. One potential limiting side effect is the presence of hallucinations, which may not be necessary for psilocybin's clinical effectiveness and create challenges in experimental design. Thus, there is a need to determine if other tryptamines have similar effects and share molecular targets but do not cause hallucinations. A novel *E. coli*-based method for synthesizing various novel tryptamines in addition to psilocybin has been identified. These novel tryptamines may have potential antidepressant efficacy and elicit common behavioral responses (i.e., head twitch response) in preliminary testing. In this study, we tested the potential discriminative properties of each of these tryptamines by establishing interoceptive effects of a training drug (e.g., psilocybin) as the cue to perform a nose poke. A cohort of 30 male Long Evans rats were trained for 50-60 days to nose-poke into one of two ports when given 1mg/kg psilocybin, and into the other port when given saline. After "acclimation" to the drug port (85% correct responding), rats given PsyBio-11040, PsyBio-11141, or PsyBio-11143 (1 mg/kg) prior to testing, to determine the discriminative properties of these novel tryptamines. We found that rats given these tryptamines had significantly increased response to the reinforced nose-poke holes during testing, thus suggesting the rats can discriminate when they are given these tryptamines. These results suggest these tryptamines have discriminative properties at 1 mg/kg, and suggest they have possible pharmacological differences despite their similarity of structure.

Future studies will examine dose-response curves for novel tryptamines and determine the discriminability of other routes of administration.

Disclosures: **O. Sandoval:** None. **B. Roberts:** None. **Z. Platow:** None. **J. Sciortino:** None. **J. Jones:** None. **M. McMurray:** A. Employment/Salary (full or part-time):; full. 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.; PsyBio Therapeutics.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.06

Topic: F.03. Stress and the Brain

Support: R01MH122742
T32GM008688
T32NS105602
1 R01 MH124777-01

Title: Psilocybin-induced stress response supports anxiolytic effects

Authors: *N. JONES[1], J. RAZIDLO[3], Z. ZAHID[4], L. WAGNER[2], C. J. WENTHUR[5]; [1]Mol. and Cell. Pharmacol., [2]Pharm., Univ. of Wisconsin, Madison, WI; [3]Neurosci., Univ. of Wisconsin - Madison, Neurosci. Training Program, Madison, WI; [4]Anesthesiol. and Pharm., Univ. of Wisconsin Madison, Madison, WI; [5]Pharm., Univ. of Wisconsin - Madison. Psychoactive Pharmaceut. Investigation Program, Madison, WI

Abstract: Investigation into the clinical use of the serotonin 2A receptor agonist psilocybin in conjunction with psychotherapy has shown promising results in the treatment of psychiatric disorders. Although, these anxiolytic effects from psilocybin are often acutely anxiogenic in humans. Despite the potential relevance of psilocybin-induced stress responses to the clinical effects of psilocybin, few studies have assessed the impact of drug-induced corticosterone release on the behavioral effects of psychedelics. A time course for psilocybin to induce anxiolysis in the absence of any non-pharmacologic intervention using C57/BL6 mice was conducted using LC-MS/MS. Serum samples tested at doses of 0.3, 1, and 3 mg/kg found peak concentrations at 15 min post-injection. In the open field test 15 minutes post-injection, mice that received 3mg/kg psilocybin demonstrated decreased center time, while the 0.3 mg/kg exhibited a dose-dependent increase. However, at 4 h post-injection, the reverse was found, in that 0.3mg/kg psilocybin decreased center time. This dose-dependent interaction correlates with psilocybin-induced increases in corticosterone levels that peak at 15 min and return to baseline by 4 h. In addition, the non-hallucinogenic compound lisuride and NDMAR antagonist ketamine were used and demonstrated a transient increase in corticosterone concentrations at 15 min and returned to

baseline by 4 h. When tested in the novelty suppressed feeding test, all three compounds reduced the latency to feed 4 h post-injection. Although following exposure to chronic oral corticosterone and when administered the glucocorticoid antagonist, mifepristone, both psilocybin and ketamine lost this anxiolytic effect. Chronic corticosterone and mifepristone suppressed the psilocybin-induced stress response and increase corticosterone release. At 7 days post-injection, when assessed in the OFT psilocybin demonstrated an anxiolytic effect, whereas ketamine and lisuride did not. Notably, this long-term behavioral effect of psilocybin was found to interact with corticosteroid status at the time of treatment, where chronic corticosterone exposure caused psilocybin to be anxiogenic at 7 d. These results suggest that psilocybin-induced stress response, and increased corticosterone, are supportive of the observed anxiolytic effects. Interestingly, psilocybin's long-term anxiolysis is sensitive to elevated corticosterone concentrations at the time of drug administration. Together, these observations highlight critical distinctions between drug-induced and exogenous manipulations of corticosterone concentrations on psilocybin’s behavioral effects.

Disclosures: **N. Jones:** None. **J. Razidlo:** None. **Z. Zahid:** None. **L. Wagner:** None. **C.J. Wenthur:** None.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.07

Topic: G.05. Mood Disorders

Support: Transpharmation Poland Ltd

Title: Psychoactive substances in the treatment of depressive disorder: Which screening test to choose?

Authors: *J. KWIATKOWSKA, M. DOMżALSKA, E. SOKOŁOWSKA;
Transpharmation Poland Ltd., Olsztyn, Poland

Abstract: More than half of depressed patients are resistant to standard treatments available on the market. Recent research has shown, that psychoactive substances, such as psilocybin, which is a nonselective 5-HT2A receptor agonist, present both short- and long-term therapeutic properties even after a single administration . Therefore, there is a growing need for a rapid testing platform adjusted for new generations of drugs with a similar mechanism of action. Here we aim to determine which of the commonly used rapid pharmacodynamic response tests may be useful in assessing the antidepressant properties of the 5HT2A receptor agonists. Briefly, naive 10-13 week old C57BL/6J mice were treated with either vehicle, ketamine (3, 10 mg/kg), buspirone (0.3 mg/kg), or psilocybin (3, 5, 10 mg/kg) and underwent either the open field (OF) and tail suspension test (TST) or sucrose preference (SP) test and forced swim test (FST). Behavioural response to the treatment was measured at 24 hr and 7-day time points in order to

assess short- and long-term efficacy. In the OF test, only psilocybin at the highest dose (10mg/kg) showed anxiolytic efficacy 24h post-administration by decreasing ($p<0.5$) the latency time to the first entry to the centre area. However, none of the tested doses had a significant effect on time spent in the centre, periphery or corners. Moreover, psilocybin at 5mg/kg is affecting locomotor activity 24h post-administration ($p<0.01$). For comparison, our buspirone treated group showed a clear anxiolytic effect, reduced time in the periphery ($p<0.05$) and increased ($p<0.005$) time in the centre. We did not observe any anxiolytic efficacy from either of the drugs 7 days post-administration. In the SP test, vehicle-treated mice demonstrated high sucrose preference leaving us with no therapeutic window for potential drug effects. In the FST test, both ketamine ($p<0.0001$) and two highest doses of psilocybin (5 mg/kg; $p<0.005$, 10 mg/kg; $p<0.5$) presented antidepressant efficacy 24hr post-administration. Moreover, 7 days post-administration ketamine effect on immobility time was still visible ($p<0,05$). Neither ketamine nor any dose of psilocybin presented antidepressant efficacy in the TST at any of the timepoints Taken together, this data shows that not all of the commonly used pharmacodynamic response tests are applicable to evaluate the antidepressant properties of the 5HT2A receptor agonists. Careful consideration is required for the selection of the appropriate testing platform.

Disclosures: **J. Kwiatkowska:** A. Employment/Salary (full or part-time):; Transpharmation. **M. Domżalska:** A. Employment/Salary (full or part-time):; Transpharmation Poland Ltd. **E. Sokołowska:** A. Employment/Salary (full or part-time):; Transpharmation Poland Ltd.

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.08

Topic: G.05. Mood Disorders

Support: Transcend Therapeutics

Title: Circuit- and behavioral- level investigation of plasticity after administration of psychedelics and entactogens in mice

Authors: *A. L. YU[1], A. BASU[4], M. DIBBS[1], M. STONGIEW[5], B. MANDELL[5], J. WARNER-SCHMIDT[5], C. J. PITTENGER[2], A. C. KWAN[6], A. P. KAYE[3];
[2]Psychiatry, [3]Dept. of Psychiatry, [1]Yale Univ., New Haven, CT; [4]Psychiatry, Yale Univ. Interdepartmental Neurosci. Program, New Haven, CT; [5]Transcend Therapeut., New York, NY; [6]Biomed. Engin., Cornell Univ., Ithaca, NY

Abstract: Psychedelic compounds and entactogens appear to have therapeutic effects in human psychiatric disorders such as depression (e.g., psilocybin) and posttraumatic stress disorder (e.g., MDMA). Classic hallucinogenic psychedelics such as psilocybin are serotonin 2A (5-HT2A) agonists, while others (sometimes called entactogens, such as MDMA) strongly induce serotonin release. In order to understand how these mechanisms may relate to therapeutic effects, we

attempted to map a phenethylamine psychedelics and entactogens onto behavioral and circuit-level phenotypes. In this study, we have evaluated the behavioral effects of these compounds through studying the head twitch response as a proxy for hallucinogenic 5-HT2A effects of psychedelic drugs. In addition, we identified patterns of fear extinction acceleration, which may relate to PTSD treatment. While a 5-HT2A agonist had a large head twitch response similar to the classic hallucinogen DOI, entactogens showed a small response or no response similar to previous studies of MDMA. These results were divergent from the fear extinction responses to the same drugs, which appeared uncorrelated to 5-HT2A agonism. Entactogens showed acute enhancement of fear extinction that dissociated from their limited or absent head twitch responses. Further studies using optical biosensors may better relate mechanisms of pharmacological agonism and monoamine release to structural plasticity. Overall, entactogen drugs appear to express fear extinction-related effects which may enhance functional plasticity, although the relationship between functional and synaptic plasticity is not well understood. This may result from serotonin- or other monoamine-releasing effects rather than activation of the 5-HT2A receptor. Optical interrogation of neural circuits underlying these effects may help to validate and improve future development of treatments for PTSD and other anxiety disorders.

Disclosures: **A.L. Yu:** None. **A. Basu:** None. **M. Dibbs:** None. **M. Stongiew:** A. Employment/Salary (full or part-time):; Transcend Therapeutics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics Inc. **B. Mandell:** A. Employment/Salary (full or part-time):; Transcend Therapeutics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics Inc. **J. Warner-Schmidt:** A. Employment/Salary (full or part-time):; Transcend Therapeutics Inc.. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Transcend Therapeutics Inc. **C.J. Pittenger:** 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.; Transcend Therapeutics Inc.. F. Consulting Fees (e.g., advisory boards); Biohaven, Teva, Lundbeck, Brainsway, Ceruvia Lifesciences, Freedom Biotech. **A.C. Kwan:** 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.; Transcend Therapeutics Inc.. F. Consulting Fees (e.g., advisory boards); Empyrean Neuroscience, Freedom Biosciences. **A.P. Kaye:** 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.; Transcend Therapeutics Inc..

Poster

068. Neural and Behavioral Effects of Psychoactive Drugs

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 068.09

Topic: G.05. Mood Disorders

Support: Transpharmation Poland Ltd.

Title: Long term and delayed effects of ketamine and psilocybin in the preclinical chronic social defeat mouse model of depression.

Authors: *M. DOMZALSKA, J. KWIATKOWSKA, E. SOKOŁOWSKA;
Transpharmation Poland Ltd., Olsztyn, Poland

Abstract: Chronic social defeat (CSD) is a stress-induced psychosocial model of depression in mice that resemble depressive illness and additionally is selectively sensitive to clinically effective antidepressants. It is involving daily physical interaction and 24h sensory contact with an unfamiliar aggressive male. Following CSD, mice can be divided into stress-susceptible (SUS) or stress-resilient (RES) based on social avoidance behaviour. Growing evidence suggests that non-competitive NMDA antagonist - ketamine as well as non-selective 5-HT2AR agonist - psilocybin and its derivatives exert antidepressant efficacy. In this study, we aim to determine social avoidance behaviour in mice subjected to CSD and to assess the antidepressant effect of acute (24 hr) and long-lasting (7 days) ketamine (10 mg/kg, s.c.) and two doses of psilocybin (3 and 10 mg/kg, i.p.) after a single administration. Briefly, 7 weeks old C57BL/6J male mice were subjected to 10 days of CSD procedure followed by the first social preference test (SP) to assess social avoidance phenotype distribution in mice when exposed to an “unfamiliar” CD-1 male mouse. After that both SUS and RES groups were treated with either: vehicle (0.9% NaCl, n=9), ketamine (10 mg/kg, n=8) or psilocybin at two doses (3 mg/kg, n=9 or 10 mg/kg, n=9). All animals were re-submitted to the second SP test 24 hr and again to the third one 7 days post-treatment. A SUS phenotype was identified in 51% of mice subjected to the procedure as indicated by a significant ($p<0.0001$) decrease in social preference score in comparison to RES and control animals. A single administration of ketamine resulted in a significant reduction of social avoidance in SUS mice both 24 hr ($p<0.001$) and 7 days ($p<0.0001$) post-administration. Psilocybin at a dose of 3 mg/kg significantly ($p<0.05$) reduced social avoidance in SUS animals but only 24 hr post-administration, whereas 10 mg/kg resulted in a significant reduction of social avoidance in SUS mice 24 hr ($p<0.001$) as well as 7 days ($p<0.05$) post-administration. Together, in this study we have shown that the CSD procedure induced depressive-like behaviour in a subset of mice which was reversed by a single dose of ketamine as well as both doses of psilocybin. Here we have shown for the first time that the antidepressant effect of ketamine, as well as psilocybin, can persist over prolonged period of time after just a single administration. Thus, our CSD protocol represents a translational model to make efficacy comparisons between traditional and novel antidepressants and has the potential to identify new, personalized avenues for depression therapy, including novel compounds based on psilocybin derivatives.

Disclosures: **M. Domzalska:** A. Employment/Salary (full or part-time):; Transpharmation Poland Ltd. **J. Kwiatkowska:** A. Employment/Salary (full or part-time):; Transpharmation Poland Ltd. **E. Sokołowska:** A. Employment/Salary (full or part-time):; Transpharmation Poland Ltd..

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.01

Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA049531
Cummings School of Veterinary Medicine Office of Research and Graduate Education

Title: Oxycodone Self-Administration during Pregnancy : Do Neonatal Ultrasonic Vocalizations Predict Adult Risk of Substance Use Disorder

Authors: *C. C. A. AARON[1], K. R. GILDAWIE[2], J. F. CELATKA[2], K. E. BUDGE[2], S. B. ISGATE[2], K. R. FLEMING[2], F. M. VASSOLER[2], E. M. BYRNES[2];
[1]Neurosci., Tufts Univ. Grad. Sch. of Biomed. Sci., North Grafton, MA; [2]Comparative Pathobiology, Tufts Univ. Cummings Sch. Vet Med., North Grafton, MA

Abstract: Oxycodone Self-Administration during Pregnancy: Do Neonatal Ultrasonic Vocalizations Predict Adult Risk of Substance Use Disorder
Authors:Chantal Aaron, Kelsea Gildawie, Jillian F. Celatka, Kerri E. Budge, Sara B. Isgate, Katie R. Fleming, Fair Vassoler, Elizabeth M. Byrnes
Abstract:The use and abuse of opioids has increased dramatically over the past decade, resulting in a fivefold increase in the number of infants experiencing neonatal opioid withdrawal syndrome (NOWS). To date, the factors influencing NOWS severity as well as the relationship between NOWS and long-term neurodevelopmental effects remain unknown. We previously reported alterations in ultrasonic vocalizations (USVs) in oxycodone-exposed pups and disrupted maternal behavior in rat dams following daily 1h oxycodone self-administration (SA) during pregnancy. In the current study we used a long-access SA model (6h daily) as well as cross-fostering to determine the relationship between prenatal oxycodone intake and USV number/quality in that absence of alterations in maternal care. In addition, we examined whether maternal intake level and/or USV parameters are associated with increased risk of substance use disorders in adult offspring. Female Sprague-Dawley rats were surgically implanted with a jugular catheter and trained to self-administer oxycodone 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 were pregnant, they had daily access (7 days/week) to operant chambers and were allowed to self-administer oxycodone for 6h/day. On postnatal day 1 (PND1) litters were culled to 4 females and 4 males, and cross-fostered to time-mated drug naïve donor mothers. Pup USV, body weight and activity levels were analyzed on PND3, 6, 9, and 12. As adults, male and female offspring were trained on either oxycodone or cocaine SA with progressive ratio schedules used to assess motivated responding. Additional offspring were examined for expression of the epigenetic regulator MeCP2 during postnatal development. Results indicate alterations in USVs are intake dependent during early postnatal development and preliminary data suggest effects on oxycodone but not cocaine SA in adult offspring. Analyses in progress will determine whether USVs and/or intake correlate with adult outcomes

and whether changes in MeCP2 expression during development play a role.
This work is supported by the National Institute on Drug Abuse R01 DA049531 andCummings School of Veterinary Medicine Office of Research and Graduate Education.

Disclosures: C.C.A. Aaron: None. **K.R. Gildawie:** None. **J.F. Celatka:** None. **K.E. Budge:** None. **S.B. Isgate:** None. **K.R. Fleming:** None. **F.M. Vassoler:** None. **E.M. Byrnes:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.02

Topic: G.09. Drugs of Abuse and Addiction

Support: R01DA043982

Title: Maternal inflammation and perinatal cannabinoid exposure both impair cognitive behavior in adult offspring

Authors: *H.-T. CHEN, K. MACKIE;
Dept. of Psychology and Brain Sci., Indiana Univ., Bloomington, IN

Abstract: Maternal immune activation (MIA) is a ubiquitous response to infection during pregnancy and may have severe neurological consequences, especially if it occurs during key periods of fetal brain development. MIA increases cytokine levels and causes an inflammatory response. Marijuana use and exposure to cannabinoids can also be proinflammatory. Use of marijuana during pregnancy is increasing. In this study we sought to compare the deficits and underlying mechanisms by which perinatal THC and MIA worsen neurological outcomes. Thus, we compared perinatal cannabinoid exposure (PCE) of both wildtype and CB1 cannabinoid receptor (CB1R) knock out mice from gestational day 5 (GD5) with acute Poly (I:C) injection (at GD16.5) eliciting MIA. We then tested the emotional and cognitive behaviors of offspring as adults. Adult offspring after maternal PCE or MIA were impaired in the novel object recognition and delayed alternated working memory tasks. This impairment was absent in the CB1KO PCE group but present in the CB1 KO MIA group. We also found that neither MIA nor PCA affected offspring anxiety or locomotor behaviors. Our findings suggests that both PCE and MIA impair proper development of circuits mediating working memory, but only in the case of PCE is this deficit CB1R-mediated.

Disclosures: H. Chen: None. **K. Mackie:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.03

Topic: G.09. Drugs of Abuse and Addiction

Support: National Institute on Minority Health and Health Disparities (U54-MD012393 [subproject 5378], PIs: Trucco, Sutherland)

Title: Amygdala-insula functional connectivity and its mediating role between hopelessness, depression, and adolescent substance use.

Authors: *B. D. SUTHERLAND[1], J. S. FLANNERY[2], M. C. RIEDEL[1], L. D. HILL-BOWEN[1], P. M. VIERA PEREZ[1], K. CROOKS[1], A. R. LAIRD[1], E. M. TRUCCO[1], M. T. SUTHERLAND[1];
[1]Florida Intl. Univ., Miami, FL; [2]Psychology, Univ. of North Carolina, Chapel Hill, NC

Abstract: Introduction: Adolescent substance use (SU) is a public health concern. Use rates of e-cigarettes, marijuana, and alcohol remain high, and elucidating factors leading to use is critical. Hopelessness is a risk factor for SU. Yet, other mechanisms such as neurobiological factors and depression may explain this association. Amygdala-insula resting-state functional connectivity (rsFC) is associated with internalizing symptoms and depression is linked to SU. As such, we examined the interrelations between hopelessness, amygdala-insula rsFC, depressive problems, and teen SU. **Methods:** A sample of 146 adolescents (48% female, 88% White, 85% Hispanic/Latinx, M_{age}=14.9 at initial visit) from a larger multi-wave study were assessed. Adolescents completed a 10-minute rs-fMRI scan at wave 1 (W1). We used a left laterobasal amygdala (lbAMY) subregion seed given associations between lbAMY and anterior insula rsFC with mental health outcomes. We separately considered ventral (vI) and dorsal (dI) anterior insula given distinct subregional functionality. The Substance Use Risk Profile Scale assessed hopelessness (collected during an eligibility screen). Depressive problems (using the Youth Self Report's DSM scale), e-cigarette, marijuana, and alcohol use were assessed at wave 2 (~15 months after MRI scanning). Serial mediation models estimated the influence of hopelessness on SU via lbAMY-anterior insula (i.e., vI, dI) rsFC and depression when controlling for sex, age, ethnicity, average framewise displacement, depression, and SU at W1. **Results:** Indirect effects were observed when considering lbAMY-vI in the e-cigarette (indirect effect=0.01, CI [0.0003,0.02]) and marijuana (indirect effect=0.01, CI [0.001,0.02]) models. Hopelessness levels predicted high rsFC, high rsFC predicted greater depressive problems, which in turn were associated with more use. A similar indirect effect linking these variables was not observed when considering lbAMY-dI rsFC. Further, indirect effects were not observed for the alcohol models. **Conclusions:** These outcomes highlight amygdala-insula rsFC and depression as potential factors linking self-reported hopelessness to e-cigarette and marijuana use. While prior work suggests hopelessness can be predictive of future depression, in this study we did not observe a direct relation between hopelessness and depression; rather; elevated amygdala-insula rsFC explained this link. We also observed a dissociation within anterior insula subregions consistent with prior work linking the vI with emotional processing and the dI with cognitive tasks. These findings highlight potential intervention points to reduce teen SU.

Disclosures: **B.D. Sutherland:** None. **J.S. Flannery:** None. **M.C. Riedel:** None. **L.D. Hill-Bowen:** None. **P.M. Viera Perez:** None. **K. Crooks:** None. **A.R. Laird:** None. **E.M. Trucco:** None. **M.T. Sutherland:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.04

Topic: G.09. Drugs of Abuse and Addiction

Support: Roche Fellowship
Williams College

Title: Enduring Immune Consequences of Early-Life Opioid Exposure

Authors: *G. E. REYNOLDS, N. DAVIDSON, M. E. HOLTZE, Y. ISHIKAWA, L. MASSAC, S. A. ROBINSON;
Neurosci., Williams Col., Williamstown, MA

Abstract: Maternal opioid use more than doubled between 2010 and 2017. Despite this dramatic increase, the developmental and long-term outcomes of early life opioid exposure, in addition to the mechanisms underlying its severity, are not well understood. Recent studies have provided novel insights into the neuroimmune consequences of opioid exposure; however, this work has largely been conducted in adult rodent models. Our lab sought to investigate the long-term neuroimmune effects of early life opioid exposure using a neonatal opioid exposure paradigm in which mice pups are administered saline (4 litters/*n*= 30 pups) or 10 mg/kg morphine (6 litters/ *n* = 36 pups) bidaily from postnatal days (PNDs) 1-14, a developmental period equivalent to the third trimester of a human pregnancy. A repeated measures ANOVA revealed that morphine treatment significantly delayed weight gain over time ($p < .01$), consistent with findings of delayed coordination and time-appropriate development. Indeed, on PND 7 morphine pups showed significantly reduced surface righting ($p < .0001$, n = 30-36 per group) and negative geotaxis ability ($p < .01$, n=30-36 per group) when compared to saline controls. Such observations persisted into PND 14, with morphine pups showing a substantial deficit in an open field/extinguishment of pivoting behavior task ($p < .05$, n = 22-25 per group). Further, precipitated withdrawal experiments conducted on PND 14 demonstrated morphine pups showed significant naloxone-precipitated withdrawal behavior when compared to saline pups ($p < .0001$, n = 13-17 per group). A subset of saline and morphine exposed pups were aged to adulthood (PND 60) and administered a single injection of 0.1 mg/kg lipopolysaccharide (LPS) or saline. Preliminary data suggests that morphine exposed mice exhibit increased sickness behavior (as measured by reduced locomotor activity) 24 hrs following an LPS challenge when compared to saline controls ($p < .05$, n = 6 per group). Future work in the lab aims to further study the enduring inflammatory consequences—particularly in the neuroimmune system—of early life opioid exposure by molecularly profiling neuroinflammatory markers at multiple developmental

timepoints (PNDs 14, 21, 60), thus developing a longitudinal profile of altered immune function in our mouse model of neonatal morphine exposure.

Disclosures: G.E. Reynolds: None. **N. Davidson:** None. **M.E. Holtze:** None. **Y. Ishikawa:** None. **L. Massac:** None. **S.A. Robinson:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.05

Topic: G.09. Drugs of Abuse and Addiction

Support: Roche Fellowship, Williams College

Title: The effects of perinatal morphine exposure on adolescent cognition and drug sensitization

Authors: S. R. VEALE, A. OROZCO, G. REYNOLDS, V. SALTZ, D. HAHN, K. STERN, *S. A. ROBINSON;
Williams Col., Williamstown, MA

Abstract: Opioid use among pregnant women in the U.S. has increased drastically over the past years. However, the long-term neurobehavioral consequences associated with early opioid exposure in humans are not well understood. The goal of this study was to determine how early-life opioid exposure affects sensitivity to drugs of abuse later in life. Pregnant dams were given either saline (n = 8 dams/ 63 pups) or morphine (10 mg/kg, n= 9 dams/ 58 pups) injections from gestational day 0 to postnatal day (PND) 7, equivalent to a full trimester human pregnancy. There were no statistically significant differences between treatment groups in weight gain from PND1-21, however, a chi-square test revealed morphine-induced delays in development on the surface righting test on PND7 ($p = 0.03$). We first evaluated the effects of a second morphine exposure during adolescence (PND 30-40) on recognition memory using a novel tactile recognition task. Male and female mice were given a single dose of saline or morphine (20 mg/kg) immediately after habituation and tested in the novel condition 24 hrs later (n= 10/group). A two-way ANOVA revealed a significant Perinatal x Adolescent drug exposure interaction ($p =0.017$). Perinatal morphine treatment coupled with adolescent morphine exposure increased time exploring the novel tactile stimulus compared to mice treated with saline perinatally and morphine as adolescents ($p < .05$). In a separate study, we examined adolescent locomotor sensitization. Male and female mice exposed to either perinatal saline (n = 30) or morphine (n =30) were administered 4 repeated doses of saline (n = 10/group), methylphenidate (n =9-11/group), or oxycodone (n = 10/group) and assessed for locomotor activity. A three-way ANOVA revealed a main effect of adolescent exposure in the methylphenidate-treated animals ($p=0.001$) in addition to a Day x Adolescent Exposure interaction ($p=0.002$). Sensitization was observed in animals perinatally exposed to saline ($p=0.026$), however, there was no effect in animals perinatally exposed to morphine ($p=0.82$). There was also a Day x Adolescent Exposure

interaction effect for oxycodone treatment ($p < 0.0001$). However, sensitization was observed in morphine exposed animals ($p = 0.01$) but not saline ($p = 0.99$). Overall, these data suggest that perinatal morphine exposure delays the formation of developmental milestones, protects against the effects of adolescent morphine exposure on recognition memory, and induces drug-specific effects on locomotor sensitization in adolescence. These findings highlight the nuanced effects of early-life opioid exposure and support further investigation into the long-term effects.

Disclosures: **S.R. Veale:** None. **A. Orozco:** None. **G. Reynolds:** None. **V. Saltz:** None. **D. Hahn:** None. **K. Stern:** None. **S.A. Robinson:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.06

Topic: G.09. Drugs of Abuse and Addiction

Title: Morphological assessment of microglia after adolescent Δ^9-tetrahydrocannabinol and adulthood heroin exposure

Authors: *M. X. MARTINEZ, C. M. RUIZ, V. C. INSHISHIAN, E. CASTILLO, S. V. MAHLER;
Univ. of California, Irvine, Irvine, CA

Abstract: Adolescence is a critical developmental window for prefrontal cortex (PFC), and exposure to drugs of abuse during this period can have long-term consequences on PFC and connected neural circuits. Accordingly, studies in humans and rodents have shown that cannabinoid drug exposure during adolescence may lead to alterations in cognitive function, as well as opioid intake and other addiction-relevant behaviors. Microglia are the resident macrophages of the brain, but they also play other roles in the brain, such as engaging in activity-dependent synaptic pruning, and influencing synaptic plasticity. A recent study (Lee et al., 2022, Biol Psychiatry) found that adolescent Δ^9-tetrahydrocannabinol (THC) exposure produced a state of microglial dyshomeostatis, and persistently impaired their normal responses to immunological insult with lipopolysaccharide or social stress during adulthood. Drugs of abuse including opioids can also activate microglia, but whether adolescent THC also persistently alters microglial responses to abused drugs is poorly understood. Here, we exposed female Long-Evans rats to THC (5mg/kg i.p.) or vehicle daily during adolescence, from postnatal day 30 to 43. After they grew into adulthood (PD70+) we administered to both groups escalating doses of heroin (1, 3, 5, and 8 mg/kg i.p.), or saline only, twice a day over a four-day period. Twelve hours after the last injection, when rats were in acute, spontaneous heroin withdrawal, we perfused and harvested brains. We stained PFC sections with the general microglia marker IBA1 to allow for quantification of microglia structural characteristics, using Imaris software. We conducted multiple assessments of microglial structural morphology including number of microglia, soma size and volume, process length, process volume, and Sholl analyses. We found several

microglia metrics were altered in heroin-treated rats with a history of adolescent THC treatment. Results will provide further insights into how adolescent THC exposure persistently impacts microglia responses to drug insults and provide clues for further investigations into the functional implications of these findings for augmented opioid addiction susceptibility.

Disclosures: M.X. Martinez: None. **C.M. Ruiz:** None. **V.C. Inshishian:** None. **E. Castillo:** None. **S.V. Mahler:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.07

Topic: G.09. Drugs of Abuse and Addiction

Title: Exposure to cannabinoids in adolescence shortens cocaine conditioned reward and alters cocaine induced brain activation and dopamine release in adulthood

Authors: L. S. ESTRADA[1], C. M. DE ANDA GAMBOA[1], M. A. FRANCO[1], S. L. CABILING[1], V. M. AYVAZIAN[2], L. ZHANG[3], *J. M. WENZEL[1], J. F. CHEER[4]; [1]Univ. of San Diego, San Diego, CA; [2]Univ. of Maryland Baltimore, Baltimore, MD; [3]Anat. & Neurobio., Univ. of Maryland Sch. of Medicin Program In Neurosci., Baltimore, MD; [4]Anat. and Neurobio., Univ. of Maryland Sch. of Med., Baltimore, MD

Abstract: Cannabinoids (CBs) are the most commonly abused illicit drugs among adolescents, and use during this sensitive period is associated with the development of psychiatric disease, including cocaine (COC) use disorder. The cause of any vulnerability, however, remains unclear. Given the well-documented role of dopamine (DA) in COC conditioned reward and reinforcement along with the ability of CBs to augment DA release, it is possible that CB exposure during this critical window affects DA system maturation resulting in long-term effects on neurobiological and behavioral responses to COC. To examine this, adolescent rats received daily CB administration. In adulthood rats were tested for the development of COC conditioned place preference (CPP) and aversion (CPA). Human and animal studies show that COC administration produces initial reward which then gives way to dysphoria and anxiety. Indeed, rats develop a CPP to the immediate effects of COC (0-5 min after administration) and a CPA to the delayed effects of the drug (15 min after). We found that adolescent, but not adult, CB exposure dose dependently attenuates CPP for COC, and, in fact, adolescent treatment with a moderate CB dose results in CPA in adulthood. It is counterintuitive that adolescent exposure would abolish COC reward, considering the increased incidence of abuse in individuals with CB experience. By shortening the conditioning session (from 5 to 2 min), we revealed that both CB-treated and control rats developed COC CPP, suggesting that CB treatment does not abolish COC reward in adulthood, but hastens the switch from positive to negative experience of the drug. Additional studies confirm altered brain activation following cocaine administration and a

blunted and shortened DAergic response to COC in CB-treated rats, which likely underlies this behavioral effect.

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Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

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Program #/Poster #: 069.08

Topic: G.09. Drugs of Abuse and Addiction

Support: F32AA027702
R01AA024798
R01AA027653

Title: Embryonic ethanol exposure induces ectopic neurons expressing hypocretin/orexin and melanin-concentrating hormone: Role of the Cxcl12/Cxcr4 chemokine system.

Authors: *A. COLLIER, N. YASMIN, G.-Q. CHANG, N. KHALIZOVA, M. FAM, B. YU, A. ABDULAI, S. F. LEIBOWITZ;
Rockefeller Univ., New York, NY

Abstract: Embryonic exposure to ethanol increases the risk for alcohol use disorder in humans and stimulates alcohol-related behaviors in different animal models. Evidence in both rats and zebrafish suggests that this phenomenon induced by ethanol at low-moderate concentrations involves a stimulatory effect on the density of hypothalamic neurons expressing the peptides hypocretin/orexin (Hcrt) and melanin-concentrating hormone (MCH), known to promote alcohol consumption. Building on our studies in zebrafish showing that embryonic ethanol exposure stimulates Hcrt migration and induces ectopic Hcrt expression outside the hypothalamus, we investigated here whether ethanol also induces ectopic peptide neurons in rats, if these ectopic neurons have a role in ethanol-induced disturbances in behavior, and if the neuroimmune chemokine Cxcl12/Cxcr4 system mediates these effects. We demonstrate here in rats that ethanol at a low-moderate dose, in addition to increasing Hcrt and MCH neurons in the lateral hypothalamus where they are normally concentrated, induces ectopic expression of these neurons further anterior, in the nucleus accumbens core and ventromedial caudate putamen, where they have not been previously observed. Similar to rats, ethanol exposure in zebrafish stimulates Hcrt neurons in the hypothalamus where they are normally concentrated and induces ectopic Hcrt neurons further anterior in the preoptic area, and laser ablation of these ectopic Hcrt neurons blocks behaviors induced by ethanol, including increased anxiety and locomotor activity. Ethanol's stimulatory effects on hypothalamic and ectopic Hcrt neurons and behavioral disturbances are blocked by embryonic administration of an antagonist of the chemokine

receptor Cxcr4. Ethanol also increases *cxcl12a* and *cxcr4b* transcripts and internalization of Cxcr4b receptors throughout the brain, and these effects occur specifically in Hcrt neurons stimulated by ethanol in the most anterior hypothalamic region and anterior ectopic Hcrt neurons in the preoptic area, but not in more posterior Hcrt neurons unaffected by ethanol. These results support a role of the ethanol-induced ectopic Hcrt neurons and Cxcl12/Cxcr4 system in these ectopic neurons in contributing to the behavioral disturbances induced by ethanol.

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Poster

069. Addictive Drugs Affecting Development

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.09

Topic: G.09. Drugs of Abuse and Addiction

Title: Sex differences in approach behavior toward a port that delivers nicotine plumes in an electronic vapor delivery system

Authors: *V. E. ESPINOZA[1], P. GINER[1], I. LIANO[1], I. A. MENDEZ[2], L. E. O'DELL[1]; [1]Psychology, [2]Pharmaceut. Sci., The Univ. of Texas At El Paso, El Paso, TX

Abstract: The goal of our laboratory is to utilize rodent models to study the underlying mechanisms of nicotine use in humans, particularly in vulnerable populations. To more closely mimic human use patterns, the present study employed passive exposure to nicotine vapor for 14 days in adolescent and adult female and male rats. We examined age and sex differences in approach behavior toward a port that delivered nicotine plumes on Day 1 and 14 of our vapor exposure regiment. Group differences in physical signs of withdrawal and plasma levels of the nicotine metabolite cotinine were assessed on Day 14. The results revealed that over time, female rats displayed a larger increase in approach behavior than males, an effect that was significantly larger in adolescent rats. The female adolescent rats displayed higher cotinine levels than all other groups, an effect that was likely related to higher nosepoke responses in the port that delivered nicotine vapor. These findings suggest that nicotine vapor produces greater motivational effects in adolescent female rats versus males. This work provides a foundation for future studies examining the mechanisms that modulate age and sex differences produced by chronic nicotine vapor exposure, particularly under conditions that involve voluntary self-administration of nicotine vapor.

Disclosures: V.E. Espinoza: None. **P. Giner:** None. **I. Liano:** None. **I.A. Mendez:** None. **L.E. O'Dell:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.10

Topic: G.09. Drugs of Abuse and Addiction

Support: PNSD 2019I012

Title: Effects of dronabinol administration during gestation and lactation in the offspring

Authors: *D. NAVARRO[1,2,3], A. GASPARYAN[4,2,3], F. NAVARRETE[1,2,3], J. MANZANARES[5,2,3];
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Abstract: There has been a worrying increase in cannabis use during pregnancy and lactation. It is known that the amount of THC in recreational cannabis has increased by more than 10% in recent years. The studies conducted on the impact of exposure of the fetus or newborn to THC suggest significant brain and behavioral alterations. However, it is still unknown how certain aspects related to perinatal cannabis exposure, such as the amount consumed, the consumption pattern, and the initiation and duration of consumption, especially during fetal development, may affect the development and performance of the newborn. This study aimed to develop a new animal model of perinatal exposure to dronabinol, evaluating the alterations produced in the offspring. Male and female C57BL/6J mice were crossed, and after confirming gestation in the females, the administration of dronabinol (10 mg/kg/12 h, from gestational day 5 (GD5) to postnatal day 21 (PND21) was started. In the offspring exposed to dronabinol, traits of anxiety, depression, and cognitive, as well as the level of alcohol consumption and motivation, were evaluated. Relative gene expression of corticotropin-releasing factor (CRF) in the paraventricular nucleus (PVN) was analyzed by real-time PCR. On the other hand, NeuN and GFAP protein expression in the cortex was studied by immunohistochemistry. THC-exposed offspring were characterized by increased traits of anxiety, depression, and cognitive problems, as well as an increased vulnerability to alcohol consumption and motivation found in males. Notably, these behavioral alterations were accompanied by a significant reduction of CRF gene expression in PVN. Likewise, exposed offspring show alterations in cortical lamination and the number and distribution of astrocytes in the hippocampus, thus showing that the normal process of brain maturation is affected. These preliminary results provide relevant information about the severe consequences of perinatal THC exposure on different behavioral aspects. These may result from alterations in cortical development mechanisms and stress axis regulation. Further research is needed to demonstrate the brain alterations that justify the behavioral changes.

Disclosures: D. Navarro: None. **A. Gasparyan:** None. **F. Navarrete:** None. **J. Manzanares:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.11

Topic: G.09. Drugs of Abuse and Addiction

Support: F31AA026498
R01AA023508
T32AA0013525
UTHSC/UAMS CORNET Award.

Title: Identification of Ethanol-Induced Expression Changes in Genes Related to QTLs that Underlies Strain-Specific Vulnerability to Apoptosis in the Hippocampus in Neonatal BXD Recombinant Inbred Mice.

Authors: J. A. BAKER[1], *K. HAMRE[2];
[1]Ctr. for Behavioral Teratology, San Diego State Univ., San Diego, CA; [2]Univ. of Tennessee Hlth. Sci. Ctr., Memphis, TN

Abstract: Fetal Alcohol Spectrum Disorder (FASD) is a leading neurodevelopmental disorder with symptoms that include behavioral alterations and cognitive impairments that present in childhood and continue throughout life. Genetics have been shown to play a role in the severity of alcohol's teratogenic effects on the developing brain, including levels of neuronal death in brain regions important for learning and memory, such as the hippocampus. Using BXD recombinant inbred mouse strains, we previously identified quantitative trait loci (QTL) that may mediate strain-specific vulnerability to ethanol-induced apoptosis in the developing hippocampus. Subsequent microarray analysis identified significant differentially-expressed genes in the hippocampus after neonatal ethanol exposure in select BXD strains that were labeled as high or low cell death strains. The present study aimed to determine if these significant gene expression changes were related to the previously identified QTLs. To test this, we analyzed genes that are within 1 LOD interval of the significant QTL on chromosome (Chr) 12 and the suggestive QTL on Chr3. Within the QTL on Chr12, there were 42 genes that were significant for a strain x treatment interaction, including *Rps6kl1* and *Tgfb3,* that showed ethanol-induced fold changes greater than 1.5. On Chr3, there were 17 genes that were significant for strain x treatment interactions including *Fxr1* and *Usp13*. To identify other potential members of the molecular networks behind our cell death phenotype, we used tools available at GeneNetwork.org and identified 296 genes that were significantly correlated with our hippocampal cell death phenotype including growth factors (*Igf2* & *Tgfb3*) and genes involved in apoptosis (*Bcl2* & *Nkg7*). Moreover, expression of genes such as *Igf2, Klf4,* and *Usp1* have been previously linked to alterations due to alcohol exposure during development. Additional analysis found *Tgfb3* was significantly correlated with developmental apoptosis and neurogenesis as well as relevant behavioral phenotypes such as those related to learning and memory, or ethanol-induced responses. This study identified candidate genes and genetic pathways that could be

crucial in mediating ethanol-induced hippocampal neuronal death following neonatal ethanol exposure; these will be evaluated in future experiments. Support: F31AA026498, R01AA023508, T32AA0013525, & UTHSC/UAMS CORNET Award.

Disclosures: J.A. Baker: None. **K. Hamre:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.12

Topic: G.09. Drugs of Abuse and Addiction

Support: Miami University URA 2021
Miami University USS
Miami University URA 2022

Title: Effects of Adolescent Lorazepam Exposure on Adult Alcohol Preference and Response

Authors: *Z. L. PLATOW, O. SANDOVAL, E. A. BOGDANSKI, M. S. MCMURRAY; Psychology, Miami Univ., Oxford, OH

Abstract: Benzodiazepines such as lorazepam are strong anxiolytic drugs that act at GABA receptors, resulting in overall inhibition of the CNS. Benzodiazepines are one of few drugs that can be both prescribed by a doctor, but also recreationally abused. One in five high schoolers report using benzodiazepines either medically or recreationally. However, the longitudinal effects of benzodiazepine use during adolescence have not been well-investigated. The purpose of this experiment was to determine the effects of benzodiazepine exposure during adolescence on immediate stress levels and later adult alcohol self-administration in rats. A total of 48 rats (12/sex/group) received injections of either lorazepam (3.2mg/kg) or saline every other day during adolescence (postnatal days 30-50). Anxiety-associated behaviors were assessed in adolescents using Open Field and Elevated Zero Maze tests, which showed age-dependent effects, with lorazepam-exposed females demonstrating increased stress-reactivity on postnatal day 100, long after lorazepam exposure had ceased. In adulthood (postnatal day 100-141), voluntary alcohol self-administration was then assessed using a two-bottle choice, drinking in the dark paradigm. Results showed that adolescent exposure to lorazepam increased adult alcohol intake and preference in females only. Lastly, neural activity associated with acute intoxication was then assessed on postnatal day postnatal day 143. Rats were given an intraperitoneal injection of ethanol (1mg/kg), euthanized one hour later, and c-fos immunoreactivity assessed in the ventral tegmental area and the nucleus accumbens. Preliminary results suggest that lorazepam-exposed females demonstrated increased c-fos expression in both regions, providing a possible mechanism for their increased alcohol self-administration. Combined, these results suggest that early-life exposure to benzodiazepines may induce lifelong increases to stress levels and alcohol preference in a sex-dependent manner. The presence of such a significant sex-based

difference in side effects warrants further research to better elucidate mechanisms and may indicate a need to revise prescription guidelines and legal restrictions regarding benzodiazepines.

Disclosures: Z.L. Platow: None. **O. Sandoval:** None. **E.A. Bogdanski:** None. **M.S. McMurray:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.13

Topic: G.09. Drugs of Abuse and Addiction

Title: Effects of prenatal cannabis vapor exposure on nucleus accumbens projecting medial prefrontal cortical neurons

Authors: *D. GINDER[1], H. R. WRIGHT[2], H. WEIMAR[1], A. N. MALENA[4], Z. D. G. FISHER[3], T. G. FREELS[5], J. H. PETERS[1], R. J. MCLAUGHLIN[1];
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Abstract: Maternal cannabis use is a growing public health concern, yet the cognitive impacts of prenatal cannabis exposure and its underlying neural substrates remain largely unknown. Using a cannabis vapor delivery approach in pregnant rat dams, we have recently uncovered impairments in cognitive flexibility in cannabis-exposed offspring. Inputs from the medial prefrontal cortex (mPFC) to the nucleus accumbens (NAc) critically orchestrate decision-making under dynamically shifting environmental demands. However, the long-term impact of prenatal cannabis exposure on the function of this corticostriatal circuit has yet to be examined.To address this gap, we developed a novel model of maternal cannabis self-administration that employs response-contigent delivery of vaporized cannabis extracts. We used this model together with slice electrophysiology recordings in adult offspring to determine whether prenatal cannabis exposure alters basal excitatory and inhibitory currents in NAc-projecting mPFC neurons. Dams were trained to self-administer cannabis (69.9% THC; 150mg/ml, 1-hr sessions) or vehicle (80:20 propylene glycol: vegetable glycerol) vapor twice daily prior to and during gestation. On postnatal day (PND) 80-90, fluorescent retrobeads (200 nl/side) were injected into the NAc core and after a 1-2 week incubation period, the frequency and amplitude of spontaneous excitatory and inhibitory currents (sEPSC and sIPSC, respectively) were measured in retrobead-positive mPFC neurons. Cannabis self-administering dams showed greater discrimination for the cannabis-paired operandum and earned more vapor reinforcers than vehicle dams. Accordingly, cannabis-exposed pups weighed less than vehicle- and air-exposed pups on PND 1, which is in line with data from humans exposed to cannabis during pregnancy. Electrophysiology data indicate that NAc-projecting mPFC neurons exhibit a significant increase in the frequency of sEPSCs in cannabis-exposed offspring relative to vehicle-exposed offspring.

Although a treatment x sex interaction was not significant at the time of analysis, the increase in sEPSC frequency was primarily driven by effects in males. Data for sIPSCs is still in the process of being analyzed.Altogether, these data support the use of a response-contingent vapor delivery model in pregnant rat dams and indicate significant alterations in excitatory transmission within NAc-projecting mPFC neurons. Thus, a disrupted balance of excitatory and inhibitory transmission within this corticostriatal circuit may be a mechanism underlying cognitive flexibility impairments previously seen in cannabis-exposed offspring.

Disclosures: D. Ginder: None. **H.R. Wright:** None. **H. Weimar:** None. **A.N. Malena:** None. **Z.D.G. Fisher:** None. **T.G. Freels:** None. **J.H. Peters:** None. **R.J. McLaughlin:** None.

Poster

069. Addictive Drugs Affecting Development

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 069.14

Topic: G.09. Drugs of Abuse and Addiction

Support: WSU ADARP Grant
WSU Office of Research

Title: Impacts of maternal cannabis vapor self-administration on emotional reactivity, drug seeking, and cortical parvalbumin expression in offspring

Authors: *H. V. WEIMAR[1], A. N. MALENA[1], A. M. RICHARDS[1], H. S. AREY[1], D. E. GINDER[2], R. J. MCLAUGHLIN[1];
[1]Integrative Physiol. & Neurosci., [2]Psychology, Washington State Univ., Pullman, WA

Abstract: Cannabis use during pregnancy is becoming more common, yet the risks of prenatal cannabis exposure remain largely unknown. We developed a novel model of maternal cannabis use that employs response-contingent delivery of vaporized cannabis extracts in pregnant rat dams. We used this model to investigate emotional reactivity, cannabis self-administration, and patterns of cortical parvalbumin interneuron expression in offspring.
Female Sprague Dawley rats self-administered Δ^9 tetrahydrocannabinol (THC)-rich cannabis vapor (70% THC; 150 mg/mL) or vehicle vapor twice daily beginning one week before mating until 24-48 hr before the expected birth date. A third group of control dams were included that did not self-administer any vapor. Emotional reactivity was assessed in neonates using isolation-induced ultrasonic vocalizations (USVs). In adulthood (postnatal day (P) 90), anxiety-like behavior was assayed using the elevated plus maze and novelty suppressed feeding tasks. To investigate cannabis-seeking behavior, offspring were assigned to self-administer cannabis or vehicle vapor over 21 days using an increasing schedule of reinforcement followed by a 3-hour progressive ratio challenge. Finally, parvalbumin interneuron expression was measured in the medial prefrontal cortex (mPFC) using immunohistochemistry.
Dams self-administered cannabis vapor throughout gestation at higher rates than vehicle dams.

Cannabis-exposed offspring weighed less on P1, which mirrors findings of low birthweight in human offspring. During the neonatal period, cannabis-exposed offspring emitted more USVs than controls on P6, but not on P10 or P13. In adulthood, no differences in anxiety-like behavior were observed. With respect to vapor self-administration, male cannabis-exposed offspring made fewer nosepokes on the vapor-paired operandum and received fewer vapor deliveries than vehicle-exposed offspring, regardless of assigned vapor. Conversely, female offspring self-administered cannabis vapor significantly more than vehicle vapor and had significantly higher break points during the progressive ratio test, irrespective of prenatal exposure condition. There were no significant differences in the number or distribution of parvalbumin interneurons in the mPFC of cannabis-exposed offspring.
These results support the use of the cannabis vapor self-administration approach to investigate long-term effects of maternal cannabis use in developing offspring. Moreover, our data indicate transient effects of prenatal cannabis exposure on emotional behavior and sex-specific impacts on vapor self-administration specifically in male offspring.

Disclosures: H.V. Weimar: None. **A.N. Malena:** None. **A.M. Richards:** None. **H.S. Arey:** None. **D.E. Ginder:** None. **R.J. McLaughlin:** None.

Poster

070. Reward and Addiction

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 070.01

Topic: G.02. Reward and Appetitive Learning and Memory

Support: Washington State University Alcohol and Drug Abuse Research Program

Title: Impact of ketamine on perineuronal nets and parvalbumin in the medial prefrontal cortex and on cocaine-induced reinstatement after novel memory reactivation

Authors: *A. E. GONZALEZ[1,2], B. A. SORG[2];
[1]Washington State Univ., Portland, OR; [2]Dow Neurobio., Legacy Res. Inst., Portland, OR

Abstract: Strong drug-associated memories are difficult to disrupt. Amnestic agents presented along with novel information during memory retrieval may disrupt reward memories, and perineuronal nets (PNNs) in the medial prefrontal cortex (mPFC) help maintain cocaine memories. Ketamine preferentially acts on parvalbumin (PV) cells, most of which are surrounded by PNNs. We hypothesized that ketamine would reduce PNN and PV intensity in the mPFC and, if given before novel memory retrieval, reduce cocaine reinstatement. Rats were trained to self-administer cocaine on a fixed-ratio 1 (FR1) schedule and given no retrieval, a cocaine-reinforced memory retrieval on an FR1 schedule, or a novel variable-ratio 5 (VR5) schedule. Saline or ketamine (6 mg/kg, i.p.) was administered 10 min before retrieval. The following day, rats had 30 min of extinction followed by a 30 min cue reinstatement. Reinstatement was not reduced with FR1 or VR5 retrieval, suggesting no impact of ketamine on

memory updating. In a second experiment, one of two doses of ketamine (20 or 100 mg/kg, i.p.) or saline was administered every 72 hr over 7 days. Rats were euthanized 2 hr later, and the mPFC was immunostained for PV and *Wisteria floribunda* agglutinin (WFA) to label PNNs. Both doses of ketamine decreased PNN intensity by 15-20%, and the high dose decreased PV intensity by about 15%, with no changes in the number of cells. These findings suggest that a repeated subanesthetic dose of ketamine may be used in future studies to modify PNNs in the mPFC and disrupt cocaine memories.

Disclosures: A.E. Gonzalez: None. **B.A. Sorg:** None.

Poster

070. Reward and Addiction

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 070.02

Topic: G.02. Reward and Appetitive Learning and Memory

Support: R21 MH123926

Title: Role of ventral midbrain dopamine neurons in familiarity

Authors: *R. KOLARIC[1], S. FLEURY[1], J. TOMAIO[1], A. T. SÖRENSEN[2], U. GETHER[3], S. MINGOTE[4];
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Abstract: It is well established that dopamine (DA) neurons signal novelty by increasing their bursting activity. After repeated presentations of the same stimulus, the activity of the DA neurons decreases providing a physiological signature that a stimulus has become familiar. However, whether or not it plays an important role in facilitating familiarity is not clear. To test this idea, we used a Novel Object Recognition (NOR) paradigm where mice were presented with two identical objects (A, A) during a familiarization session, then presented with a new and previously seen object (A, B) during the subsequent NOR test session. Time spent exploring the new object provides an index of recognition for the familiar object. We first show that the ability to recognize the novel object is associated with 1) the amount of pre-exposure to the familiar objects and 2) level of DA activity during familiarization as measured by c-fos expression. We found that the activity of DA neurons remains high during the first encounter with the A,A objects, and that one familiarization session is not sufficient to produce robust novel object recognition. However, after 3 familiarization sessions, the activity of DA neurons during the last A,A exposure is significantly decreased, and the mice show robust novelty recognition during the NOR test session. We then investigated a causal role of DA in familiarity, and tested if decreasing DA neuron activity during the first encounter with the A,A is sufficient to drive rapid familiarization. We used chemogenetics to inhibit DA neurons in the ventral tegmental area

(VTA). TH-flp mice received injections of an AAV8 flp-dependent hM4Di. We then stimulated hM4Di receptors and found that inhibiting DA neurons during familiarization increased subsequent novel object exploration. Inhibiting DA neurons after the familiarization session did not improve NOR. These results suggest that suppressing DA neuron activity enhances familiarity recognition. Ongoing experiments suggest that this DA-dependent effect on familiarity may be due to a facilitation of object-context association.

Disclosures: R. Kolaric: None. **S. Fleury:** None. **J. Tomaio:** None. **A.T. Sörensen:** None. **U. Gether:** None. **S. Mingote:** None.

Poster

070. Reward and Addiction

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 070.03

Topic: G.02. Reward and Appetitive Learning and Memory

Support: CONACyT grant FOINS 474

Title: The anterior insular cortex modulates the nucleus accumbens activity through comunication with ventral tegmental area during reward memory formation

Authors: *A. CORTÉS, E. HERNÁNDEZ-ORTIZ, F. BERMÚDEZ-RATTONI;
Univ. Nacional Autonoma de Mexico, México, Mexico

Abstract: The consumption of substances of abuse activates diverse areas and circuits of the brain that include the dopaminergic mesolimbic system. This reward circuit includes the dopaminergic projections of the ventral tegmental area (VTA) towards the nucleus accumbens (NAc). However, recent studies have shown a relevant role of the insular cortex (IC) due to the processing of interoceptive and exteroceptive signals related to the consumption of substances of abuse. In this sense, the functional participation of the anterior insular cortex (aIC) in rewarding memory formation through its communication with the VTA to NAc circuit is unknown. Our results show that the aIC modulates the NAc activity through communication with the VTA during reward memory formation. To validate the communication and functionality of this tripartite pathway, we used anterograde transsynaptic labelling from the aIC, and the real time conditioned place preference (rtCPP) model was associated with optogenetic stimulation of the NAc terminals. To demonstrate the anatomical and neuronal projection of the pathway, we used confocal microscopy and found positive projections to eYFP from the VTA to the NAc that came, selectively, from the aIC. Finaly, we used immunohistochemistry against tyrosine hydroxylase and observed colocalization with eYFP positive neurons, which in accordance with the measurement through in vivo microdialysis of the extracellular concentration of the neurotransmission shows there is neuronal activity of the dopaminergic type during the optogenetic stimulation of the NAcc terminals. Our results suggest that aIC requires VTA dopaminergic release to modulate the NAc activity and enhance a rtCPP. In contrast, the activity

of the anatomical projection sent by the aIC to the Nacc is not sufficient to induce the reward memory formation.

Disclosures: A. Cortés: None. **E. Hernández-Ortiz:** None. **F. Bermúdez-Rattoni:** None.

Poster

070. Reward and Addiction

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 070.04

Topic: G.02. Reward and Appetitive Learning and Memory

Title: The Effect of Noncontingent Methamphetamine on Reward Learning in Rats

Authors: *A. VERGHESE[1], I. HOANG[2], M. SHARPE[2];
[2]Psychology, [1]UCLA, Los Angeles, CA

Abstract: Environmental cues predicting the arrival of specific rewards are used to govern adaptive behavior. For example, if you're walking down the street and smell the aroma coming from a fast food restaurant, it may entice you to go in to order a burger. Drugs of abuse can hijack these learning pathways. Indeed, drug addiction is thought to be driven by drug-paired cues having increased control over behavior associated with drug seeking. It is unclear whether the increases in the ability of drug-paired cues to exert control over behavior is due to the repetitive action to consume the drug (creating aberrant learning of drug contingencies), or whether the drug itself sensitizes circuits involved in reward learning. To test this, we examined whether non-contingent methamphetamine would impact cue-governed reward-seeking. Rats first received experimenter-administered injections of methamphetamine (drug group) or saline (control group) according to a schedule determined by rats that had self-administered methamphetamine in prior experiments in our lab. Then, rats underwent a Pavlovian-to-instrumental (PIT) procedure. This involves first teaching rats that two cues (e.g.tone and click) lead to two different rewards (e.g. sucrose pellets and maltodextrin liquid). Subsequently, rats separately learned to press levers to obtain these rewards (e.g. left lever→pellet, right lever→liquid). Finally, we test what rats have learnt about these associations by playing the cues in a test phase, and examining how this influences lever-pressing behavior in the absence of reward feedback. Usually, rats will show that they have learnt the specific contingencies by increasing responding on the lever producing sucrose when the cue paired with sucrose is played. This shows that they have formed a sensory-specific association between the cue and sucrose, and lever and sucrose, and are using that information to guide adaptive behavior. However, rats given prior injections of methamphetamine show an enhancement in cue-invigorated lever-press responding for specific rewards. This demonstrates that methamphetamine promotes reward-paired cues to exert heightened control over behavior directed towards specific rewards, and that this is due to influences of the drug on the circuit rather than changes in drug-reward contingencies. Given recent findings for hypothalamic-midbrain circuitry in this form of reward

learning, we are interested in further investigating whether this circuit underlies the effect of methamphetamine on cue control of behavior.

Disclosures: **A. Verghese:** Other; University of California, Los Angeles. **I. Hoang:** Other; Department of Psychology, University of California, Los Angeles. **M. Sharpe:** Other; Department of Psychology, University of California, Los Angeles.

Poster

070. Reward and Addiction

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 070.05

Topic: G.02. Reward and Appetitive Learning and Memory

Support: NIDA 5T32DA024635-14

Title: Methamphetamine and midbrain-hypothalamic control of cue-guided behavior

Authors: *I. B. HOANG[1], J. J. MUNIER[2], Z. GREER[2], S. J. MILLARD[1], L. E. DIFAZIO[1], K. M. WASSUM[1], A. IZQUIERDO[1], M. J. SHARPE[1];
[1]Psychology, [2]Sch. of Dent., UCLA, Los Angeles, CA

Abstract: Through life experiences, we learn to associate certain cues with particular outcomes. These cue-outcome memories help to inform our everyday decisions, such as choosing our dinner plans or picking what snack to get at the grocery store. While normally an adaptive process, individuals with a substance use disorder (SUD) can be extremely vulnerable to the influence of drug and other reward-predictive cues in controlling behavior. This excessive control of cues over behavior can ultimately lead to resumption of drug use while trying to abstain and perpetuate the cycle of addiction. Though a breadth of studies has firmly established that previous drug experiences can heighten responding to cues predictive of reward, the specific nature of these drug-induced enhancements in cue-guided behavior and the neural substrates responsible have yet to be characterized. Here, we find that rats with a history of methamphetamine self-administration show enhanced specific cue-guided behavior, tightly correlated with their prior drug intake, and that this drug experience appears to sensitize the hypothalamic-midbrain pathway. Previously, we have shown that the lateral hypothalamus (LH) is critical for learning about reward-paired cues, via a circuit with the midbrain dopamine system (Sharpe et al., 2017, *Current Biology*; Sharpe et al., 2021, *Nature Neuroscience*). Thus, changes in this circuitry could underlie the enhanced control that reward-paired cues have over behavior following drug abuse. In particular, one mechanism for how this region could be regulating cue-reward learning is via receipt of phasic dopamine prediction errors from the ventral tegmental area (VTA_{DA}). To probe the functional role of VTA_{DA} input to LH in mediating this specific cue-reward learning, we phasically stimulated this pathway in drug naïve rats to unblock learning for a reward-paired cue. Subsequently, we devalued the outcome for the unblocked cue and found that rats reduced responding to the cue, suggesting the original cue-reward association unblocked

by VTA_{DA}-LH stimulation was sensory-specific. Altogether, these data suggest that the LH may be harboring specific cue-outcome memories via dopaminergic input from the VTA, which, following drug experience, could be heightening the function of LH in learning these associations that then produces the enhancements in specific cue-guided behavior.

Disclosures: I.B. Hoang: None. **J.J. Munier:** None. **Z. Greer:** None. **S.J. Millard:** None. **L.E. DiFazio:** None. **K.M. Wassum:** None. **A. Izquierdo:** None. **M.J. Sharpe:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.01

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant R01 AG072898

Title: Impact of Alcohol use disorder on cognition, memory and amyloid plaque burden

Authors: *H. ESSA[1], P. MIN[2], V. LOWE[2], R. PETERSEN[3], E. LUNDT[4], C. MESTER[4], D.-S. CHOI[5];
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Abstract: Little is known about the impact of alcohol use disorder on cognitive and memory function as well as the impact of AUD on amyloid plaque burden with conflicting results from different literature. Here we report the effect of AUD on cognition, memory, and amyloid plaque burden in community-based aged population. Mayo Clinic Study of Aging (MCSA) is an observational study aiming to estimate the prevalence of cognitive decline and dementia longitudinally. With a special interest in developing tools to predict risk factors associated with cognitive impairment, dementia, and Alzheimer's disease (AD). For cognitive and memory tests, a trained psychometrist conducted neuropsychological tests as per the wechsler adult intelligence scaled revised (WMS-R) which included executive function which included trail making test B and digit symbol substitution test, language assessment including Boston naming test and category fluency test, memory assessment which included logical memory II test, and visuospatial assessment which involved picture completion and block design. The global cognition measure, which is the average over these four domains, was an outcome as well as a particular interest in the memory domain given that existing literature indicates memory can be especially affected by alcoholism. We assessed the amyloid plaque burden by amyloid positron emission tomography (PET) imaging using the Pittsburgh Compound B tracer (PiB). Our data has demonstrated a significant effect of AUD on both global cognition as well as and memory

function. AUD+ group did have lower cognitive z-scores than the AUD- group. AUD- group had a mean cognitive z-score of -0.29, range of (-5.52 - 2.96) and SD of 1.28 compared to AUD+ which had a mean score of -0.42, range of (-4.74 - 2.35) and SD of 1.26. Additionally, AUD+ have 0.2 z lower memory on average across all ages. This happens to be equal to nearly 2 years less education, similar to our finding with global cognition scores. We also calculated the odds ratio for developing amyloid plaque burden comparing AUD+ groups to a reference group of AUD- under 65 year old. AUD+ under 65 was not significantly different from our reference group (OR=1.0, 95% CI 0.9 to 1.2; P = 0.87). However, AUD+ over 65 had (OR=1.6, 95% CI 1.3 to 1.8; P<0.001). We conclude in our study that AUD has a significant effect on cognitive and memory function as well as an impact on amyloid plaque burden.

Disclosures: H. Essa: None. **P. Min:** None. **V. Lowe:** None. **R. Petersen:** None. **E. Lundt:** None. **C. Mester:** None. **D. Choi:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.02

Topic: G.09. Drugs of Abuse and Addiction

Support: K99/R00-AA025393

Title: Chronic Alcohol Exposure and Abstinence Induces Protracted Impairments in Cognitive Performance That Are Sex-Dependent

Authors: *L. A. ASKINS[1], S. A. COLLINS[1], S. B. FLORESCO[2], L. A. NATIVIDAD[1]; [1]Col. of Pharmacy, Div. of Pharmacol. and Toxicology, Univ. of Texas, Austin, TX; [2]Dept. of Psychology and Djavad Mowafaghian Ctr. for Brain Hlth., Univ. British Columbia, Vancouver, BC, Canada

Abstract: Detoxification from chronic ethanol (EtOH) exposure impairs cognitive function, including behavioral flexibility and working memory. Our lab has shown that male rats exhibit deficits in cognitive flexibility during EtOH abstinence. It is unclear if females would exhibit similar EtOH-induced deficits. Here, we used two operant models of cognitive function to compare the effects of protracted EtOH abstinence across sexes. Long-Evans rats (n=24/sex) were first trained to lever press for food pellets in operant chambers, followed by EtOH vapor exposure (14 hours daily) for 6 weeks. Dependent rats and their naïve counterparts were trained on an operant model of strategy set-shifting, beginning with a visual discrimination task in which levers were presented along with a cue light that signaled the reinforced lever. On days 10-12 of abstinence, rats were introduced to an automated sequence beginning with the “visual cue” rule, followed by a switch to the spatial location task, in which only one lever was reinforced. Rats were then examined for retention of the “spatial location” rule, followed by reversal of the reinforced lever to the alternate side. During the reminder trials of the set-shift task, females

displayed lower accuracy for the “visual cue” rule than males (p=0.031). No sex differences were observed in trials or errors to criterion during the set-shift, but all EtOH rats displayed fewer errors during the retention of the “spatial location” rule (p=0.019). Interestingly, EtOH females required more trials to complete the reversal task than naïve controls (p=0.037). Working memory was assessed in the same rats using the delay match-to-sample task. Rats were introduced to a single lever during trial initiation, followed by a variable delay period ranging from short (0-12 sec) to long (16-24 sec) intervals. Rats were then tasked to recall the correct lever that was presented earlier. Overall, females displayed lower accuracy than males (p=0.010), but also exhibited high omissions during the task (p=0.005). Females were unaffected by EtOH abstinence, while males displayed lower accuracy in the long-delay probe (p=0.031). A correlation of performance in the two models revealed a negative association between total trials in the set-shift and percent accuracy in the long delay for EtOH males only (R^2=0.82, p=0.001). The data suggest that chronic EtOH exposure may differentially impact cognitive function across sexes. While males displayed evidence of cognitive-based perturbations, females were somewhat resilient to the protracted effects. EtOH may have further imposed different gradations of abstinence-induced dysfunction that were more prominent in males.

Disclosures: L.A. Askins: None. **S.A. Collins:** None. **S.B. Floresco:** None. **L.A. Natividad:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.03

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA R01AA025380

Title: Contribution of Sex and Stress Hormones to the Effects of Repeated Binge Alcohol

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Abstract: Binge alcohol leads to degeneration and immune activation in the brain, in humans and animal models. We have previously shown significant cell loss and microglial activation from once-weekly binge exposure in rats, and are now investigating potential contributions of sex and stress hormones on these measures. Exercise attenuates binge damage, potentially by reducing the stress hormone cortisol (CORT) in response to alcohol. Sex hormones influence other types of brain injury, such as stroke, but their influence on binge-induced damage is not known. In the present study, cohorts of both male and female rats were gavaged once weekly for 5 weeks with either 5 g/kg alcohol or isocaloric control solution. The focus of one cohort was exercise effects, half of the animals had access to running wheels 4 d/wk. The focus of the other

cohort was the influence of sex hormones; these animals underwent gonadectomy (GDX) or sham surgery in early adulthood prior to binge alcohol exposure. Outcome measures included behavioral intoxication, CORT output and hippocampal cell loss. In the exercise cohort, binge exposure resulted in significant cell loss in the dentate gyrus (DG) of the hippocampus in sedentary rats of both sexes, but not in those that exercised. Interestingly, binge-exposed rats of both sexes ran less than control diet rats. Binged groups, regardless of exercise, had significantly increased CORT levels from week 1 to 5, suggesting that exercise-induced neuroprotection is not due to attenuation of CORT. Moreover, exercise did not alter behavioral intoxication, suggesting that it does not influence alcohol metabolism. Preliminary data for the sex hormone cohort suggest a role of circulating androgens in protection from binge-induced damage, as binged GDX males had fewer remaining DG neurons than intact binged males. Additionally, GDX binged males showed increased behavioral intoxication across binge exposures, whereas intact males showed decreased. Binged GDX females were not different from binged intact. This suggests that circulating androgens may buffer the negative effects of repeated binge exposure. Overall, our results to date suggest a potential role for androgens in the neurobehavioral effects of repeated binge alcohol exposure. In addition, although increased binge doses were associated with higher CORT levels, the increase was not attenuated by exercise, suggesting that exercise neuroprotection is not due to effects on stress hormones.

Disclosures: **A.L. Beck:** None. **E. Met Hoxha:** None. **M.J. Spinetta:** None. **D. Bravo:** None. **K.H. Gehm:** None. **C.G. Kennedy:** None. **J.L. Leasure:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.04

Topic: G.09. Drugs of Abuse and Addiction

Support: NIH/NCATS Colorado CTSA UL1 TR002535

Title: Subjective Effects of Co-administered Oral Cannabidiol and Alcohol

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Abstract: Preclinical data suggest that cannabidiol (CBD) reduces alcohol intake and markers of dependence in rodents, but limited research exists evaluating the effectiveness of CBD as a potential treatment option for humans who wish to reduce their alcohol intake. Only three prior human studies have explored the impact of CBD on alcohol intake, and these have yielded mixed results. The present study aimed to assess the potential impact of CBD on alcohol-induced motor and balance impairment, intoxication, alcohol craving and subjective responses to alcohol in humans. These assessments are important factors in determining if CBD could be a helpful tool in alcohol use disorder (AUD) treatment. Inclusion criteria required self-reported heavy drinking

and no recent or regular cannabis use. Participants completed 3 sessions in which they were randomly assigned to consume either 30mg, 200mg, or placebo CBD prior to drinking a standardized dose of alcohol. Measures of psychomotor impairment, alcohol craving, subjective responses to alcohol, and breath alcohol content (BrAC) were measured every 30 minutes for 4-hours after consuming the CBD and alcohol. Blood was drawn at the start of the experiment, 25 minutes post-CBD administration (estimated peak blood-CBD content) and 60-minutes post-alcohol administration (estimated peak BAC). Differences across the three CBD conditions were explored using multilevel models. Interactions between CBD condition and sex were also tested. There were no effects of CBD condition on BrAC, psychomotor impairment or alcohol craving. We observed a significant effect of CBD dose on the slope of self-reported alcohol-related stimulation, suggesting CBD sustains the stimulating effects of alcohol compared to placebo. Similarly, CBD also lengthened the time of reported sedative effects of alcohol. A sex by condition interaction was also seen, with a stronger relationship in males compared with females. The results of the present study further our understanding of the role CBD may have in moderating both subjective sedative and stimulating effects of alcohol in humans. Subjective responses to alcohol are known to impact AUD risk. Thus, these findings offer preliminary insight into one mechanism through which CBD may impact AUD phenotypes in humans.

Disclosures: M.L. Drennan: None. **L. Zulic:** None. **M. Prince:** None. **H.C. Karoly:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.05

Topic: G.09. Drugs of Abuse and Addiction

Title: Adolescent alcohol consumption in rats leads to sex-dependent differences in probabilistic discounting, affective-like behavior, and drinking in adulthood

Authors: A. N. TEJADA, D. GARCIA, J. C. BEZENAH, M. GONZALES, C. CUETO, D. OVERTON, C. BENICTA, *L. R. AMODEO;
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Abstract: Alcohol use commonly begins during early adolescence. While exploration is developmentally appropriate, early initiation is frequently associated with the development of alcohol dependence and is frequently co-occurring with affective disorders in adulthood. Preclinical studies have shown that alcohol exposure during adolescence can increase alcohol consumption in adulthood. However, exposure alone does not always increase alcohol preference. Therefore, it is important to consider the many factors that may contribute to these differences. Since the brain continues to develop throughout the adolescent period into early adulthood, ethanol exposure during this period may have unique and deleterious consequences including changes in disinhibitory, cognitive, and affective behaviors. Preclinical models have provided valuable insight into the neurobiology of adolescent alcohol use and risk factors for

later dependence. To this end, adolescent male and female rats (PD 28-56) were given intermittent night-time access to either 10% ethanol in a gelatin solution or a gelatin only solution (control) for a total of 15 sessions. Consumption levels were recorded after each session and ethanol rats were divided into low and high ethanol consumers via median split. Rats were then tested on a modified open field, forced swim test, and probabilistic discounting. For probabilistic discounting, rats choose between a small-certain reward and a large-risky reward which paid off either 0%, 16%, 33%, 67% or 100% of the time, depending on the session. After completion of the behavioral tasks, all rats were given night-time access to 20% ethanol intermittently for 10 sessions. Preliminary results suggest that high consuming adolescent rats show sex-dependent effect on affective-like behavior and risk preference which is correlated with future drinking behaviors.

Disclosures: **A.N. Tejada:** None. **D. Garcia:** None. **J.C. Bezenah:** None. **M. Gonzales:** None. **C. Cueto:** None. **D. Overton:** None. **C. Benicta:** None. **L.R. Amodeo:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Program #/Poster #: 071.06

Topic: G.09. Drugs of Abuse and Addiction

Support: ISCIII - MICINN - Grant PI21/00488

Title: Behavioral and gene expression changes induced by CBD in a new model of alcohol spontaneous withdrawal in mice

Authors: J. MANZANARES, A. GASPARYAN, D. NAVARRO, F. NAVARRETE RUEDA; Miguel Hernández Univ., San Juan de Alicante, Spain

Abstract: Alcohol consumption and dependence is one of the most devastating health problems worldwide, with high personal, family, economic and social impacts. A significant percentage of patients who achieve remission of consumption and abstinence relapsed a few months ago. Adequate pharmacological agents are necessary to manage the abstinence phase, reduce relapse and increase the success of the recovery. Therefore, the main goals of this study were 1) to establish an animal model of spontaneous alcohol withdrawal, analyzing the time course of behavioral changes and gene expression modifications associated with the peak of its presentation, and 2) to evaluate the efficacy of CBD in modulating these alterations. C57BL/6J male mice were exposed to increasing doses of alcohol (2.5-3-3.5 g/kg, p.o., every dose for 5 days) during a period of 15 days. Alcohol spontaneous withdrawal-induced anxiety and somatic withdrawal signs were evaluated 6, 12, 24, and 72 hours after the last administration. This time course evaluation allowed the selection of the time point with higher behavioral affectation. This time point was used to analyze the ability of different doses of CBD (10, 20, and 40 mg/kg, i.p.) to modulate spontaneous withdrawal-induced behavioral alterations and gene expression changes

in different brain targets by qPCR. Alcohol withdrawal-exposed mice showed increased affectation on withdrawal somatic signs at all time points, which were the most significant at 6 and 12h after the last ethanol administration. However, only the time point of 6h revealed increased anxiety-like behaviors. In addition, gene expression studies in microdissected brain nuclei showed reduced gene expression of cannabinoid receptor 1 (CB1r), whereas increased gene expression of cannabinoid receptor 2 (CB2r) and mu-opioid receptor (MOR) in the nucleus accumbens. Interestingly, CBD administration normalized CB1r gene expression alterations but induced an additional increase in the MOR and CB2r gene expression. Therefore, the results obtained in this study indicated that the new animal model of spontaneous alcohol withdrawal simulated the behavioral alterations associated with alcohol abstinence and revealed the involvement of cannabinoid and mu-opioid receptors. In addition, CBD modulated these behavioral and gene expression changes, suggesting that this cannabinoid might result in a new therapeutic agent for the treatment of alcohol withdrawal.

Disclosures: J. Manzanares: None. **A. Gasparyan:** None. **D. Navarro:** None. **F. Navarrete Rueda:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Program #/Poster #: 071.07

Topic: G.09. Drugs of Abuse and Addiction

Support: NIMH Grant R01MH116156

Title: The relationship between psychedelic use and alcohol use disorders in the United States

Authors: S. C. JUNGERS[1], A. LARSON[3], T. KIRSH[2], B. PIERCE[1], ***J. L. NIELSON**[1,2]; [1]Psychiatry & Behavioral Sci., [2]Inst. for Hlth. Informatics, Univ. of Minnesota, Minneapolis, MN; [3]Amherst H. Wilder Fndn., St. Paul, MN

Abstract: Background: Due to current modest success rates for treatment of alcohol use disorders (AUD), development of alternative therapies is warranted. There is a growing body of evidence from clinical trials suggesting efficacy of psychedelics within a therapeutic context in treating substance use disorders (SUDs). Interestingly, the self-reported use of psychedelics in nonclinical settings is also associated with significant decreases in problematic substance use, previously reported by Pisano et al (2017), reporting significantly lower rates of past-year opioid use disorders (OUD) in those with a history of psychedelic use. Garcia-Romeu et al (2019) also found statistically significant rates of alcohol cessation and reduction following psychedelic use from an online survey study. The current study aims to replicate the methods of Pisano et al (2017) to determine if self-reported use of psychedelics was also associated with decreases in AUD in a nationally representative population in the United States.
Methods: Utilizing anonymized data from the National Survey on Drug Use and Health

(NSDUH, N = 615,364, years 2002-2019), controlling for sociodemographics, risky behavior, and use of other illicit substances, we analyzed rates of AUD (abuse and dependence) with the past year in people who did (n=102,958) or did not (n=510,648) report past psychedelic use. Building upon statistical models of weighted risk ratios (RR) used in Pisano et al, we tested the hypothesis that a history of psychedelic use decreases the risk of AUD, while using the same model and data for OUD reported from Pisano et al as a positive analytical control to replicate their findings.
Results: We successfully replicated the findings of Pisano et al, by demonstrating a significantly lower risk of past year opiate abuse (RR = 0.65, p= 0.015) and dependence (R = 0.69, p = 0.006), in those with past psychedelic use, with weighted RR within +/- 0.05 of their findings. For AUD, we found that those who self-reported psychedelic use did not have a significantly higher risk of developing alcohol abuse (p=0.072) or dependence (p=0.939) in the past year, compared to those with no history of psychedelic use. We also found that those who self-reported marijuana use had a much higher risk of developing AUD in the past year for alcohol abuse (RR = 2.23, $p < 0.001$) and dependence (RR = 2.51, $p < 0.001$).
Conclusion: The current findings are inconsistent with previous survey studies and small clinical trials examining the use of psychedelics to treat AUD. Further clinical research is needed to determine causal relationships and therapeutic potential of psychedelics to treat and/or prevent AUD.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: División de Investigación y Posgrado, Universidad Iberoamericana Ciudad de México
Sistema Nacional de Investigadores from the Consejo Nacional de Ciencia y Tecnología of México.
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Title: Effects of different maternal separation protocols on alcohol consumption in adult rats

Authors: S. MUÑOZ-SÁNCHEZ[1], L. RODRÍGUEZ-SERRANO[3,1], C. HERNÁNDEZ GUERRERO[2], C. CRUZ-FUENTES[4], *M. BUENROSTRO-JAUREGUI[1];
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Abstract: Maternal separation (MS) induces stress in pups' rodents. It has been reported that mother rats change their behavioral repertoire of maternal care in the face of chronic separation from their pups. This model allowed to outline new theories on the development vulnerability for the acquisition of mental and behavioral pathologies such as substance abuse, depression, and anxiety disorders. The maternal separation produces an affectation on the reward system, which would lead to a greater vulnerability to alcohol consumption in adulthood. The aim of this research was to characterize the alcohol consumption during adulthood of different protocols of maternal separation (15 or 21 days). Twenty-seven rats were nested with a normal light cycle. We separated animals into three groups: 15 days of SM (15MS), 21 days of SM (21MS), and a control group without MS. The maternal separation procedure for 15MS was carried out during postnatal days 2 (2 PND) to 15 PND (for 3 hours), and for the 21MS group during postnatal days 2 PND to 21 PND (for 3 hours). The animals had free access to food and water in their home. For four days, starting on 50 PND, the water bottle (500 mL) was removed from each cage and replaced by two smaller bottles with water (100 mL). The animals were exposed to alcohol under two different conditions: gradually increasing concentrations (2%-8%) and high concentration (8%). Alcohol was prepared each day by mixing the corresponding ethanol solution with tap water. In the first condition, four concentrations were used (2%, 4%, 6%, and 8% v/v), and the animals had access to each concentration for four consecutive days. In the high concentration protocol, the animals had access to a single concentration (8%) for 16 consecutive days. The position of water and alcohol bottles in the cage was changed daily to avoid preference, and they were weighed every 24 hours. The normalized alcohol (g/kg) consumed was calculated individually. The alcohol preference ratio was also determined as the alcohol intake between the alcohol plus water intake. The results showed that 15 MS animals consumed more alcohol than 21 MS and the control group in both gradually increasing concentrations (2%-8%) and high concentration (8%). We did not observe differences in alcohol consumption between concentrations inside the MS groups.Our data suggest that the duration of maternal separation can generate changes at the central level that change vulnerability to alcohol consumption in adulthood. However, we must be cautious when making inferences from animal models to humans because behavioral developmental dynamics are different between species.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH T32 AA007462

Title: Low alcohol preferring mice have reduced task engagement during a waiting task for alcohol, which is enhanced by intermittent alcohol drinking

Authors: *P. A. STARSKI[1], F. W. HOPF[2], D. MAULUCCI[3];
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Abstract: Alcohol use disorder (AUD) is related to excessive binge alcohol consumption, and there is considerable interest in associated factors that promote intake. AUD has many behavioral facets that enhance inflexibility toward alcohol consumption, including impulsivity, motivation, and attention. Thus, it is important to understand how these factors might promote responding for alcohol and can change after protracted alcohol intake. Previous studies have explored such behavioral factors using responding for sugar in the 5-Choice Serial Reaction Time Task (5-CSRTT), which allows careful separation of impulsivity, attention, and motivation. Importantly, our studies uniquely focus on using alcohol as the reward throughout training and testing sessions, which is critical for beginning to answer central questions relating to behavioral engagement for alcohol. Alcohol preference and consumption in C57BL/6 mice were determined from the first 9 sessions of 2-hour alcohol drinking which were interspersed among 5-CSRTT training. Interestingly, alcohol preference but not consumption level significantly predicted 5-CSRTT responding for alcohol. In contrast, responding for strawberry milk was not related to alcohol preference. Moreover, high-preference (HP) mice made more correct alcohol-directed responses than low-preference (LP) during the first half of each session and had more longer reward latencies in the second half, with no differences when performing for strawberry milk, suggesting that HP motivation for alcohol may reflect "front-loading." Mice were then exposed to an Intermittent Access to alcohol paradigm and retested in 5-CSRTT. While both HP and LP mice increased 5-CSRTT responding for alcohol, but not strawberry milk, LP performance rose to HP levels, with a greater change in correct and premature responding in LP versus HP. Overall, this study provides three significant findings: 1) alcohol was a suitable reward in the 5-CSRTT, allowing dissection of impulsivity, attention, and motivation in relation to alcohol drinking, 2) alcohol preference was a more sensitive indicator of mouse 5-CSRTT performance than consumption, and 3) chronic alcohol drinking promoted behavioral engagement with alcohol, especially for individuals with less initial engagement.

Disclosures: P.A. Starski: None. **F.W. Hopf:** None. **D. Maulucci:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH Grant AA028265
NIH Grant AA007611
NIH Grant AA029409

Title: Assessing aversion-resistant alcohol consumption in head-fixed mice

Authors: N. M. TIMME, C. ARDINGER, S. WEIR, C. C. LAPISH;
Indiana University-Purdue Univ. Indianapolis, Indianapolis, IN

Abstract: Aversion-resistant drinking is a key symptom of alcohol use disorder (AUD) that is particularly relevant in advanced stages of the disease when treatment is more difficult. During this maladaptive form of consumption related decision-making, the subject decides to consume alcohol despite negative consequences/punishment. Aversion-resistant alcohol drinking has been observed previously in freely moving mice. This study sought to determine if head-fixed mice also engage in aversion-resistant drinking in order to assess the feasibility of studying this key behavioral phenotype using powerful neural monitoring and manipulation techniques that are best utilized in head-fixture. Adult male and female C57/BL6 mice were implanted with head-fixation bars and then acclimated to alcohol using a 5-day limited access home-cage drinking protocol (drinking in the dark). Next, mice were trained to consume 20% ethanol in a head-fixed Pavlovian task. After acclimating to head-fixed consumption over multiple sessions, aversion-resistant consumption was assessed using quinine adulterated alcohol and air puff. For quinine adulteration, ethanol was adulterated with various concentrations of quinine (up to 3.6 g/L). For air puff punishment, a brief (0.1-0.5 sec, 10-25 PSI) air puff was administered to the animal's neck posterior to the head-bar implant. Most subjects readily consumed 20% alcohol during head-fixture and many reached pharmacologically relevant blood ethanol concentrations (greater than 80 mg/dl). Consumption decreased during quinine adulterated consumption and air puff punished consumption. A dose response curve was fit to consumption values during quinine adulteration to quantify the aversion-resistance of each animal. A wide range of alcohol consumption and aversion-resistance was found across animals, indicating that it is possible to phenotype individual mice prior to other head-fixed experiments. Furthermore, mice drank higher concentrations of quinine when head-fixed than when freely moving and preliminary data indicated female mice may be more aversion-resistant in head-fixture than male mice. These studies demonstrate that head-fixed mice will engage in aversion-resistant drinking of alcohol. In the future, these procedures will be refined to allow for the implementation of advanced neural monitoring and manipulation techniques (e.g., high-density neural probe electrophysiology, calcium imaging, and optogenetic manipulation).

Disclosures: N.M. Timme: None. **C. Ardinger:** None. **S. Weir:** None. **C.C. Lapish:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Program #/Poster #: 071.11

Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant K01-AA027833

Title: Contribution of Treatment of Anxiety Disorders to Remission from Alcohol Use Disorder

Authors: K. VASHKEVICH, *N. MALEKI;
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Abstract: Alcohol is a well-known and widely available central nervous system depressant. Many people with early symptoms of anxiety consume alcohol socially and naturally get mild depressant effect on the central nervous system which can relieves anxiety symptoms and provoke relief of some other central nervous system symptoms such as chronic pain. Since usually, patients do not even understand they have anxiety, and they identify it as a complaint that they seek help for relatively late, in this study we aimed to determine the incidence of anxiety disorders in individuals with a history of alcohol use disorders (AUD) and determine the prevalence of use of anxiety medication in management of AUD. Two hundred subjects with possible history of alcohol use disorders were randomly identified. Upon detailed reviewing of their clinical data, 37 subjects were kept in the analysis with confirmed history of alcohol abuse and 3 years or more of follow up with at least 3 visits related to their alcohol abuse. All, except 3, of the participants were confirmed to be in remission. Of the total subjects, 27 (73%) had also reported anxiety symptoms but only 9 (24%) of those had received specific medical attention for anxiety. Nevertheless, the use of medication anti-anxiety medication as well as medication with anti-anxiety effects (e.g., antidepressants) was highly prevalent and 85% of the patients with reported symptoms had received different classes of anxiety medication. For the majority of the patients (74%) anxiety symptoms were recognized years prior to the treatment/management of AUD. Anxiety with time may lead to excessive alcohol drinking, abuse or addiction because alcohol has a strong affinity to brain cell receptors, and " remembering fast" the effects of depressants such as alcohol, and in a period of being sober may exacerbate the desire to have a drink again. This might become a vicious circle for a patient. Our results suggest that treating anxiety in this patient population enables patients to remain in remission. Properly in time diagnosed anxiety disorders in patients with alcohol use disorder may help adjust treatment options, prevent excessive drinking, and improved treatment outcomes. Long-term undiagnosed or under-treated anxiety overstimulates the central nervous system and overwhelms the sympathetic nervous system response.

Disclosures: K. Vashkevich: None. **N. Maleki:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01AA024109

Title: Double shot of alcohol 20% as a new rat model of binge drinking

Authors: *T. DE OLIVEIRA SERGIO, R. JANE SMITH, S. WEAN, E. ENGLEMAN, F. HOPF;
Indiana Univ., Indiana Univ., Indianapolis, IN

Abstract: Binge drinking (BD) has serious consequences for the drinker as well as society and has been increasing in the last decade. The NIAAA defines BD as the consumption of a large amount of alcohol in a short period of time, bringing blood alcohol concentration (BAC) to 0.08mg%. Most rat preclinical models do not result in such high BAC levels, unless using forced administration of alcohol, alcohol dependence, and/or genetically selected rat lines to reach higher BACs. Developing relevant BD models in outbred strains is of major importance for a better characterization of individual and overall differences in the neurobiological mechanisms underlying this dangerous and harmful behavior, especially in rats, which allow broader behavioral investigations. Here we developed a new rat binge alcohol model, exposing females and males Wistar rats to "two shots" of ethanol. Rats had access to alcohol 20% in the IA20E schedule for 3 months, then drank in two closely spaced short intake sessions, under scheduled access conditions, reaching BACs >0.08mg%. We then proceeded with a pharmacological validation with an opioid receptor antagonist, naltrexone (1mg/kg), an antagonist of β adrenergic receptors, propranolol (5 and 10 mg/kg) and an antagonist of α1 adrenergic receptors, prazosin (0.75 and 1.5mg/kg). Our results showed that naltrexone decreased binge consumption in both sexes. Also, propranolol at 5mg/kg decreased the alcohol binge intake only in females and with no effect in males, while the high dose of propranolol (10mg/kg) decreased the binge intake in both sexes. Like propranolol, the low dose of prazosin (0.75mg/kg) was effective only in females, while the higher dose (1.5 mg/kg) was effective in both sexes. Taken together, ours results showed that the expose of rats two brief "shots" of ethanol 20% can be used as a new model of rat binge drinking.
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Disclosures: T. De Oliveira Sergio: None. **R. Jane Smith:** None. **S. Wean:** None. **E. Engleman:** None. **F. Hopf:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.13

Topic: G.09. Drugs of Abuse and Addiction

Support: This work was supported by the Polish National Science Centre (Research grant 2018/31/B/NZ7/03690 to RR).

Title: Trait sensitivity to negative and positive feedback determines various aspects of alcohol addiction in rats.

Authors: *A. CIESLIK, K. NOWORYTA, J. SOLICH, A. KORLATOWICZ, A. FARON-GÓRECKA, R. RYGULA;
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Abstract: Alcohol use disorder (AUD) is one of the most common psychiatric disorders and a leading cause of mortality worldwide. It has been previously demonstrated that people with AUD are abnormally sensitive to the outcomes of their actions and are less able to use performance feedback to guide and adjust ongoing behavior. However, far less is known about the role of aberrant processing of performance feedback before the onset of AUD. In this study, we investigated the theoretical claim that sensitivity to feedback as a stable and enduring cognitive trait can predict subsequent vulnerability to the development of compulsive alcohol consumption in rats. For this, we initially tested the animals in a series of probabilistic reversal learning tests, and based on this "feedback sensitivity screening", we classified each rat as insensitive/sensitive to negative and positive feedback. Subsequently, in the intermittent access two-bottle choice paradigm, we measured the consumption of alcohol in the animals classified as described above. In the next step, using the instrumental second-order chained schedule of the alcohol reinforcement task, we examined the influence of insensitivity/sensitivity to negative and positive feedback on the development of compulsive alcohol-seeking behavior. Subsequently, we also measured how trait sensitivity to feedback affects the extinction of alcohol-seeking and the reinstatement of this behavior following a period of abstinence. The behavioral studies were complemented by analyses of the levels of stress hormones and differences in the expression of potentially involved genes. The results of our study demonstrated, for the first time, that trait sensitivity to feedback might determine the vulnerability of rats to the development of compulsive alcohol seeking, motivation to drink, propensity to extinguish alcohol-seeking behaviors following termination of alcohol availability, and reinstatement of these behaviors. They also demonstrated that trait sensitivity to feedback interacts with the levels of stress hormones and the expression of genes related to serotonergic and dopaminergic neurotransmission.

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Poster

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5R25GM061151-19

Title: Role of temperature in the regulation of the ethanol tolerance

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Abstract: Alcoholism is the third leading cause of deaths. One of the key components in the development of alcoholism is the gradual increase in resistance to alcohol over repeated exposures. This adaptation is also known as tolerance, which is thought to involve many neural adaptations and plasticity within the brain leading to alcohol addiction. Even though it was evident that higher ethanol doses increases the tolerance, the effects of temperature and doses, and their interaction on tolerance has not been previously studied. We are inclined to hypothesize the tolerance might increase with higher interaction of dose just as doses alone to tolerance, though we aren't sure if that will be the trend. Therefore, we were motivated to fill this knowledge. One of our focuses is to understand the relation of temperature with ethanol sensitivity when wild-type control canton-S(CS) flies were exposed to 50% ethanol. Subsequently ethanol sedation scores were recorded and analyzed. To further understand how the relation between temperature (18°C and 28°C) and ethanol doses (50%, 75%, and 100%), their interaction, affect tolerance, we generated targeted expressions of GFP control within hemocytes using UAS/Gal4 binary expression. F1 age matched female GFP expressed control flies were subjected to a 2-day alcohol treatment paradigm. Subsequently ethanol sedation scores were recorded and analyzed for tolerances. Our findings represent that, in wild type CS flies sensitivity to ethanol increases with temperature at low doses (50%). When tested how the relationship between temperature and dose, their interaction can alter ethanol tolerance, we found at low ethanol doses (50%) higher temperatures stimulates ethanol tolerance, in contrast at high doses (100%), higher temperatures negatively affects tolerance. Whereas at moderate doses, temperature has no or little effect on tolerance. Suggesting that, neuronal adaptation mechanisms underlying ethanol tolerance might be different from the perspective of dose only vs interaction of doses and environmental temperature. More specifically, at colder temperatures, one needs high doses of ethanol to be able to develop tolerance, suggesting the nervous system is less excitable and therefore needs more alcohol to be able to trigger plasticity mechanisms. In Contrast, high doses will not develop tolerance as much as at lower, suggesting with high temperatures and high ethanol doses too much excitability is expected and might result in blokage of plasticity mechanisms.

Disclosures: M. Kuchibhotla: None. **A. Montes-Mercado:** None. **J.L. Rivera:** None. **A. Ghezzi:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIH(AA028175-01)
Department of veterans Affairs Merit Research Award(I01BX002661

Title: Chronic alcohol exposure differently affects sleep-wakefulness in male and female mice.

Authors: *M. PARIKH[1], R. SHARMA[2], M. M. THAKKAR[3], P. SAHOTA[4];
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Abstract: Title: Chronic alcohol exposure differently affects REM sleep in male and female mice. **Authors:** Meet Parikh, MS, Rishi Sharma, PhD, Pradeep Sahota, MD and Mahesh M. Thakkar, PhD.**Affiliations:** Harry S. Truman Memorial Veterans Hospital and Department of Neurology, University of Missouri-School of Medicine, Columbia MO
Background: Alcohol consumption (also known as high-risk drinking) is on the rise among women. In addition, as compared to men, women advance faster from the onset of drinking to compulsive drinking and display more severe psychosocial consequences of excessive alcohol consumption. Alcohol consumption plays a crucial role in Sleep disturbance. Even though there are animal studies that suggest females display sleep disturbances during alcohol withdrawal, studies showing the effect of alcohol on sleep during chronic alcohol consumption are lacking. Hence, we used the Lieber-DeCarli method (mimicking the compulsive alcohol drinking) to investigate the effects of active alcohol consumption on sleep among the male and female mice. We hypothesize that female mice display more sleep problems than males when exposed to chronic alcohol consumption**. METHODS:** To test our hypothesis, adult male and female C57BL/6J mice, instrumented for sleep recording, were exposed to alcohol (6.8% v/v alcohol; Alcohol Group) or control (Control Group) diet for 20 days via Lieber-Decarli liquid diet method. Sleep-wakefulness was examined on days 1, 5, 10, 15 and 20. **RESULTS:** It was found that chronic excessive alcohol consumption has differential effects on sleep in males and females. Both male and female mice display an escalation in the amount of alcohol consumed across days. Sleep-wakefulness was comparable between males and females exposed to a control liquid diet. However, as compared to males, female mice display more sleep-wake disturbances as evident by a significant increase in the REM sleep on Days 5, 10, 15 and 20. No significant changes were observed during wakefulness and NREM sleep.
CONCLUSIONS: We believe this is the first evidence to suggest that chronic excessive alcohol differentially affects sleep-wakefulness in male and female mice, mainly affecting REM sleep.

Disclosures: M. Parikh: None. **R. Sharma:** None. **M.M. Thakkar:** None. **P. Sahota:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Program #/Poster #: 071.16

Topic: G.09. Drugs of Abuse and Addiction

Support: MERITUS Program of the UTEP COURI
UTEP Vicepresident for Student Affairs
Orville Edward Egbert, M.D. Endowment Fund

Title: Synergistic effect of social environment and dopamine-signaling on alcohol-induced locomotor behavior

Authors: *D. MURILLO[1], J. ALVARADO[2], B. HERNANDEZ[1], P. SABANDAL[1], K.-A. HAN[1];
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Abstract: Alcohol consumption occurring in a social or solitary setting often yields different behavioral responses in human subjects. For example, social drinking is associated with positive effects including euphoria and increased sociality while solitary drinking is linked to negative effects such as depressive symptoms and social anxiety. However, the neurobiological mechanism by which the social environment during alcohol intake impacts on behavioral responses remains understudied. We investigated whether distinct social environments affect behavioral responses to ethanol and the role of the dopamine system in this phenomenon in the fruit fly *Drosophila*. We analyzed the locomotor responses of flies to ethanol in either a socially-isolated or socially-enriched setting. We found that the wild-type *Canton-S* (*CS*) flies exhibited a small increase in locomotor response when exposed to ethanol in a group setting than alone. We next investigated the role of dopamine in this phenomenon. Dopamine is a neurotransmitter that mediates ethanol-induced hyperactivity in flies and mammals, and also has a role in social interaction. We first tested the *fumin* flies lacking dopamine transporter thus having enhanced dopamine neurotransmission. When exposed in a group to ethanol, *fumin* flies showed an exaggerated hyperactivity compared to singly exposed *fumin* as well as single- and group-exposed *CS.* Together, these findings indicate that a socially enriched environment together with hyper-dopamine intensifies the ethanol-induced behavioral response. To investigate the receptor(s) mediating the social environment and hyper-dopamine interaction, we are examining the flies with mutations in both dopamine transporter and individual dopamine receptors. This study will provide important information regarding the mechanism by which genetic (dopamine system) and non-genetic (social context) factors interact to influence behavioral responses to alcohol use and addiction.

Disclosures: D. Murillo: None. **J. Alvarado:** None. **B. Hernandez:** None. **P. Sabandal:** None. **K. Han:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

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Program #/Poster #: 071.17

Topic: G.09. Drugs of Abuse and Addiction

Title: Maternal stress as a vulnerability factor for alcholism: focus on sex differences

Authors: S. MORLEY-FLETCHER[1], H. BOUWALERH[3], G. VAN CAMP[2], M. CHOULLI[4], M. KABBAJ[5], F. NICOLETTI[6], **S. MACCARI**[1];
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Abstract: In order to improve the identification of alcohol users at risk and promote their treatment, studies on the factors involved in individual vulnerability are important. The contribution of a preclinical animal model of maternal stress (PRS rat model) represents a good tool for revealing the establishment of fragilities in the development of alcohol addiction observed later in adolescence and adulthood. Indeed, PRS-induced chronic stress and sleep abnormalities are associated with increased vulnerability to psychostimulants and natural reward. Gender differences are also an important factor that has to be taken into account and males and females are unequally affected by the PRS. We have previously shown that PRS increases the preference for ethanol in heavy drinking females after severe stress. In PRS males, activation of the stress axis is reduced in response to alcohol consumption, a phenomenon also observed in heavy drinkers as well as in alcohol-dependent rats. The aim of this study was to investigate the link between early life stress, stress in the adulthood and alcohol consumption/sensitivity in both sexes as well as the strategies implemented at the behavioural level, in particular the disruption of the sleep-wake cycle by exposing PRS rats to chronic intermittent alcohol consumption (20%) in a two-bottle choice paradigm either during adolescence or adulthood. We show that 1) adolescent PRS rats consume more alcohol than non-stressed controls with a greater effect in PRS females; 2) in adult PRS water drinking rats, mGlu 1 and 2/3 receptor expression was increased with respect controls; 3) adult females present a higher alcohol consumption than males which is associated to a greater reduction of striatal mGlu 1 and 2/3 receptors in females than in males; 4) the analysis of sleep architecture shows a higher sleep fragmentation in males compared to females while PRS animals have higher fragmentation than controls; 5) after 2 months of intermittent alcohol intake, PRS drink less alcohol than control alcohol drinking rats and all groups reduce their sleep fragmentation except PRS males who show a further increase in sleep fragmentation. In conclusion, our results support the evidence of interplay between glutamate and early life stress on alcohol dependence, sex dependent. Indeed, high levels of glutamate in the ventral striatum of mice have been shown to lead to high alcohol consumption and, male PRS rats are characterised by a hippocampal 'hypo-glutamatergic' state that could be explain with the data presented here on mGlu receptors in the striatum, the reduced alcohol preference in PRS animals of both sexes compared to controls.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: National Science Center 2019/35/B/NZ4/04077

Title: Sociability shapes alcohol addiction-like behavior in female mice living in social group in IntelliCage

Authors: *M. STEFANIUK[1], M. BARANSKI[2], L. KACZMAREK[3];
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Abstract: Many of the models of alcohol use disorder omit the social context as they rely on training of individually housed animals. The aim of this study was to analyze social interaction between the mice living in group and verify whether social position shapes further alcohol addiction-like behaviors. For this we used a fully automated IntelliCage where animals can be tracked 24/7 with minimal contact with experimenter. We introduced C57Bl6 female mice (n=11) to the IntelliCage where they were allowed to explore the cage freely. Animals had access to experimental corners where drinking bottles were placed. Adaptation period served as a base to establish the initiation of social network formation. Next animals were trained to use the automated corners and given 20% ethanol for 2 hours daily for three weeks. They were further tested for motivation to alcohol, persistence in alcohol seeking in withdrawal and consumption in relapse was measured. Overall animals spend 60 days together. We evaluated their Sociability Rank (SR), i.e. interactions between pair of animals including both following and being followed events. On the basis of alcohol consumption, motivation and persistence we assessed an Addiction-like Score (AS) for each mouse. While some animals exhibited both increased alcohol consumption and motivation reaching a high AS, other refrained from drinking alcohol. Thus depending on their performance we divided animals into low (AS<-1.5), medium (-1.5<AS<1.5) and high drinking (AS>1.5). We have observed how social network formed and have found out that social structure evolved dynamically. Some animals were strikingly more followed than the others. Interestingly animals having higher SR were also more prone to have a higher AS ($r=0.3476$, $p<0.05$). These animals were also more followed in training sessions were alcohol was available as measured by visits with the interval of less than 60 seconds. Our study offers an insight into how addiction-like behaviors form when social context is preserved. It further provides an important factor of intensity of sociability for individuals in the context of their AS. We highlight the importance of the fact that not all mice are equal in their social context and that this factor must be taken into account in addiction studies.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 DA042499
R01 DA041781
R01 DA045463

Title: Effect of inflammatory pain on the maintenance of alcohol-drinking behaviors in male and female rats

Authors: *Y. CAMPOS-JURADO, A. D. BALLARD, J. MORON-CONCEPCION;
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Abstract: During the last years, multiple clinical and epidemiological studies have revealed that thepresence of chronic pain is closely related to Alcohol Use Disorder (AUD). In fact, previous preclinical studies have shown that inflammatory pain induces relapse-like behavior in female rats whereas only male mice under inflammatory pain increase their alcohol intake during an acquisition phase. However, there is only a few number of studies approchig this problematic and therefore the global effect of pain on AUD remains not fully discern. In this study we aimed to deeply explore wether the development of an inflammatory pain condition induced by the intraplantar injection of the Complete Freud Adjuvant (CFA) could impact alcohol related behaviors in animals with a previous history of alcohol exposure. For that, male and female rats (n=15/group) were exposed to a non-operant intermittent alcohol consumption paradigm. After 4 weeks of alcohol intake acquisition, rats were injected with CFA or saline into their hindpaws and the drinking patterns were monitored for 3 more weeks. Finally, the the response to different doses of ethanol was evaluated during in the following 3 weeks. Moreover, we corralate our behavioral data with the study of neural activation of different regions in the mesocorticolimbic system measured by fluorescence immunohistochemistry of delta-Fos B. Our results show that females drink signifficatntly higher aumounts of alcohol throughout the acquisition period. Interestingly, our preliminary results also show that inflammatory pain differentially affects total alcohol intake depending on the sex and the alcohol dose available. These findings may contribute to the better understanding of the intersection between pain and AUD and to development of more individualized treatments for chronic pain patients with a history of alcohol abuse.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: NIAAA Grant K01-AA027833

Title: How social media algorithms may be potentially harmful to individuals seeking recovery from alcohol use disorders

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Abstract: Due to lack of stigma, exposure to alcohol use related content is unavoidable on social media (SM). As such, the content pushed by SM algorithms are of great significance in recovery from Alcohol Use Disorders (AUD), as exposure may be triggering or cueing for recovering individuals. The level of exposure to alcohol content to recovery seeking users on Twitter and Instagram (IG) and the effectiveness of strategies to mitigate exposing content is currently unknown. In a novel experiment, we sought to discover how much photographic content someone who is seeking recovery from AUD may see on certain SM platforms using fictious social media accounts. We created 4 fictitious accounts on IG and Twitter, 2 males aged 30 (M), and 2 females aged 30 (F). All 4 accounts followed 19 alcohol brand accounts, with 2 accounts (1 male, 1 female) following only those alcohol brand accounts (Alcohol Only, AO group) while the other two (1 male, 1 female), additionally followed recovery resources (Alcohol Recovery, AR group). 2 accounts accidentally followed 2 different IG accounts of the same brand, but all had about the same number of posts. All accounts were checked on the same iPhone at 5pm every day for 1 month with the same default ad/app privacy settings. 2 more weeks of data collection got conducted with different ad and privacy settings. With default ad settings on IG, after a month, MAO totaled 155 alcohol brand posts, MAR 139, FAO 140, and FAR 140. With privacy/ad settings on, after 2 weeks, MAO totaled 55 alcohol brand photos and MAR totaled 54. FAO and FAR both received 57 alcohol brand photos. On Twitter, the recovery accounts received less photographic alcohol content. With default settings, MAO received 180 alcohol brand photos while MAR only received 119. Similarly, FAO received 194 while FAR received 117. With privacy settings on, MAO received 93, MAR 50, FAO 110, and FAR 49. While the privacy settings did not help much for blocking the photographic content on either platform, Twitter algorithms were more successful in profiling someone seeking recovery, resulting in suppressing potentially triggering content, though not totally effective. Implications of these results suggest if a patient is seeking recovery for AUD, risks of exposure to triggering/cueing photographic alcohol content may outweigh benefits of SM apps. Furthermore, results suggest that Twitter's algorithms work more effectively in suppressing alcohol content than IG. The results suggest a lack of control with filters and no privacy on SM. The results finally suggest that it may be beneficial for providers to prioritize patients finding alternative ways for human connection like recovery groups, clubhouses, et cetera.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: Instituto de Neurobiología UNAM DGAPA-PAPIIT 113.A

Title: Stress increases ethanol consumption in the acute phase but not in the chronic phase of an intermittent access 2-bottle choice model in Wistar rats

Authors: *J. RASGADO-TOLEDO, D. ANGELES-VALDEZ, C. J. CARRANZA-AGUILAR, J. P. MAYA-ARTEAGA, D. E. ORTUZAR, A. LOPEZ-CASTRO, E. A. GARZA-VILLARREAL;
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Abstract: Alcohol use disorder (AUD) has been described as a recurrent disorder where a compulsion to seek and ingest alcohol, loss of control in its consumption and a state of dysphoria, anxiety and irritability during abstinence (Koob et al., 2019). Stress has been suggested as a motivator in the generation of AUD through synergistic effects in the reward circuit. It is suggested that negative neuro-adaptations manifested as reduced signaling in the HPA axis and cortical connections to the PFC lead to excessive alcohol consumption (Becker, 2017). However, studies have only focused on short drinking sessions (~10 days) rather than chronic sessions (~45 days). The present study seeks behavioral and brain phenotyping of the influence of stress on drinking behavior in a murine model of chronic AUD using longitudinal MRI. For this, 31 Wistar rats (14 female) were exposed to a 20% ethanol (EtOH) solution using the intermittent access 2-bottle choice model (IA2BC), and we exposed them to stress using 3-hour chronic restraint movement (Str). The 4 groups of study were: 1) EtOH+/Str+, 2) EtOH+/Str-, 3) EtOH-/Str+, 4) EtOH-/Str-. Our preliminary results showed a higher consumption of ethanol in the EtOH+Str in contrast to the EtOH+/Str- group over only the first 5 IA2BC sessions. After which, both groups increased ethanol consumption similarly for 15 following sessions. Elevated plus maze test indicated a similar non-anxiety behavior of EtOH+Str and Str but not for EtOH group after 20 IA2BC sessions. However, preferency of EtOH obtained by conditioned place preference was higher for EtOH+Str+ than EtOH+/Str- group. The EtOH+/Str+ (956 ± 485 nmol/L) group showed higher plasma levels of corticosterone than the EtOH-/Str+ (583 ± 219 nmol/L) group, while the other groups showed normal levels of corticosterone. Overall, our preliminary results suggest that stress increases ethanol consumption in the acute drinking phase, but not in the chronic phase in an AUD model in Wistar rats, even with higher plasma levels of corticosterone. Longitudinal MRI as well as behavioral measures of the full sample are pending.

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Poster

071. Cognitive and Behavioral Effects of Alcohol

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Topic: G.09. Drugs of Abuse and Addiction

Support: R01 AA029258

Title: Chronic binge-like ethanol vapor exposure decreases attention and motivation during the five choice serial reaction time task

Authors: *L. PEYTON[1], B. LEON[1], H. ESSA[2], D.-S. CHOI[3];
[2]Neurobio. of Addiction and Alcoholism, [1]Mayo Clin., Rochester, MN; [3]Mol. Pharmacol. and Exptl. Therapeut., Col. of Med., Rochester, MN

Abstract: Chronic alcohol use leading to alcohol use disorder is a growing concern, but much is still unknown how continuous binge-like ethanol exposure impacts sucrose reward seeking. We used the 5-choice serial reaction time task (5CSRTT) in C57BL6/J male (n=15) and female (n=15) mice subjected to daily 4 hours chronic ethanol vapor exposure for 5 times a week, totaling 15 weeks of ethanol vapor exposure versus air exposed male (n=15) and female (n=15) counterparts. We found that chronic binge-like ethanol exposure decreases attentional performance and dampens motivation during the 5CSRTT. Moreover, these effects were obtained without change to sucrose preference or anxiety-like behavior. Seven days withdrawal from chronic binge-like ethanol vapor exposure did not rescue attention or motivational performance in the 5CSRTT. Next, we used light sheet microscopy in combination with immunolabeling-enabled three-dimensional imaging of solvent cleared organs (iDISCO) technique to investigate c-Fos expression through the entire mouse brain of mice exposed to chronic binge-like ethanol vapor exposure and sacrificed 1 hr. after behavior performance in the 5CSRTT. Our results suggest that chronic binge-like ethanol exposure may affect brain-regions governing attention and motivational processes.

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Poster

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Topic: G.09. Drugs of Abuse and Addiction

Support: R21AA028602

Title: Infant footshock increases aversion-resistant alcohol drinking selectively in female mice

Authors: *T. PERRY[1], A. REICHERT[1], E. A. SNEDDON[2], N. SHAND[1], H. CARVOUR[1], C. THACH[1], J. J. QUINN[4], A. K. RADKE[3];
[2]Psychology - Brain, Cognition, and Develop. Program, [3]Psychology, [1]Miami Univ., Oxford, OH; [4]Psychology, Miami Univ. Dept. of Psychology, Oxford, OH

Abstract: In humans, early life stress (ELS) is associated with an increased risk for developing both alcohol use disorder (AUD) and post-traumatic stress disorder (PTSD). We have previously used an infant footshock model to explore this shared predisposition. Infant footshock produces stress-enhanced fear learning (SEFL) in rats and mice and increases aversion resistant alcohol drinking in rats. The goal of the current study was to extend this model of comorbid PTSD and AUD to male and female C57BL/6J mice. We also explored whether social buffering could mitigate the effects of infant footshock on adult drinking behaviors. Social buffering is a phenomenon in which the exposure to conspecifics ameliorates the long-term effects of a stressful experience. Acute ELS was induced using 15 foot-shocks on postnatal day 17. In the 90 minutes following stress exposure, mice were placed in a cage alone, with their littermates, with their dam, or with their littermates and dam. Fear conditioning was performed in all mice on PND 60. After PND 90, mice were given intermittent access to 20% ethanol and water for five weeks Consumption levels were measured throughout the experiment both 30 minutes into and at the end of each 24-h drinking session. In the fifth week of ethanol drinking, increasing concentrations of quinine (10 mg/L, 100 mg/L, 200 mg/L) were added to the ethanol to test aversion-resistant drinking. We found that, similar to rats, infant footshock did not affect ethanol-only drinking. However, infant footshock had a sex dependent effect on consumption of ethanol adulterated with quinine. In males, and in females who had not been exposed to footshock, quinine reduced consumption of ethanol at the 100 mg/L and 200 mg/L. concentrations. In females who received footshock, quinine did not significantly reduce ethanol consumption at any concentration. Investigation of social buffering influences on ethanol drinking behaviors is ongoing. Together, our findings suggest that the infant footshock model of ELS may be useful for investigating the neural underpinnings of comorbid PTSD and AUD in vulnerable individuals, particularly females and those exposed to ELS.

Disclosures: T. Perry: None. **A. Reichert:** None. **E.A. Sneddon:** None. **N. Shand:** None. **H. Carvour:** None. **C. Thach:** None. **J.J. Quinn:** None. **A.K. Radke:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.24

Topic: G.09. Drugs of Abuse and Addiction

Support: PAPIIT-DGAPA 201420
CONACYT 1085633

Title: Effect of chronic sugar consumption on ethanol intake in adolescent and adult rats

Authors: *M. RESÉNDIZ-FLORES, M. I. MIRANDA, G. VERA, A. RANGEL;
Dept. de Neurobiología Conductual y Cognitiva, Inst. De Neurobiología, UNAM, Querétaro, Mexico

Abstract: Exacerbated sugar consumption and its cognitive processing involve activation of the brain reward system, in which areas such as the prefrontal cortex (PFC) mediate learning and behavior related to reinforcing consumption of sweet substances. However, few studies have analyzed the impact of high sugar consumption and its relationship with the consumption of addictive substances, such as ethanol, mainly when it is present in sugary alcoholic mixtures. On the other hand, late maturation of PFCs during adolescence is related to early intake of addictive substances such as ethanol. Given that alcohol intake usually begins in adolescence through sugary alcoholic mixtures, the objective of this study was to evaluate, in adolescent and adult rats, the intake of sugary ethanol after chronic and permanent sugar exposure.In a reverse light-dark cycle, male and female adolescent and adult Wistar rats were housed individually and kept at 23°C. Food was available ad libitum. Rats were randomly assigned to the groups: Naive (N) or Chronic (C), with access to water or sugar, respectively, as the only liquid available for 24 h/21 days. Bodyweight, fluid, and food intake were recorded daily. After 21 days, each group was divided into two sub-groups, four groups total: NaiveW and NaiveS, ChronicW, and ChronicS. “W” and “S” indicate access to water or sugar between each of the four sugary ethanol (S-EtOH: 10%ethanol-10% sugar) vs. water preference tests (24 h each). Access to sugar in adult rats (NaiveS, ChronicW, ChronicS) decreased subsequent S-EtOH intake compared to control rats (NaiveW); however in adolescent rats, only chronic and continuous access to sugar (ChronicS) decreased S-EtOH intake compared to the NaiveW group; furthermore, adolescent male rats consumed more than female rats.In addition, a longitudinal evaluation from adolescence to adulthood was performed, where adult rats were exposed to a new series of four preference tests (S-EtOH vs. Water). The results suggest that chronic sugar consumption and water intake between S-EtOH preference tests (ChronicW) during adolescence increased later S-EtOH intake in adulthood compared to rats only evaluated in adulthood (i.e., without any manipulation during adolescence). Altogether, these results suggest that adolescent rats are more susceptible to sugar deprivation after chronic consumption, independently of sex. The susceptibility could be related to late brain maturation and its implications for increased impulsive and risk-seeking behaviors, such as initiating addictive substance use. Moreover, consuming sugary ethanol during adolescence can facilitate its intake during adulthood.

Disclosures: M. Reséndiz-Flores: None. **M.I. Miranda:** None. **G. Vera:** None. **A. Rangel:** None.

Poster

071. Cognitive and Behavioral Effects of Alcohol

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 071.25

Topic: F.03. Stress and the Brain

Title: Measuring Sympathetic Nervous System Effects in Alcohol Withdrawal: the Severity of Ethanol Withdrawal Scale (SEWS) Versus Clinical Institute Withdrawal Assessment Alcohol Scale, Revised (CIWA-Ar)

Authors: *T. BERESFORD[1], P. J. RONAN[2];
[1]RMRVAMC-SOM U Colorado, Denver, CO; [2]Sioux Falls VA/USD Sch. of Med., Sioux Falls, SD

Abstract: BACKGROUND: Alcohol Withdrawal Syndrome (AWS) is a toxic recovery phenomenon referable to the autonomic nervous system (ANS), more specifically to the Sympathetic Nervous System (SNS) that mediates the fight-or-flight response. Most view SNS hyperactivity during AWS as a rebound phenomenon: heavy, sustained alcohol use produces a marked exaggeration of normal Parasympathetic Nervous System (PNS) activity. On sudden release of the PNS agonism, a symptomatic SNS response occurs as the two opposing systems (SNS and PNS) act to reset their normal equilibrium of function. OBJECTIVE: Following our small sample pilot report, we hypothesized that our new AWS measure, the Severity of Ethanol Withdrawal Scale, or SEWS, would lessen the Time On Medication Protocol (TOMP) in symptom-driven AWS medication treatment as compared to the Clinical Institute Withdrawal Assessment Alcohol Scale, Revised, or CIWA-Ar in common usage at present. METHOD: Since both scales purport to measure SNS hyperactivity symptoms in AWS, we sought to test them in a head-to-head comparison using Quality Assurance outcome data in our hospital. We harvested electronic clinical record data on two samples--both SEWS-driven and CIWA-Ar-driven medication treatment protocols. The data were generated prospectively using each scale. AWS case data were then entered into digitized clinical records as they presented in real time; there were no randomization or blind procedures. Means for TOMP were calculated for each sample and compared using Student's t-test and the Wilcoxon test of mean differences. RESULTS: The SEWS-driven medication treatment (n=244) reduced TOMP treatment course to 2.2 days, compared to 3.4 days with the CIWA-Ar (n= 137). Significance is $p<0.0001$. CONCLUSION: Novel data on the SEWS from this head-to-head Quality Assurance comparison strongly suggests that the SEWS is a superior clinical measure of AWS-related SNS symptoms than the much less effective, and likely inaccurate, CIWA-Ar. In this real-world practice experience, the SEWS shortens the clinical course of acute AWS. This implies the high likelihood of lessened healthcare costs due to a shorter AWS treatment course.

Disclosures: T. Beresford: None. **P.J. Ronan:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.01

Topic: H.01. Attention

Support: PHS grant R01DA045063

Title: Striatal processing of attention-demanding signals in rats with opposing attentional control styles.

Authors: *E. DONOVAN[1], M. SARTER[2];
[1]Univ. of Michigan, Ann Arbor, MI; [2]Psychology, Univ. of Michigan Dept. of Psychology, Ann Arbor, MI

Abstract: Cortical cholinergic activity mediates the detection of behaviorally significant cues in attention-demanding contexts. The transfer of cue information via cortico-striatal projections allows for the integration of attentional and motor functions to initiate goal-directed action. Here we wished to demonstrate the role of cortico-striatal inputs in coding and inserting information about the attentional cue into striatal circuitry. Moreover, we investigated the impact of individual variations in the degree of top-down versus bottom-up attentional control on cortico-striatal activity. In rats with opposing cognitive styles as traits, goal-trackers (GTs) and sign-trackers (STs), differences in attentional control are due in part to the presence of a high- (GTs) versus low-capacity (STs) cortical cholinergic input system. Specifically, aberrant intracellular choline transporter translocation in STs limits presynaptic acetylcholine synthesis and release. Using glutamate-sensitive microelectrode arrays (MEAs) for *in vivo* amperometric recordings, we measured glutamatergic signaling in the dorsomedial striatum of STs and GTs performing a Sustained Attention Task (SAT). SAT consists of a series of cued and non-cued trials, in which the animal must differentiate between trial types by engaging one of two presented levers. Findings indicate that cues leading to hits evoked sharp glutamatergic transients averaging 8.4 µM above baseline prior to behavioral response in GTs but not STs. Conversely, glutamate signals in STs followed within 600 ms of reward delivery in both cued and non-cued trials, with average peak amplitudes of 8.8 and 8.9 µM, respectively. This data indicate the relative absence of cortico-striatal transfer of cue information in STs. Additionally, our findings are consistent with the general view that, in STs, performance is primarily influenced by motivational variables, and that this bias may be mediated in part by dopaminergic, reward-encoding neurons. In contrast, striatal cue-directed action in GTs is primarily controlled by the detection of cues via cortical circuitry and the transfer of information about the presence of cues into the striatum. These findings not only inform emerging theories of variations in attentional control of actions, but they also support hypotheses focusing on the cognitive-motivational contributions to the relative vulnerability for addiction-like behaviors in STs.

Disclosures: E. Donovan: None. **M. Sarter:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.02

Topic: H.01. Attention

Title: Structural and Functional Connectomes of the Frontal Eye Field and Inferior Frontal Junction

Authors: *M. BEDINI[1], E. OLIVETTI[1,2], P. AVESANI[1,2], D. BALDAUF[1];
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Abstract: The attention networks comprise regions that span the parietal, temporal and frontal lobes, but their underlying long-range connectivity remains not well understood. In the prefrontal cortex, the frontal eye field (FEF) and the inferior frontal junction (IFJ) are specialized in the control of spatial vs non-spatial visual attention and working memory, respectively. We hypothesized that their connectivity fingerprints may support these specialized roles. We first performed an activation likelihood estimation (ALE) fMRI meta-analysis to accurately infer the localization of the FEF and IFJ in MNI152 space. We selected only saccadic functional localizers to localize FEF (35 studies), and oddball/cueing, n-back, Stroop and task-switching paradigms to localize IFJ (30 studies). We then employed the ALE peak coordinates as seeds to perform a data-driven meta-analytic connectivity modeling (MACM) analysis to uncover their fMRI coactivation patterns within the Brainmap database and decoded these patterns to characterize significant associations with behavioral domains. The same seeds were also used for confirmatory surface-based probabilistic tractography using 3T diffusion MRI data from the Human Connectome Project from 56 unrelated subjects (age: 22-35, mean = 28.66, std = 3.81). We tracked streamlines to the dorsal and ventral visual streams parcellated using the atlas by Glasser et al. (2016). The ALE technique revealed four main clusters whose peaks were localized at the junctions of the superior precentral sulcus and superior frontal sulcus (for FEF: x = -28, y = -6, z = 54, and x = 30, y = -6, z = 50), and the inferior precentral sulcus and inferior frontal sulcus (for IFJ: x= -42, y = 6, z = 30. and x = 46, y = 12, z = 28). MACM showed that FEF coactivates with the IFJ, the superior/inferior parietal lobule and the supplementary/cingulate eye fields, whereas the IFJ additionally coactivates with the insular and inferotemporal cortices, and its coactivations are significantly associated with greater behavioral domains ($p<.05$, Bonferroni correction) than FEF. Using surface-based probabilistic tractography, we found predominant structural connectivity from FEF to regions of the dorsal visual stream (n=14/34; $p<.01$, FDR, $q=.05$), and from IFJ to regions of the ventral visual stream (n=13/32), irrespective of seed-to-region distance. The connectomes of the FEF and IFJ provide converging evidence of their specialization in the control of spatial vs non-spatial selection mediated by long-range pathways within the attention networks. From a broader perspective, our results suggest that the classic two visual stream architecture extends to the prefrontal cortex.

Disclosures: **M. Bedini:** None. **E. Olivetti:** None. **P. Avesani:** None. **D. Baldauf:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.03

Topic: H.01. Attention

Support: Department of Psychology and Neuroscience Research Fund

Title: The causal role of frontal gamma oscillations in visual attention: a tACS study

Authors: *H. L. MORGAN, J. RIDDLE, J. DONG, J. B. HOPFINGER;
Univ. of North Carolina Chapel Hill, Univ. of North Carolina Chapel Hill, Chapel Hill, NC

Abstract: Attention is vital for selecting and prioritizing information during successful task performance. When presented with conflicting visual information, executive control processes are required to override automated processing, and prior electroencephalography (EEG) studies have correlated these processes with theta activity (4-8 Hz) over frontal regions. Other evidence, however, has correlated visual conflict resolution and executive control processes with frontal gamma (30-120 Hz). While observational studies find both frontal theta and gamma activity during executive control processes, transcranial alternating current stimulation (tACS) can test causal relationships and can be designed to target neural oscillations in a frequency-specific manner. This study aimed to dissociate the role of frontal theta and gamma oscillations in human attention and executive control by delivering stimulation to the right lateral prefrontal cortex at either theta (6 Hz) or gamma (60 Hz) frequency. The right hemisphere was stimulated due to prior data showing its dominance in visuospatial control processes. Healthy participants (N=32, age 18-28), in a within-subject crossover design, completed three separate tACS sessions, including a control (sham) session that mimics the feeling of tACS. Condition orders were randomized and counterbalanced, and participants were successfully blinded to the stimulation conditions. To investigate the contributions of frontal theta and gamma oscillations to attentional processes, participants performed the lateralized attention network task (LANT) while receiving tACS. This task manipulates lateralized attention between visual fields, and it includes measures of alerting, orienting, and executive control. The LANT quantifies executive control as the speed of identifying a target stimulus in the presence of either incongruent or congruent flankers. Repeated-measures ANOVA for reaction time showed an interaction between the stimulation type and flanker congruency ($F=4.46$, $p=0.04$). Post-hoc t-tests found that the interaction was driven by impaired processing of incongruent targets in the visual
field ipsilateral to stimulation during gamma tACS, relative to sham ($t=2.2$, $p=0.03$), and relative to congruent flankers ($t=2.7$, $p=0.01$). By contrast, theta tACS did not significantly modulate performance compared to sham. These results show that frontal gamma oscillations play a causal role in visual conflict processing. Moreover, the laterality of this effect suggests that frontal gamma oscillations bias attentional resources towards the contralateral visual field at the expense of the ipsilateral visual field.

Disclosures: H.L. Morgan: None. **J. Riddle:** None. **J. Dong:** None. **J.B. Hopfinger:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.04

Topic: H.01. Attention

Support: NIH Intramural research program at 1ZIAMH002783

Title: Whole-brain Laminar connectivity-based Evidence for Feedforward-Feedback Interactions within the attention and Visual Processing System

Authors: *R. KLEIN[1,2], P. BANDETTINI[1];
[1]Section on Functional Imaging Methods, Natl. Inst. of Mental Hlth., Bethesda, MD;
[2]Georgetown Univ., Washington, DC

Abstract: Attention is a critical component of visual sensory processing. Attention enhances task-relevant signal, suppresses distractor signals, and reduces noise among populations of neurons. Area V4 is one such region that is heavily modulated by attentional mechanisms (Reynolds 2004). Areas including the frontoparietal cortex are hypothesized to have a role in generating top-down modulatory processes considering their involvement in attention, working memory, and visual tasks (Buschman 2007). Here we use laminar-dependent fMRI and a movie-watching task to evaluate laminar functional connectivity across frontoparietal and area V4 networks. Based on anatomical studies, we assume that top-down feedback occurs through neurons terminating in superficial and deep cortical layers while feedforward connections terminate in middle layers (Fig 1; Felleman 1991). Fig. 2 shows the V4 layer profiles produced by using each seed ROI time course as the regressor in an FSL FEAT analysis. Visual ROIs produce an inverted-U layer profile across V4 with the highest Z statistic values occurring within the middle layers indicating feedforward inputs from these visual ROIs. Prefrontal and parietal ROIs yield an upright-U or superficial layer biased layer profiles at V4 with the highest Z statistic values occurring within the superficial and deep layers indicating V4 is receiving feedback from these frontoparietal ROIs.
Fig. 3 shows a whole-brain laminar connectivity analysis using five principal components from the left frontal-eye field as seeds in separate univariate GLM analyses. The b-weights were averaged and layer-specific smoothing was applied. Feedback profiles are seen in parietal and occipital regions.

Fig 1

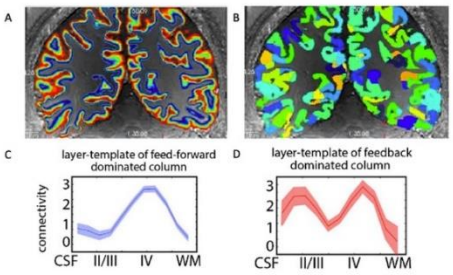


Fig 2

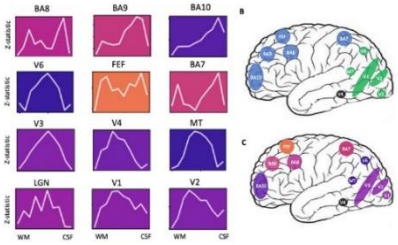
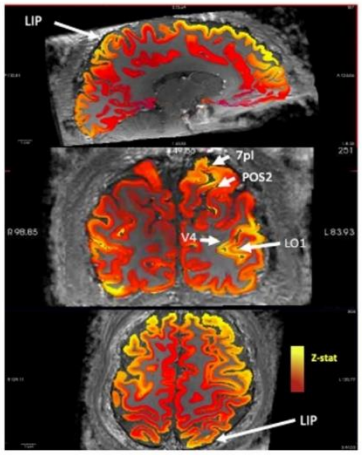


Fig 3



Disclosures: **R. Klein:** None. **P. Bandettini:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.05

Topic: H.01. Attention

Title: Inhibition of Cholinergic Basal Forebrain Neurons Disrupts Salience Detection and Subsequent Defensive Action Selection

Authors: *D. HEREFORD, E. LE, A. RESENDEZ, J. FADOK;
Psychology, Tulane Univ., New Orleans, LA

Abstract: Attending to stimuli that predict aversive outcomes is critical in initiating defensive action selection, and maladaptive defensive responses can arise from misattribution of salience to innocuous stimuli. Salience driven attention is known to be influenced by activity in the basal forebrain (BF), specifically the substantia innominata (SI), and this region has previously demonstrated a direct effect on the strength of fear memory acquisition (Jiang et al., 2016). Cholinergic populations in the SI are known to project to the basolateral amygdala (BLA), a primary coordinator of defensive responses. It remains unknown what role this cholinergic pathway plays in salience assignment during acquisition of a conditioned fear response and how it modulates defensive action selection. We used histological assessment of c-Fos to examine the neuronal activation of cholinergic cells in the SI of male and female C57BL/6J mice (N=6) using Serial Compound Stimulus (SCS) fear conditioning. SCS conditioning uses two distinct auditory stimuli (tone and white noise [WN]) presented sequentially and terminating with a 1s foot shock

(0.9mA). Instances of colocalization of c-Fos and choline acetyltransferase (ChAT) in the SI was greatest in the paired (n=94) when compared to unpaired (n=14) and no shock control groups (n=23). Peak expression of colocalized c-Fos and ChAT was found between -0.8 and -0.55 mm from bregma, the region with the densest cholinergic projections to the BLA. Next, we chemogenetically inhibited cholinergic neurons in the SI of ChAT-IRES-Cre mice (N=4) during acquisition of SCS fear conditioning. Following bilateral injection of a Gi inhibitory DREADD into the SI, subjects were injected i.p. with 5 mg/kg of Clozapine-N-Oxide 30 minutes prior to the first day of SCS conditioning. When compared with control (N=4) animals, inhibiting the cholinergic population lead to higher levels of freezing to the white noise segment of the SCS during trials 2, 3 and 4 (p=.125, .104, .070) on conditioning day 2. This represents a deviation from the canonical expression of active flight behavior as seen in the controls. These findings indicate that inhibition of cholinergic SI signaling leads to defensive behavior selection inconsistent with the salience of threat predictive cues. Our results support our hypothesis that cholinergic cells in the SI are active in response to stimuli predictive of imminent threat, and that inhibition of this population leads to the abnormal expression of freezing to a normally flight inducing stimulus. Future studies will explore the function of SI projections to downstream targets using an intersectional viral approach.

Disclosures: D. Hereford: None. **E. Le:** None. **A. Resendez:** None. **J. Fadok:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.06

Topic: H.01. Attention

Support: NIH 1K99GM140215-01

Title: Booting up the brain: intracranial electroencephalography reveals cognitive network dynamics during anesthesia emergence

Authors: *D. A. PURGER[1], S. EAGLEMAN[1], L. ARIKAN[1], B. REID[1], N. KABOODVAND[1], C. HALPERN[2], D. DROVER[1], B. RAZAVI[1], K. MEADOR[1], V. P. BUCH[1];
[1]Stanford Univ., Stanford, CA; [2]Univ. of Pennsylvania, Philadelphia, PA

Abstract: The dynamic interplay between several large-scale brain networks is thought to underlie human consciousness. The default mode network (DMN) governs internal thought during rest, the central executive network (CEN) drives goal-directed problem solving, and the salience network (SN) detects and integrates new stimuli during the transition between resting and cognitively active states. While scalp electroencephalography (EEG) imaging studies have elucidated much about these networks in steady states, intracranial EEG (iEEG) dynamics during state transitions remain largely unclear.
Seven patients with refractory epilepsy underwent stereotactic placement of depth electrodes for

seizure focus localization. Recordings from 422 iEEG contacts representing 49 distinct cognitive network nodes were obtained during anesthesia emergence. Local-field dynamics and Observer's Assessment of Alertness/Sedation (OAAS) scale state were analyzed in 20s clips for complexity, functional connectivity (FC), and graph communicability metrics.

Signal complexity as represented by mean multiscale sample entropy (MSE) of SN and DMN nodes increases significantly during emergence from anesthesia (P<0.05; fig. 1a), driven by a shift from low- to mid-frequency power in key nodes (fig.1b-c). Intermediate consciousness states demonstrated higher mean MSE and participation coefficient, representing early integrative network behavior, before reaching an optimal balance of segregation and integration at wakefulness (fig. 1d). During emergence, between-network FC increased most strongly between SN and CEN nodes (fig. 1e-f). Communicability of SN nodes increased during emergence, driven predominately by increasing communicability of right anterior insula (fig. 1g).

iEEG recording of brain activity during anesthesia emergence demonstrates a complex but quantifiable interplay of default mode, central executive, and salience network hubs. There may be a particularly greater than previously understood role of right anterior insula in gating transitions in consciousness states.

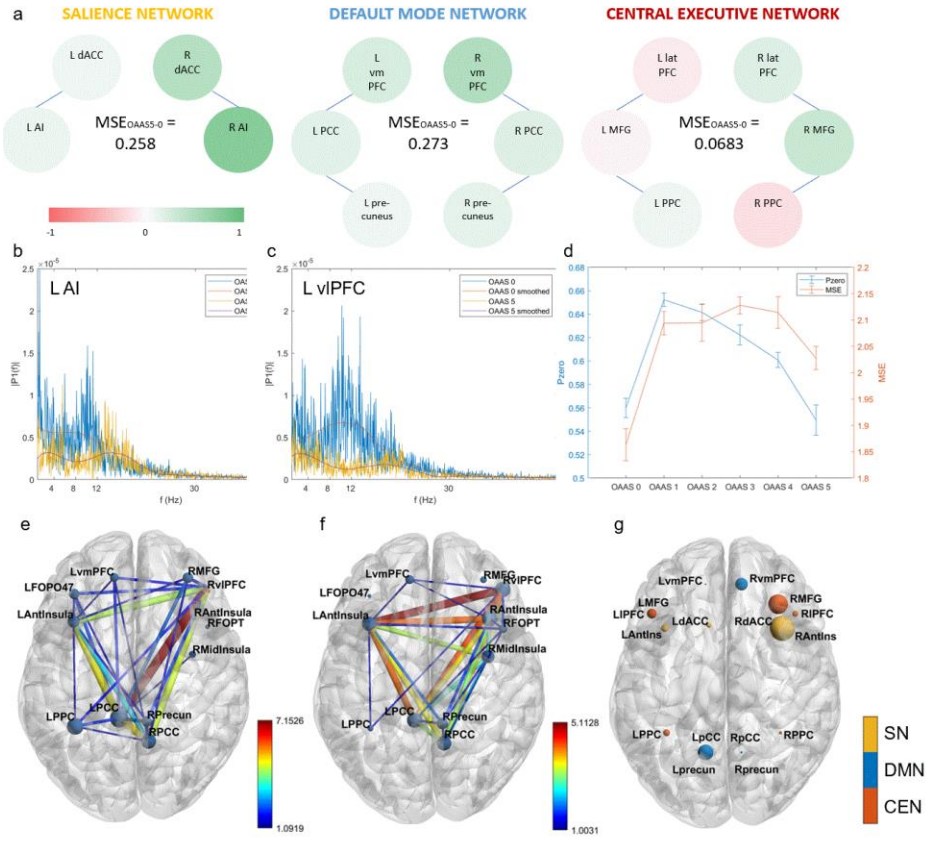


Fig 1. a: Cognitive network nodal multiscale sample entropy (MSE) change between OAAS 0 (unconscious) and 5 (awake). Strongest increases in iEEG signal complexity during emergence (darker green) occur in the right anterior insula (R AI), right dorsal anterior cingulate cortex (R dACC), and right ventromedial prefrontal cortex (R vmPFC). Mean SN and DMN signal MSE (center of each diagram) increases significantly more during emergence than CEN (P<0.05). **b-c: Representative iEEG nodal spectrograms at OAAS 0 and 5.** In left anterior insula (**b**, L AI), fast-Fourier-transformed waveform shows expected reduction of anesthesia-induced alpha-frequency power and concurrent increase in beta-frequency power between OAAS 0 and 5. Conversely, in left ventrolateral prefrontal cortex (**c**, vlPFC), a node at which signal decreases in complexity between OAAS 0 and 5, broadband power reduction (particularly at 12Hz) is evident during emergence. **d: Participation coefficient (Pzero) and multiscale sample entropy (MSE)** share similar temporal dynamics with peaks at intermediate consciousness states. **e-f: Representative functional connectivity graph at OAAS 0 and 5.** During emergence between unconscious (e) and fully awake (f) states in one patient, between-network functional connectivity increases, especially between nodes of SN and CEN, and between SN and DMN, while CEN nodal strength decreases relative to SN and DMN. Node size weighted by nodal strength; edges weighted by communicability. Edges displayed if internode communicability > 1. **g: Whole-network communicability during emergence.** Communicabilities of overlapping nodes of SN, DMN, and CEN from all patients were normalized within each patient and averaged to obtain a whole-network communicability value for SN, DMN, and CEN. This value increased most in SN, slightly decreased in DMN, and remained roughly unchanged in CEN during emergence (OAAS 0 to OAAS 5). The greatest increases in nodal communicability were seen in right anterior insula (RAntIns) and right middle frontal gyrus (RMFG), representing SN and CEN, respectively. Node size weighted by difference in nodal communicability over the OAAS 0 to 5 transition. Abbreviations: vmPFC = ventromedial prefrontal cortex. lPFC = lateral prefrontal cortex. dACC = dorsal anterior cingulate cortex. PPC = posterior parietal cortex. pCC = posterior cingulate cortex. precun = precuneus. MFG = middle frontal gyrus.

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Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.07

Topic: H.01. Attention

Support: NIA AG058748
NIA AG072328
Alzheimer's Association Award AARF-17-530186

Title: Locus coeruleus catecholamine synthesis capacity moderates the effect of resting-state network coupling on attentional switching in aging

Authors: *J. H. PARENT[1], K. CASSADY[2], C. GORDON[1], W. J. JAGUST[2], A. S. BERRY[1]; [1]Brandeis Univ., Brandeis Univ., Waltham, MA; [2]Univ. of California, Berkeley, Univ. of California, Berkeley, CA

Abstract: Attentional control, which involves shifting, reorienting, and maintaining attention to meet goals is one of the earliest cognitive domains to be affected in Alzheimer's Disease (AD). Whole-brain functional networks, specifically the cingulo-opercular network (CON) and the frontoparietal control network (FPCN) support attentional control and are dedifferentiated in AD. The LC-catecholamine system is implicated in attentional processes and is a centerpiece of current models of the early pathophysiology of AD. Here, we used [^{18}F]Fluoro-m-tyrosine ([^{18}F]FMT) PET to measure LC catecholamine synthesis capacity, and 3T resting-state fMRI to examine relationships among LC catecholamine function, attentional network coupling, and attentional switching performance (Trail Making Test: Trails B-A) in cognitively normal older adults (n = 45; 28 females, mean education: 17 mean age: 77 (range = 62-85 years); 13 beta-amyloid positive determined by [^{11}C]Pittsburgh compound B PET). We found that there were no direct relationships among LC-catecholamine synthesis capacity, CON-FPCN coupling, or performance. However, LC-catecholamine synthesis capacity and CON-FPCN coupling interacted to predict performance ($\beta_{\text{CON-FPCN coupling x LC [18F]FMT}}$ = 3443, t(38)= p = .010, adjusting for age, sex, years of education). In the context of higher LC catecholamine synthesis, lower coupling was associated with better attentional performance. The direction of coupling-cognition relationships is broadly consistent with interpretations that lower between-network coupling reflects greater network differentiation in aging. A marginal interaction with beta-amyloid status ($\beta_{\text{CON-FPCN coupling x LC [18F]FMT x [11C]PiB status}}$ =6314.5, p=.078) suggests the benefit of higher LC-catecholamine synthesis may be felt more strongly for those on the path to preclinical AD. These findings may be relevant for research implicating LC function in resilience to early AD pathology.

Disclosures: **J.H. Parent:** None. **K. Cassady:** None. **C. Gordon:** None. **W.J. Jagust:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Dr. Jagust holds an equity interest in Optoceutics. F. Consulting Fees (e.g., advisory boards); Dr. Jagust has served as a consultant to Biogen and Bioclinica. **A.S. Berry:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.08

Topic: H.01. Attention

Support: JSPS KAKENHI Grant JP19K11331

Title: Neural correlates of spatial attention bias: changes of resting-state functional connectivity in the attention networks during neglect-like deficits induced by transcranial direct current stimulation in healthy adults

Authors: *K. TSUJIMOTO[1,2], D. NISHIDA[3,2], M. TAHARA[4], M. LIU[5], T. TSUJI[5], K. MIZUNO[3,2,5];
[1]Dept. of Advanced Neuroimaging, Natl. Ctr. of Neurol. and Psychiatry, Integrative Brain Imaging Ctr., Tokyo, Japan; [2]Dept. of Physical Rehabil., Natl. Ctr. of Neurol. and Psychiatry Hosp., Tokyo, Japan; [3]Dept. of Rehabil., Tokai Univ., Kanagawa, Japan; [4]Saiseikai Higashikanagawa Rehabil. Hosp., Kanagawa, Japan; [5]Dept. of Rehabil. Med., Keio Univ. Sch. of Med., Tokyo, Japan

Abstract: It has been reported that neglect-like behavior appears with biparietal transcranial direct current stimulation (biparietal-tDCS) (left anode/ right cathode) in healthy persons. However, few studies have focused on the changes in functional connectivity (FC) within the attention networks with biparietal-tDCS. The purpose of this study was to investigate changes in FC after biparietal-tDCS in healthy adults. Participants (n=19) underwent 20 minutes of right hemisphere cathodal and left hemisphere anodal tDCS. Resting-state fMRI and two visual search tasks were recorded before and after sham-tDCS and real-tDCS. The visual search tasks were used to examine the presence of pseudo-neglect behavior. Functional brain images were acquired on a 1.5-T MR scanner. A seed-based correlation analysis was performed to investigate the FC in a dorsal attention network (DAN) (frontal eye field and intraparietal sulcus) and ventral attention network (VAN) (middle frontal gyrus and superior temporal sulcus). We found that the reaction time for targets in the left hemifield was significantly prolonged during two different types of visual search tasks, and FC of the attention networks was altered by biparietal-tDCS. Furthermore, the change in reaction times for the left visual target in the two different tasks were significantly correlated with the change in FC of either the right DAN or right VAN depending on tasks. These results suggest that biparietal-tDCS delivered to the posterior parietal cortex bilaterally induced neglect-like behavior by altering the connectivity of the entire attentional

network through excitability changes in the cortical area under the electrode. This is the first study to demonstrate that local cortical stimulation can induce not only changes in local brain function but also changes in cortical networks in healthy subjects.

Disclosures: K. Tsujimoto: None. **D. Nishida:** None. **M. Tahara:** None. **M. Liu:** None. **T. Tsuji:** None. **K. Mizuno:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 072.09

Topic: H.01. Attention

Support: MOE2017-T3-1-002 from the Singapore Ministry of Education

Title: The basolateral amygdala excites the claustrum by preferentially innervating specific claustral subzones

Authors: *G. HAM[1], G. J. AUGUSTINE[2];
[1]Sch. of Social Sci., Nanyang Technological Univ., Singapore, Singapore; [2]Lee Kong Chian Sch. of Med., Singapore, Singapore

Abstract: In a proposed framework for salience-guided attention (*Front. Neuroanat*. 13:64), the basolateral amygdala (BLA) is thought to convey important valence information to claustrum (CLA). Though anatomical evidence suggests that the BLA may connect to the CLA, this suggestion has not been tested. We used viral-based tracing techniques, optogenetics and brain slice electrophysiology to dissect the circuit relationships between the BLA and the CLA. Our tracing results show that both BLA-projecting CLA neurons and axon fibers from the BLA are located in the CLA shell region, but are absent from the CLA core. Photostimulation of ChrimsonR-expressing BLA inputs mostly elicited excitatory postsynaptic currents (EPSCs) in CLA neurons (n = 125). These currents were small (mean amplitude 21 pA at -70 mV), fast (~2 ms rise and 5 ms decay) and variable (CV = 0.64). Contrary to the expectations from our anatomical tracing, EPSC properties were similar for neurons in both the CLA core and shell regions; presumably this occurs because the BLA innervates the distal dendrites of CLA core neurons, while innervating shell neurons more broadly. In contrast, BLA input between neurons in the dorsal and ventral CLA did differ (n = 70). EPSC response probabilities were significantly higher in the ventral CLA (0.46) compared to dorsal CLA (0.17). In all cases, BLA inputs were subthreshold, indicating that substantial summation of inputs would be required to evoke action potentials in CLA neurons. These results establish the existence of a functional BLA-to-CLA connection that preferentially excites neurons in the ventral CLA network. Our findings define how the BLA regulates CLA activity and uncover a topology-based preference for BLA input. These results reinforce our hypothesis of topology-based segregation of CLA function (*Front.*

Neuroanat. 16:42) and represent a fundamental step toward uncovering the function of the CLA in salience and attentional processing.

Disclosures: G. Ham: None. **G.J. Augustine:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 072.10

Topic: H.01. Attention

Support: NBRC Flagship program BT/ MEDIII/ NBRC/ Flagship/ Program/ 2019: Comparative mapping of common mental disorders (CMD) over lifespan

Title: How do distracting sounds distract us while listening to target speech and non-speech audio?

Authors: *P. GHOSH, K. SALUJA, A. BANERJEE;
Cognitive Brain Dynamics Lab., Natl. Brain Res. Centre, Manesar, Gurgaon, India

Abstract: Responding to salient changes in the environment while doing routine tasks is not only important to humans (spotting a pedestrian while driving car) but is critical for survival throughout the animal kingdom (escaping from predators). Burgeoning research has shown that the Ventral Attention Network (VAN) is primarily responsible for reorientation of attention to such salient stimuli from the goal-directed stimuli in a task. Previous research from our group revealed the oscillatory and connectivity changes in VAN in response to salient distractors while performing various complex goal-directed visual tasks. We try to draw parallels with these findings in the *visual* domain by using *auditory* stimuli in the present study, designed with 3 different levels of complexity - ***static*** pure tones, ***dynamic*** FM sweeps and ***speech*** syllables. We recorded behavioral and simultaneous 64-channel scalp EEG data from 28 right-handed healthy humans (20-31 years, 16 females) who signed informed consent forms approved by the Institutional Human Ethics Committee of NBRC, India. The participants performed auditory tasks with the 3 categories of stimuli where they had to identify the longer/shorter sound (prompted before each block) from a pair of 2 identical sounds (differing only in duration) presented in succession. Trials with the target audio pairs in each category were presented with (ST) and without (WT) salient distracting sounds. Additionally, there were attention control trials where both the audio of a pair were equal in duration (NT). 2 blocks of each category (90 trials in each block; 30 each of WT, ST and NT) were presented in random order. Behavioral results indicated significantly higher ($p<0.001$) medians of reactions times (in ms) of NT>ST>WT in static (1908.6, 1692.6, 1644.9), dynamic (1890.2, 1742.9, 1663.2) and speech (1938, 1802.6, 1711) categories. Accuracies (in %) in the presence of salient stimuli (ST) dropped as compared to trials without them (WT), in static (ST-89.46, WT-93.45), dynamic (82.98, 89.05) and speech (73.15, 88.39) conditions. We further identified the neural correlates of behavior and found

significantly enhanced (p=0.02) alpha power (~10 Hz) in the presence of distractors (grand-average across 64 sensors) in case of *static* stimuli. However, the increase in alpha power was not significant for *speech* and *dynamic* stimuli (neural data analyzed for 10 subjects only). A detailed comparison of correlations between alpha powers and reaction times across the 3 scenarios, WT, ST and NT, will provide us with important insights into understanding how auditory distractors are processed while attentively engaged in listening to sounds from different categories

Disclosures: P. Ghosh: None. **K. Saluja:** None. **A. Banerjee:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

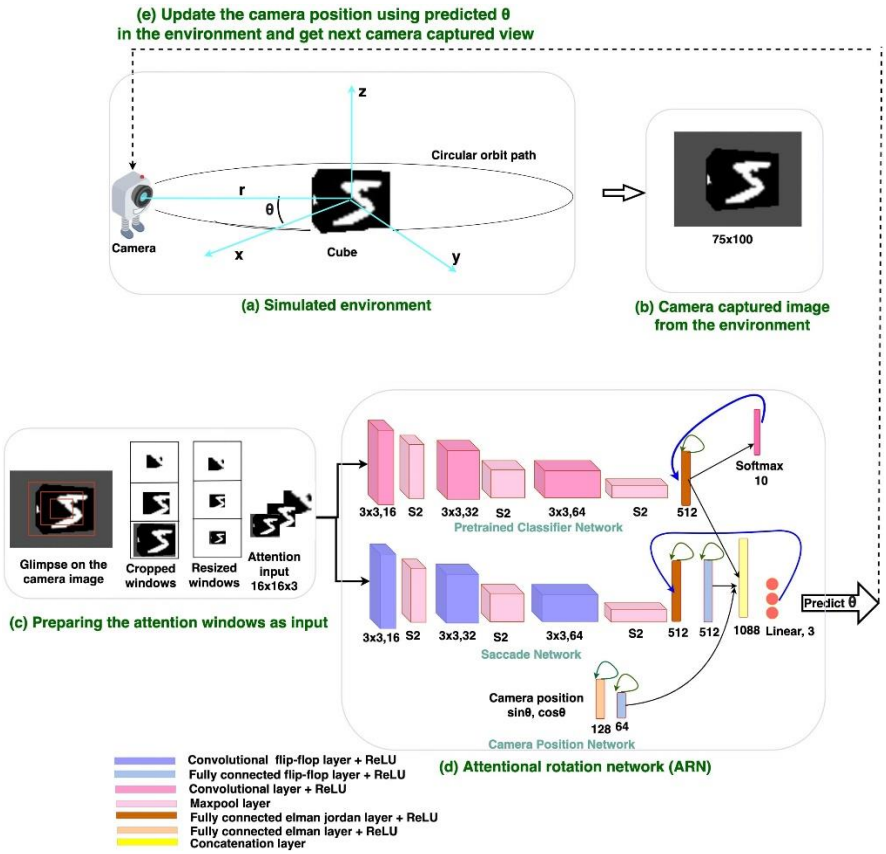
Program #/Poster #: 072.11

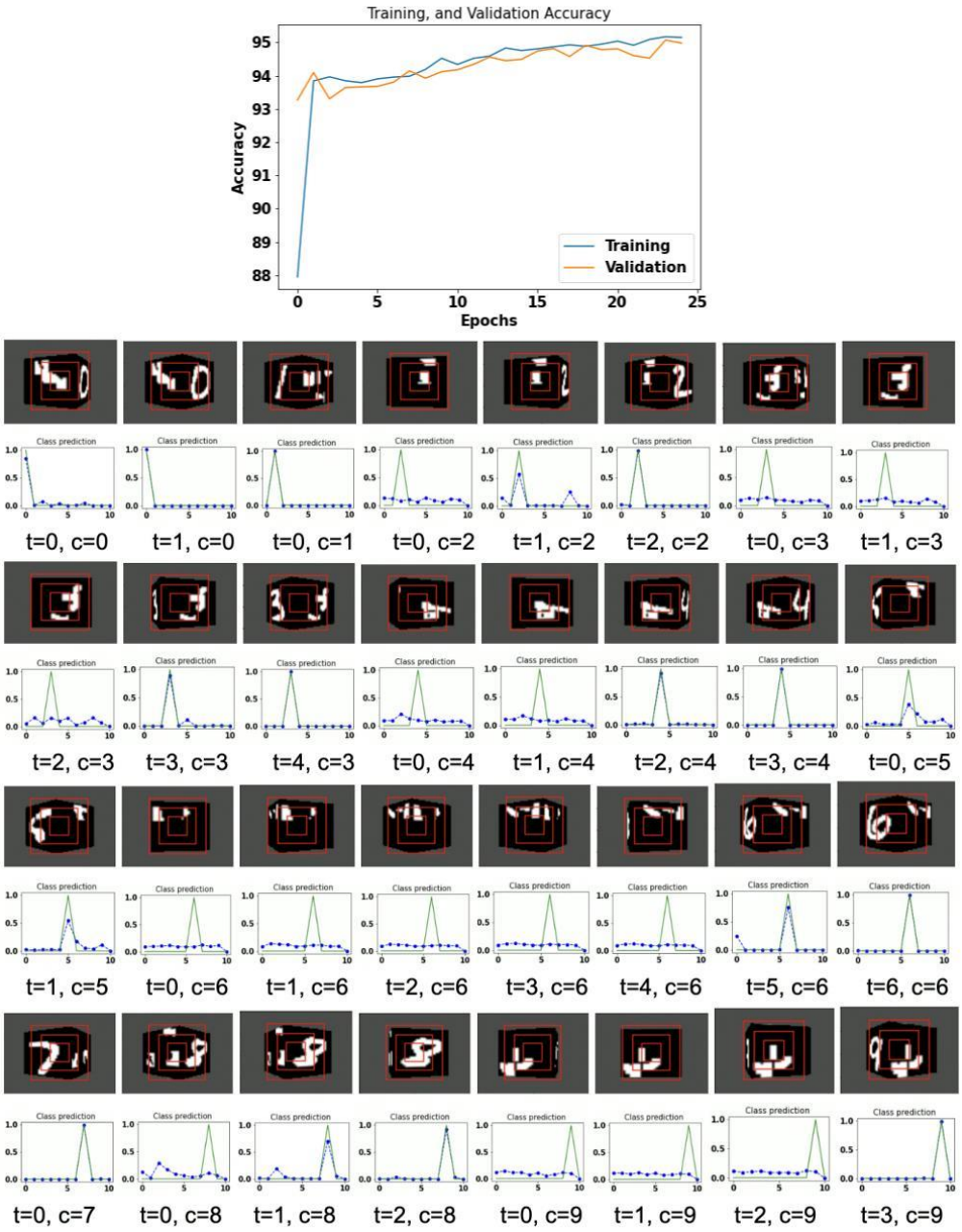
Topic: H.01. Attention

Title: An attentional network fashioned after what and where/how pathways for target search in 3D environment

Authors: *S. KUMARI[1], S. A. V. Y[2], N. M[1], S. V. CHAKRAVARTHY[1];
[1]Dept. of Biotech., IIT Madras, Chennai, India; [2]IIT BHU, Banaras, India

Abstract: The human visual system scans a scene by performing a sequence of attentional fixations on the scene. In the visual cortex, the visual information flows through what and where/how pathways to extract object identity, and spatial properties. We propose a biologically plausible attention model to target search in a 3D environment, which has a separate channel for object classification ("what") and saccade generation ("where"). We generated a 3D Cluttered MNIST Digit Cube dataset that consists of a MNIST image on one vertical face, and clutter images on the other faces. The camera goes around each cube on a circular orbit of the cube and determines the identity of the MNIST image and the face on which it is located. The attentional input of 3 concentric cropped windows resembling the high-resolution central fovea and low-resolution periphery of the retina, flows through a Classifier Network (CN) and a Saccade Network (SN) (Fig-1). The CN classifies the current view into one of the MNIST classes or clutter. The SN predicts the camera's next position on the orbit (varying the azimuthal angle or 'θ'). Here the camera performs one of three actions: move right, move left, or dont move. The SN is trained using Q-learning where the reward is 1 if the CN gives the correct classification, otherwise 0. Total loss is computed by adding the mean square loss of temporal difference and cross entropy loss, and backpropagated using Adam. Target classification accuracy achieved is 96.25% in the testing set (Fig-2).


(e) Update the camera position using predicted θ
in the environment and get next camera captured view
z
Circular orbit path
r
θ
Camera
x
Cube
y
(a) Simulated environment
75x100
(b) Camera captured image
from the environment
Glimpse on the
camera image
Cropped
windows
Resized
windows
Attention
input
16x16x3
(c) Preparing the attention windows as input
3x3,16
S2
3x3,32
S2
3x3,64
S2
512
Softmax
10
Pretrained Classifier Network
3x3,16
S2
3x3,32
S2
3x3,64
S2
512
512
1088
Linear, 3
Predict θ
Saccade Network
Camera position
sinθ, cosθ
128
64
Camera Position Network
(d) Attentional rotation network (ARN)
Convolutional flip-flop layer + ReLU
Fully connected flip-flop layer + ReLU
Convolutional layer + ReLU
Maxpool layer
Fully connected elman jordan layer + ReLU
Fully connected elman layer + ReLU
Concatenation layer



Disclosures: **S. Kumari:** None. **S.A.V. Y:** None. **N. M:** None. **S.V. Chakravarthy:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

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Program #/Poster #: 072.12

Topic: H.01. Attention

Support: BMBF grant CRCNS No. 01GQ1805A

Title: Comparison of functional connectivity of area FST in humans and macaques

Authors: *F. MOLLA, M. HIMMELBACH;
Ctr. for Neurol., Tübingen, Germany

Abstract: The temporal cortex has been recently reported to be a crucial hub in spatial attention control in both humans and monkeys. We provided evidence that area FST in humans is involved in visual attention. Replicating an experiment done in monkeys, we found increased activation concomitant to the attentional task in the MT+ complex and in FST, independently of the stimulus feature that was presented. We argued that this area was homologous to the monkey area aFST. In the current study we compared the functional connectivity of area FST in humans and monkeys to corroborate our hypothesis that FST shares a similar role in both species and show that its activity is well integrated with traditional attentional areas. We used publicly available resting state data from the HCP (n=40 humans, acquired at 7T) and from the INDI-PRIME project (n=38 macaques, acquired at 3T). We preprocessed the human data with the minimal HCP pipeline, while the monkey data were preprocessed using the C-PAC pipeline. Both included motion-correction, global mean and motion components regression, and spatial smoothing. We calculated the Pearson's correlation between the average time series of parcels taken from the Glasser parcellation (Glasser et al. 2016) for the human data and the D99 atlas [REF] for the non-human primate data. In humans and macaques, we observed a similar connectivity pattern for FST. In humans, we detected positive correlations with areas MIP, LIP, VIP, as well as MT, MST, LOC, TPO, PIT and V4. Similarly, macaque area FST showed positive correlations with MIP, LIP, VIP, MST and TEO. These findings reinforce the idea that area FST is embedded in the same cortical networks across the two species and that its activity is inherently related to that of areas traditionally linked to visual attention in both species. Further analyses will address the interplay between FST and other subcortical areas known to play a key role in visual attention, such as superior colliculus and pulvinar.

Disclosures: F. Molla: None. **M. Himmelbach:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

Location: SDCC Halls B-H

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Program #/Poster #: 072.13

Topic: H.01. Attention

Title: Neuromodulation of a thalamo-cortical cue detection circuit

Authors: *K. RUNYON, A. GREENWAY, S. MAZANEK, C. SALLEE, A. HARTLE, K. MARSCHALKO, W. M. HOWE;
Sch. of Neurosci., Virginia Tech., Blacksburg, VA

Abstract: A deficit in the capacity to use instructive cues to guide ongoing behavior, “cue detection”, is a feature of many neurodegenerative and neuropsychiatric disorders. Previous research identified a circuit whereby mediodorsal thalamic (MD) inputs to the prefrontal cortex (PFC) interact with local cholinergic systems to determine the efficacy of such cue detection. Aside from this terminal regulation, activity in PFC-projecting MD neurons is tightly controlled by the thalamic reticular nucleus (TRN). This band of GABAergic cells has been referred to as the brain's “internal attentional searchlight” (Crick, 1984) given their role to regulate thalamo-cortical interactions. Accordingly, lesions to the TRN impair the ability of attention-grabbing cues to guide behavior (Weese et al., 1999; Bucherelli et al., 1993). The TRN receives inputs from all major ascending monaminergic and cholinergic systems, which themselves have been implicated in the normal control of cue-guided behavior. Here, we describe a set of studies designed to provide insight into the unique contributions of each ascending system to TRN control of the MD-PFC cue detection circuit. Using transgenic mouse lines (Chat-Cre, DAT-Cre, Sert-Cre) and viral tracing techniques (n=2-4 per group), we first show that MD-projecting neurons are clustered in ventral TRN. Interestingly, this same part of the TRN received a relatively dense input from dopamine neurons of the substantia nigra pars compacta. In contrast, serotonergic and brainstem cholinergic inputs were most dense in dorsal TRN. We further identified a subset of cholinergic projection neurons that branched off to directly innervate the MD. To begin to understand how each of these TRN inputs modulate behavior, we have developed a 2-choice serial reaction time task to assess cue detection and attentional control in mice. Preliminary experiments (n=12) indicate mice can acquire the task after about ~8 weeks of training, and importantly, performance accuracy is signal-duration dependent. Ongoing experiments use in vivo calcium sensors to measure activity throughout the TRN-MD-PFC circuit during task performance. Future studies will use DREADDs to selectively manipulate each ascending input to the TRN to better understand how each system modulates circuit activity and behavior.

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Poster

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Program #/Poster #: 072.14

Topic: H.01. Attention

Support: NIH Grant 5U01EY025858
NIH Grant R01EY031589

Title: Determining optimal clustering of co-activation patterns in fMRI data during resting-state and task

Authors: P. STEWART[1], ***K. M. VISSCHER**[2];
[1]Neurobio., [2]Univ. of Alabama, Birmingham, Birmingham, AL

Abstract: The brain transitions between states of different activity patterns. Functional MRI can be used to quantify how these states change with time. One such approach uses K-Means clustering to find a set of co-activation patterns (CAPs), which are repeating brain states that occur within a set of fMRI data collected from multiple participants. Different sets of CAPs can be created from a single dataset by varying the number of K-Means clusters. Using existing analytical techniques (Frederick, 2017) and a dataset including fMRI data from 64 participants under a wide variety of conditions and states, including resting state data collected in both light and darkness, numerous cognitive tasks, movie watching, and retinotopic mapping sequences, we show that the specific choices of analytical parameters have limited influence on CAP outputs. Specifically, regardless of the number of clusters that are used to group the data, we find that each cluster mean has a strong correspondence to one of a set of 8 distinct CAPs. We use silhouette statistics to further reinforce the idea that the natural number of clusters that is appropriate for this approach is in the range of 6-8. This suggests that this clustering approach may not be able to find unique coactivation patterns found solely in specific tasks or experimental conditions. However, we have identified CAPs that appear significantly more or less often in given tasks or conditions, bolstering the interpretation that the CAPs reflect changes in brain state that are behaviorally relevant. By limiting the number of clusters to a distinct set of 8 CAPs, and by looking at the relative distribution of those CAPs within functional data, we can learn more about how different experimental conditions relate to ongoing dynamics of brain activity.

Disclosures: P. Stewart: None. **K.M. Visscher:** None.

Poster

072. Neural Mechanisms of Attention: Electrophysiology and Circuit Approaches

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Program #/Poster #: 072.15

Topic: H.01. Attention

Support: MRC [MR/P013031/1]
Wellcome Trust [093104]
Tehran University of Medical Sciences [grant No. 97-02-87-38826]

Title: Sharp Wave Ripples in Macaque V1 and V4 are Modulated by Top-Down Visual Attention

Authors: J. DOOSTMOHAMMADI[1], M. A. GIESELMANN[2], J. VAN KEMPEN[2], R. LASHGARI[3], A. YOONESSI[4], ***A. THIELE**[2];

[1]McGill Univ., Montreal, QC, Canada; [2]Newcastle Univ., Newcastle Univ., Newcastle upon Tyne, United Kingdom; [3]Inst. For Res. In Fundemental Sci., Inst. For Res. In Fundemental Sci., Tehran, Iran, Islamic Republic of; [4]Tehran Univ. of Med. Sci., Tehran, Iran, Islamic Republic of

Abstract: Sharp-wave ripples (SWRs) are highly synchronous neuronal activity events. They have been predominantly observed in the hippocampus during offline states such as pause in exploration, slow-wave sleep and quiescent wakefulness. SWRs have been linked to memory consolidation, spatial navigation, and spatial decision-making. Recently, SWRs have been reported during visual search, a form of remote spatial exploration, in macaque hippocampus. However, the association between SWRs and multiple forms of awake conscious and goal-directed behavior is unknown.

In hippocampus SWR frequency is modulated by PV+ and SOM+ cell activity, as well as by cholinergic input. Given specific roles of PV+, and SOM+ cell activity in spatial attention and centre surround interaction, we predicted that attention to the receptive field should increase ripple rates, a wide focus of attention (harder task) to the receptive field would increase ripple rates, and small stimuli should yield higher ripple compared to large stimuli.

To examine these possibilities, we recorded LFPs and spiking activity from laminar probes in visual area V1 and V4 of two male macaque monkeys performing a cued spatial attention task, where we manipulated the spatial predictability of target appearance, resulting in narrow and wide foci of attention.

SWRs were detected in both regions, ripples occurred more often during the sustained period of stimulus presentation, compared to pre-stimulus periods. The occurrence of ripples was increased by attention towards the receptive field, and by the size of the attentional focus. During attention to the receptive field, the monkey's reaction times in detecting behaviorally relevant stimulus changes was affected by SWRs. These results show that ripple activity is not limited to hippocampal activity during offline states, rather they occur in the neocortex, are modulated by specific aspects of active attentive states and by stimulus characteristics.

Disclosures: J. Doostmohammadi: None. **M.A. Gieselmann:** None. **J. Van Kempen:** None. **R. Lashgari:** None. **A. Yoonessi:** None. **A. Thiele:** None.

Poster

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Program #/Poster #: 072.16

Topic: H.01. Attention

Support: NIH grant MH108591
NSF grant BCS1558497

Title: Connectome-based predictions of individual performance improve when a task is more attentionally demanding

Authors: *K. R. YOO[1], Y. KWON[1], M. D. ROSENBERG[2], M. M. CHUN[1];
[1]Psychology, Yale Univ., New Haven, CT; [2]Psychology, Univ. of Chicago, Chicago, IL

Abstract: In connectome-based predictive modeling (Finn et al., 2015, *Nat Neurosci*), functional brain connectivity can predict individual differences in behavior, and the predictions are more accurate from connectomes measured while participants perform cognitive tasks than from connectomes measured at rest (Greene et al., 2018, *Nat Comms*). However, what makes task connectomes more predictive of behavior is still unknown. Here, we asked if the level of attentional demand within the same task influences the accuracy of behavioral predictions. We examined two possibilities: the same-network hypothesis vs. the different-network hypothesis. According to the same-network hypothesis, high and low levels of attention induce similar mental and brain states. Random mind-wandering is unavoidable at rest, and goal-directed attention in tasks may lessen inter-individual variation in ingoing cognition and connectomes, resulting in a similar degree of prediction improvement over rest. In the different-network hypothesis, high attentional demands engage different brain networks that can further improve predictions over both the easier condition or rest. We analyzed fMRI data from 92 subjects who completed multiple object tracking (MOT) and visual short-term memory (VSTM) tasks during fMRI scanning (Yoo et al., 2022, *Nat Hum Behav*). Each task varied task difficulty, requiring higher and lower degrees of attention, and we calculated behavioral performance and the underlying brain connectomes separately for higher and lower attention trials. We used a generalized psychophysiological interaction-like general linear model to estimate attention level-specific connectivity while controlling for task activation. We employed connectome-based predictive modeling to predict behavior. Individual task performance was significantly lower in higher attention trials in both tasks ($p<0.001$; paired t test). The spatial similarity of the rest connectome to the high-attention connectome was significantly lower than that to the low-attention connectome in both tasks ($p<0.05$; paired t test). The high- and low-attention connectomes predicted individual task performance significantly better than the rest connectome, and they exhibited distinct patterns of predictive anatomy from each other. Compared to the low-attention connectome, the high-attention connectome predicted individual behavior significantly better (for all trials, $p<0.05$ from 1,000 permutations). Overall, in support of the different-network hypothesis, these results show that higher attentional task demands improve behavioral predictions over easier versions of the same task or rest.

Disclosures: K.R. Yoo: None. **Y. Kwon:** None. **M.D. Rosenberg:** None. **M.M. Chun:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

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Program #/Poster #: 073.01

Topic: H.01. Attention

Support: Army Research Laboratory Cooperative Agreement W911NF-10-2-0022
Vannevar Bush Faculty Fellowship from the US Department of Defense (N00014-20-1-2027)

Title: Bold activity in locus coeruleus covaries with multiple neural processes in the reorienting response

Authors: *L. HONG, H. HE, P. SAJDA;
Biomed. Engin., Columbia Univ., New York, NY

Abstract: Efficient reorienting of attention is imperative for animals and humans to survive in the ever-changing environment. The ability to redirect our attention to novel and unexpected stimuli allows us to avoid danger and reap rewards in a timely manner. When this ability is impaired, even the simplest daily activity can be compromised (such is the case with neglect patients or populations with autism, depression, or posttraumatic stress disorder). Recent studies have implied the role of arousal in modulating attention reorienting. The precise nature of such modulation, however, remains unknown. In this study, we investigated how locus coeruleus (LC) based arousal interacts with attention reorienting in the human brain. We simultaneously recorded EEG and fMRI to explore the spatiotemporal dynamics between the arousal and reorienting systems underlying an auditory oddball task. We used blood oxygenation level dependent (BOLD) activity in the LC to index cortical arousal, as LC is the major brainstem nuclei responsible for releasing norepinephrine into the cortex. We then used temporally specific trial-by-trial EEG measures to model task-evoked BOLD activity in the LC, by capitalizing on the complementary temporal and spatial resolutions of EEG and fMRI, respectively. Our findings revealed that BOLD activity in the LC covaried with EEG measures from 250 to 375 ms poststimulus (range of coefficient estimate b: 0.0177 to 0.0336; range of significance level P: 0.0068 to 0.0454). These results suggest that LC activity is related to neural processes at multiple latencies of the reorienting response, and imply the impact of LC during different temporal stages of attention reorienting. Taken together, this study revealed comprehensive spatiotemporal dynamics on the interplay between arousal and attention reorienting. Our findings therefore (i) complement and extend previous work by providing additional information on how the healthy brain reorients attention under the influence of arousal; and (ii) provide insights on potential biomarkers that can be used to monitor deterioration or improvement of clinical conditions.

Disclosures: L. Hong: None. **H. He:** None. **P. Sajda:** Other; scientific advisor to Optios Inc. and OpenBCI LLC.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.02

Topic: H.01. Attention

Title: Signal detection theory as a mode to explore the full range of cognitive control:

Authors: *N. ALI;
None, London, United Kingdom

Abstract: Bilinguals are superior at the resolution of interference because they are quicker at inhibiting verbal distracting information or quicker in executive processing. This study explores conflict monitoring over time for interference (verbal; non-verbal) and speed of adjustment. Sixteen monolinguals (English) and 23 bilinguals acquired their languages before the age of 6. To explore speed of adjustment of onset or offset of incongruent conflicting information, a 2 x 2 x 2 ANOVA incongruency on or off (CI or IC) with language group and task shows significant main effect of incongruency on or off, p <.001, significant interaction between task and incongruency on/off, p < .001, and a significant interaction between task, on/off and language group, p = .001. T tests revealed longer RTs for incongruent trials for both language groups. Bilinguals RTs were lower on the Stroop task, and their RTs were significantly shorter for the offset of incongruency (IC) on the Simon task.To explore monitoring processes over time, mean RTs for each position of a run of the same 6 trials showed bilinguals were significantly faster at the Stroop, t (6) = 9.59, p < .001. Plotting mean RTs across trial types, a u curve with highest reaction times at the beginning and end of the run of trials was found in the Stroop for both language groups but an inverse u curve with lowest reaction times at the beginning and end trials in the Simon task. Bilinguals' faster release to offset of conflict within a non-verbal task suggests a bilingual efficiency in changing response strategy. Different modulatory strategies across time were found for a verbal and non-verbal task across language groups. By neighbouring modulatory behaviour over time for the Simon and then the Stroop task presents an S curve, revealing a sensory pre-motor system of conflict detection that feeds into one of conflict resolution. This method may be used to identify the various time points of the executive system and using signal detection theory, localise the cross-over point where one can detect the brain networks and the time course for change in strategies.

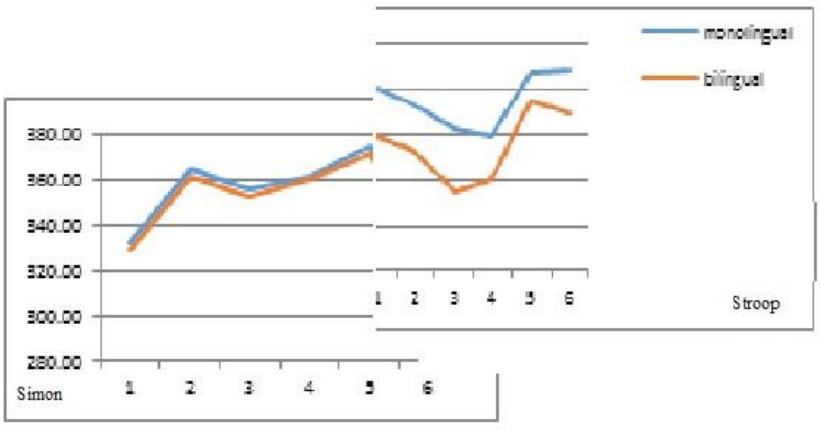

Figure 1: Average mean RT for each of the 1-6 positions of trial runs averaged across the trial types (neutral, congruent and incongruent); for the Simon (left) and Stroop (right) tasks for both monolinguals (blue) and bilinguals (right).

Disclosures: **N. Ali:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.03

Topic: H.01. Attention

Support: NIH/NIAAA Intramural Research (Y1AA3009)

Title: Methylphenidate reduces cortical synchronization and modulates central-autonomic interactions

Authors: ***E. SHOKRI KOJORI**, P. MANZA, D. TOMASI, G.-J. WANG, N. D. VOLKOW; NIH, Bethesda, MD

Abstract: Methylphenidate (MP) is a leading treatment for ADHD that enhances dopamine and norepinephrine signaling through inhibiting their presynaptic reuptake. MP is a sympathomimetic agent and affects the autonomic nervous system through peripheral adrenergic neurons. Several fMRI studies have documented that an acute MP challenge reduces low frequency (LF, < 0.1 Hz) activity and connectivity in sensorimotor and visual regions, yet the neurophysiological underpinning of this effect remains unclear. Previously we have identified significant temporal coupling (indexed by phase) between peripheral sympathetic tone (indexed by LF pulse signal changes) and sensorimotor, dorsal attention, and visual regions (an autonomic network, AN). Here, we hypothesized that reduced LF synchronization in AN with a MP challenge is associated with altered coupling between brain and peripheral measures of autonomic function. In a group of 26 healthy individuals (9 females, 22-64 years old), we measured brain resting-state activity with fMRI (7.7 min) and concurrently recorded pulse and respiratory signals in two sessions: after oral administration of MP (60 mg) or placebo (PL) in random order. Consistent with prior studies, we found a decrease in fractional amplitude of LF fluctuations primarily in visual and sensorimotor regions of AN ($p_{FWE} < 0.05$). Follow up analyses revealed that this effect was consistent with reduced LF synchrony as indexed by reduced LF power of the average signal within these regions ($p < 0.001$). MP did not significantly increase LF power of pulse and respiratory signals ($p > 0.15$). Analysis of LF phase between pulse and brain revealed significant associations in AN, medial and orbito-frontal, insular, thalamus, and pons regions in PL ($p_{FWE} < 0.05$). In MP, however, no major phase association was observed. For the respiratory signal, these were significant phase associations in the thalamus, striatum, visual, cerebellar, and midbrain regions in PL ($p_{FWE} < 0.05$). In MP, the phase associations expanded to insula, precuneus, cuneus, and cingulum regions ($p_{FWE} < 0.05$). LF power of AN in PL was significantly associated with LF phase between pulse and brain ($r(24) = -0.48$, $p < 0.02$) whereas LF power of AN in MP was significantly associated with LF phase between respiratory variations and brain ($r(24) = -0.45$, $p = 0.02$). In sum, reduced LF synchrony in AN with MP was concurrent with diminished LF brain-pulse phase coupling but

was associated with enhanced brain-respiratory phase coupling. The neuropharmacological effects of MP could provide unique insights into how interactions between autonomic and central nervous systems contribute to cortical synchronization.

Disclosures: E. Shokri Kojori: None. **P. Manza:** None. **D. Tomasi:** None. **G. Wang:** None. **N.D. Volkow:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.04

Topic: H.01. Attention

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Swiss National Science Foundation 100019_176016
Swiss National Science Foundation 100014_165884
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European Union's Horizon 2020 research and innovation program 794395
University of Zurich FK-19-020

Title: Dissociating motivation- from information-based neural salience drivers of attention

Authors: *J.-C. KIM, L. HELLRUNG, M. GRUESCHOW, S. NEBE, Z. NAGY, P. N. TOBLER;
Dept. of Econ., Univ. of Zurich, Zurich, Switzerland

Abstract: Salience optimizes behavior through attentional mechanisms. Traditionally, salience is thought to arise from the uncertainty and probability of motivationally charged (appetitive or aversive) outcomes. However, it remains unclear whether salience can also be bestowed by motivationally neutral outcomes, in line with an informational account of salience. To fill this important gap, we use human neuroimaging and a Pavlovian task where different cues predict appetitive, aversive or neutral liquids with different probabilities. We show that cue-elicited responses accelerate, and pupil sizes increase primarily for cues that predict motivationally charged outcomes with higher probability. With regard to neutral outcomes, particularly uncertainty (rather than probability) accelerates cue-induced responding and decreases pupil size. At the neural level, distinct regions of medial prefrontal cortex and occipital cortex separately process motivational or generic (i.e., motivation-related AND motivation-unrelated) salience signals. Moreover, the medial prefrontal, orbitofrontal, insular and occipital cortices dissociate probability-based motivational salience from uncertainty-based motivational salience. Thus, salience signals appear to be neurally distributed, in that purely informational forms of salience are coded separately from motivation-based forms. More generally, this dissociation suggests that for the brain not every bit of information is created equal and that there is traction in formalizing distinct forms of salience.

Disclosures: **J. Kim:** None. **L. Hellrung:** None. **M. Grueschow:** None. **S. Nebe:** None. **Z. Nagy:** None. **P.N. Tobler:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

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Program #/Poster #: 073.05

Topic: H.01. Attention

Support: ISF grant 2339/20

Title: Ecological investigation of attention to speech and the own-name advantage in a virtual cafe

Authors: A. BROWN, D. PINTO, K. BURGART, Y. ZVILICHOVSKY, ***E. ZION GOLUMBIC**;
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Abstract: Detecting that someone has said your name is one of the most famous examples for incidental processing of supposedly task-irrelevant speech. However, empirical investigation of this so-called “cocktail party effect” has yielded conflicting results. Here we present a novel empirical approach for revisiting this effect under highly ecological condition, using speech-stimuli and tasks relevant for real-life and immersing participants in a multisensory virtual environment of a café. Participants listened to narratives of conversational speech from a character sitting across from them, and were told to ignore a stream of announcements spoken by a barista character in the back of the café. Unbeknownst to them, the barista-stream sometimes contained their own name or semantic violations. We used combined measurements of brain activity (EEG), eye-gaze patterns, physiological responses (GSR) and behavior, to gain a well-rounded description of the response-profile to the task-irrelevant barista-stream.
Both the own-name and semantic-violation probes elicited unique neural and physiological responses relative to control stimuli, indicating that the system was able to process these words and detect their unique status, despite being task-irrelevant. Interestingly, these responses were covert in nature and were not accompanied by systematic gaze-shifts towards the barista character. This patterns demonstrate that under these highly ecological conditions, listeners incidentally pick up information from task-irrelevant speech and are not severely limited by a lack of sufficient processing resources. This invites a more nuanced discourse about how the brain deals with simultaneous stimuli in real-life environments and emphasizes the dynamic and non-binary nature of attention.

Disclosures: **A. Brown:** None. **D. Pinto:** None. **K. Burgart:** None. **Y. Zvilichovsky:** None. **E. Zion Golumbic:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.06

Topic: H.01. Attention

Support: Draper Labs

Title: Capturing States of Attention: Sensory ERPs and Pre-stimulus Alpha Power

Authors: *D. DISTEFANO, E. RACE;
Dept. of Psychology, Tufts Univ., Medford, MA

Abstract: Attention fluctuates across time and influences how we perceive and interact with the world around us. In the brain, neural responses to environmental stimuli are reduced during internal compared to external attentional states, indicating reduced cortical processing of the outside world ("perceptual decoupling"). For example, the amplitudes of visually-evoked P1 event related potentials (ERPs) are reduced when individuals report being internally oriented. Internal states of attention have additionally been associated with heightened levels of oscillatory power in the alpha range (~8-12 Hz) before a stimulus appears, which are thought to reflect the ongoing inhibition of neural activity in sensory processing regions. However, the precise relationship between pre-stimulus alpha power and stimulus-evoked neural responses remains a matter of debate and may depend on how these variables are measured. The current EEG experiment aimed to clarify this relationship using a novel binning procedure which measures pre-stimulus alpha power of individual trials binned by the amplitude of their stimulus-evoked responses. Participants viewed a series of faces and performed a gender discrimination task. The amplitude of evoked P1 responses to the face stimuli was used to back-sort pre-stimulus responses into low amplitude (decoupled) and high amplitude (perceptually coupled) trials, then mean pre-stimulus alpha power was calculated for each trial set. If pre-stimulus alpha power reflects states of internal attention, we predicted an inverse relationship between pre-stimulus alpha power and stimulus-evoked P1 amplitudes. In contrast to our prediction, we found that the amplitudes of evoked P1 responses were positively related to pre-stimulus alpha power within participants, with greater P1 amplitudes preceded by periods of stronger alpha power. A positive correlation between pre-stimulus alpha power and stimulus-evoked P1 amplitudes was also observed between participants. This relationship was further corroborated by a more traditional forward analysis of ERP amplitudes binned by pre-stimulus alpha power, with stronger pre-stimulus alpha power again being positively associated with greater P1 amplitudes. Together, these results suggest that increases in pre-stimulus alpha power may not always reflect decoupled internal states of attention, and that the relationship between pre-stimulus alpha power and stimulus-evoked cortical responses may vary according to task contexts or goals.

Disclosures: D. DiStefano: None. **E. Race:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

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Program #/Poster #: 073.07

Topic: H.01. Attention

Support: NSF Grant 2120539
Searle Scholars Program

Title: Independent mechanisms of anticipatory and reactive suppression during visual selective attention

Authors: *Z. V. REDDING, I. C. FIEBELKORN;
Neurosci., Univ. of Rochester, Rochester, NY

Abstract: Imagine navigating a congested street during a regular commute. In these everyday situations, we are presented with a vast array of sensory information. Given our limited capacity to simultaneously process this information, we must rely on attention, the set of filtering mechanisms by which the processing of relevant information is enhanced and irrelevant or distracting information is suppressed. Much has been learned about the neural bases of attention-related enhancement; however, there remains considerable debate over the mechanisms by which distracting information can be suppressed. Here, we used EEG to investigate the relationship between anticipatory suppression (e.g., in response to validly cued distractors) and reactive suppression (e.g., in response to invalidly cued distractors). Human participants performed a task with spatial cues that indicated the most likely location of both a low-contrast visual target and a high-contrast visual distractor. The results confirm an earlier suppression of sensory processing following the presentation of a validly cued distractor relative to invalidly cued distractors, consistent with anticipatory suppression. For invalidly cued distractors, the results revealed a later positivity—in the timeframe of the previously described distractor positivity—associated with correct trials (relative to incorrect trials). This later positivity is consistent with reactive suppression. With alpha-band activity during the cue-to-distractor delay as a proxy for the strength of anticipatory suppression, the results further revealed that this later positivity was also present in response to validly cued distractors but only when there was low anticipatory alpha-band activity (relative to trials when there was high anticipatory alpha-band activity). The present findings thus indicate that reactive suppression is engaged only when anticipatory attention is weak, providing evidence for different stages of attention-related suppression.

Disclosures: Z.V. Redding: None. **I.C. Fiebelkorn:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.08

Topic: H.01. Attention

Title: Attentional impairment in Parkinson's disease is modulated by the side of symptomatology onset: Neurophysiological and behavioral evidence

Authors: *Y. SERRATO, R. SOLÍS-VIVANCO;
INSTITUTO NACIONAL DE NEUROLOGIA Y NEUROCIRUGIA, CIUDAD DE MEXICO, Mexico

Abstract: Neurophysiological studies exploring involuntary attention (IA) through the event-related potentials (ERP) technique have reported that the P3a wave is impaired from initial stages of Parkinson's disease (PD). It is well known that automatic mechanisms of IA are regulated by the ventral frontoparietal attention network, mostly lateralized to the right hemisphere. PD generally initiates its motor symptomatology unilaterally, which has led to explore the cognitive and neurological differences between left vs. right onset PD patients. Whether IA is impaired depending on the onset side of motor symptoms in PD remains unknown. Here we compare the neurophysiological correlates of IA among a PD group with left-side onset (L-PD; n=25), a PD group with right-side onset (R-PD; n=31), and a healthy control group (HC; n=24). All participants performed an auditory IA task while a digital EEG was recorded. Our main finding was a reduction in the P3a amplitude in the L-PD group compared to the HC. Further, there was a significant correlation between P3a amplitude and disease duration in the R-PD, but not in the L-PD group. Behaviorally, both clinical groups, and in particular L-PD, showed reduced distraction effects by deviant stimuli. Our results indicate that IA is differentially impaired in patients with left side onset of motor symptoms. IA impairment might be present from initial stages of L-PD and become progressively compromised in patients with R-PD. The implications of motor symptoms onset side for attention impairment in PD are discussed.

Disclosures: Y. Serrato: None. **R. Solís-Vivanco:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

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Program #/Poster #: 073.09

Topic: H.01. Attention

Support: Vici project VI.C.181.032
Templeton World Charity Foundation, Inc. network "Survival Circuits Influences on Human Nature" to LdV, Elizabeth Phelps and Joseph LeDoux

Title: Assessing pupil size as an index of activation of subcortical ascending arousal system nuclei during rest

Authors: *B. LLOYD[1], L. DE VOOGD[2], V. MÄKI-MARTTUNEN[1], S. NIEUWENHUIS[3]; [1]Leiden Univ., Utrecht, Netherlands; [2]Donders Inst., Nijmegen, Netherlands; [3]Leiden University, The Netherlands, Leiden, Netherlands

Abstract: Neuromodulatory nuclei that are part of the ascending arousal system (AAS) play a crucial role in regulating cortical state and optimizing task performance. Pupil diameter, under constant luminance conditions, is increasingly used as an index of activity of these AAS nuclei. Indeed, task-based functional imaging studies in humans have begun to provide evidence of stimulus-driven pupil-AAS coupling. However, whether there is such a tight pupil-AAS coupling during rest is less clear. To address this question, we examined simultaneously acquired resting-state fMRI and pupil-size data from 74 participants (39 females), focusing on six AAS nuclei: the locus coeruleus, ventral tegmental area, substantia nigra, dorsal and median raphe nuclei, and cholinergic basal forebrain. Previous whole-brain fMRI studies assumed that the relationship between pupil-size and BOLD-signal changes during rest is characterized by a canonical hemodynamic response function. However, unlike cortical control regions, none of the AAS nuclei showed robust pupil-BOLD coupling under this assumption, or when we adopted region-specific hemodynamic response functions based on spontaneous events in the fMRI data. Instead, converging evidence indicated that all six AAS nuclei were optimally (and significantly) correlated with pupil size at short time lags (0-2 seconds), suggesting that spontaneous pupil changes were almost immediately followed by corresponding BOLD-signal changes in the AAS. These significant pupil-BOLD signal correlations were dominated by ultra-slow oscillation frequencies (~0.1 - 0.2 Hz). These findings raise interesting questions regarding neurovascular coupling in the AAS during rest. More in general, our results suggest that spontaneous changes in pupil size that occur during states of rest, can be used as a noninvasive index of activity in AAS nuclei, not just the locus coeruleus.

Disclosures: B. Lloyd: None. **L. de Voogd:** None. **V. Mäki-Marttunen:** None. **S. Nieuwenhuis:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.10

Topic: H.01. Attention

Support: The NIMH Intramural Research Program

Title: Emotion and Anxiety Interact to Bias Spatial Attention

Authors: *H. BACHMANN, S. JAPEE, E. P. MERRIAM, T. T. LIU;
Lab. of Brain & Cognition, Natl. Inst. of Mental Hlth., Bethesda, MD

Abstract: Emotional expressions are an evolutionarily conserved means of social communication. It is known that emotional faces influence attentional processing, but the

mechanisms by which they enhance or impair attention are unclear. Moreover, while individual variability in factors such as anxiety modulate attention, whether such factors interact with the processing of stimuli with emotional valence is uncertain. We conducted two preregistered experiments online via Amazon's Mechanical Turk to investigate the relationship between anxiety, emotional valence, and spatial attention. Stimuli were greyscale images of faces, varying in emotional expression (happy, angry and neutral), that were closely cropped and balanced for low-level image statistics and matched for arousal ratings. In the first experiment, participants (n=154) performed a visual search task, searching for a unique happy or angry face among either three or seven distractor faces. We found an attentional bias for positive over negative valence in visual search, robust at both set sizes, in search accuracy, response time, and inverse efficiency score. Anxiety ratings correlated positively with search accuracy for angry targets, but negatively with search accuracy for happy targets. We further computed a valence index of emotional attention as a difference in search accuracy between angry and happy targets. This valence index correlated strongly with anxiety, suggesting that participants with higher anxiety showed more threat-related attentional bias and less attentional bias to stimuli with positive valence. In the second experiment, participants (n=420) performed an emotional spatial cueing task. Subjects fixated a central cross while a face cue was presented for 250 ms in the periphery. After a brief interstimulus interval, a target white dot appeared in either the cued (60%) or the uncued (40%) location and participants judged the position of the dot relative to the midline. We found an interaction between anxiety and emotion such that emotional faces took longer to disengage from attention than neutral faces within the high anxiety group (n=47), but not in the low anxiety group, suggesting that the level of anxiety had a distinct impact on attentional disengagement. Together, our findings highlight the role of positively valenced stimuli in attracting and holding attention. We found that anxiety has a distinct impact on attentional capture and disengagement, suggesting that anxiety is a critical factor modulating spatial attention to emotional stimuli.

Disclosures: H. Bachmann: None. **S. Japee:** None. **E.P. Merriam:** None. **T.T. Liu:** None.

Poster

073. Attention: Neural Mechanisms and Neural Circuits

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 073.11

Topic: H.01. Attention

Support: K23ES026239

Title: Prenatal tobacco smoke and maternal demoralization alter cingulo-opercular network global efficiency in school-age children

Authors: *P. B. GREENWOOD[1,3], E. KOE[3], E. RODRIGUEZ[3], L. SALAS[3], J. HERBSTMAN[2], D. PAGGLIACIO[3], A. MARGOLIS[1,3];
[1]Dept. of Psychiatry, Vagelos Col. of Physicians and Surgeons, [2]Dept. of Envrn. Hlth. Sci. and

Columbia Ctr. for Children's Envrn. Health,, Columbia Univ., New York, NY; [3]Div. of Child and Adolescent Psychiatry, New York State Psychiatric Inst., New York, NY

Abstract: Background: Prenatal exposure to environmental tobacco smoke (ETS) is associated with adverse neurodevelopmental outcomes, including altered functional activation of cognitive control circuitry and increased attention problems. High exposure to ETS is common in urban contexts and could co-occur with maternal demoralization, which is non-specific psychological distress and inability to cope with stressful situations most likely due to socioeconomic disadvantage. Herein, we aimed to examine the combined effects of ETS exposure and maternal demoralization on general functional connectivity of the cingulo-opercular network (CO) in childhood. **Methods:** Thirty-two children (7-9 years) from a prospective longitudinal birth cohort of non-smoking mothers participated in this study. We investigated the combined effects of prenatal ETS exposure and maternal demoralization at child age 5 on the global efficiency of the CO network. Global efficiency scores were extracted from general connectivity data collected while children completed the Simon Spatial Incompatibility functional magnetic resonance imaging task. ETS was measured using prenatal maternal urinary cotinine; maternal demoralization was assessed at child age 5 using a 27-item questionnaire. The Childhood Behavior Checklist (CBCL) measured Attention and Attention Deficit Hyperactivity Disorder (ADHD) problems. Linear regression examined the associations between CBCL and global efficiency of the CO network controlling for age, sex, maternal education at prenatal visit, race/ethnicity, global correlation, and mean motion. **Results:** The prenatal ETS by maternal demoralization interaction term was associated with the global efficiency of the CO network (β=.673, $t(22)$=2.427, p=.024). Higher global efficiency of the CO network was associated with more attention (β=.472, $t(23)$=2.780, p=.011, n=31) and ADHD problems (β=.436, $t(29)$=2.567, p=.018) as measured by the CBCL. **Conclusions:** Our findings suggest that combined exposure to prenatal ETS and maternal demoralization reported at age 5 are associated with alterations in CO circuitry which affects attention and ADHD problems in childhood. These findings are consistent with prior studies showing ETS exposure is associated with altered cognitive control circuitry influencing attention and ADHD problems; however, our model identifies maternal demoralization as a significant contributor. These data highlight the combined neurotoxic effects of prenatal ETS exposure and early life stress on brain circuitry associated with error and performance monitoring likely impacting self-regulation.

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Poster

073. Attention: Neural Mechanisms and Neural Circuits

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Program #/Poster #: 073.12

Topic: H.01. Attention

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NIH NINDS F99NS120705 to A.F.
NIH R01 EY019693 to M.C.

Title: When and where does transcranial magnetic stimulation (TMS) affect presaccadic attention?

Authors: *N. M. HANNING, A. FERNÁNDEZ, M. CARRASCO;
Dept. of Psychology and Ctr. for Neural Sci., New York Univ., New York, NY

Abstract: Shortly before a saccadic eye movement, visual sensitivity is enhanced at the upcoming fixation because presaccadic attention shifts there during saccade preparation. Another type of attentional orienting is covert exogenous attention, which likewise automatically enhances sensitivity, yet is deployed in the absence of eye movements. Some behavioral and neural correlates of presaccadic and covert attention are similar, which led to the notion that the two processes may be functionally equivalent and rely on the same neural circuitry. A recent TMS study showed that covert exogenous attention relies on early visual areas. Here, in a similar protocol, human participants received TMS near the occipital pole and marked their perceived phosphene. Subsequently, they performed saccades to their phosphene region or the opposite hemifield and discriminated oriented gratings presented prior to saccade onset. We applied double pulse sub-threshold occipital TMS at different times during saccade preparation. When TMS was applied within the last 100 ms before saccade onset (the peak of presaccadic attention) visual sensitivity at the saccade target -an index of presaccadic attention- was reduced when the stimulation site matched the saccade target. This demonstrates a causal role of early visual areas in presaccadic attention. Critically, unlike covert exogenous attention, the effect is time sensitive and locked to the period right before saccade onset, indicating that occipital regions are recruited only during later stages of saccade programming -in line with the view that presaccadic attention modulates perception through feedback signals from oculomotor structures (e.g., FEF, SC) to occipital cortex. Our result provide further evidence dissociating presaccadic and covert attention.

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Poster

073. Attention: Neural Mechanisms and Neural Circuits

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Program #/Poster #: 073.13

Topic: H.01. Attention

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ANR-10-IAIHU-06
AVC grant FR-AVC-017

EPIRES- Marie Laure PLV Merchandising
Fund from Dassault Systèmes

Title: Fronto-parietal networks shape human conscious report through attention gain and reorienting

Authors: *J. LIU[1], D. BAYLE[2], A. SPAGNA[3], J. D. SITT[1], A. BOURGEOIS[4], K. LEHONGRE[5], S. FERNANDEZ-VIDAL[5], C. ADAM[6], V. LAMBRECQ[1], V. NAVARRO[1], T. SEIDEL MALKINSON[1], P. BARTOLOMEO[1];
[1]ICM - Hôpital Pitié Salpêtrière, Paris, France; [2]Licae Lab, Univ. Paris Nanterre, Nanterre, France; [3]Dept. of Psychology, Columbia Univ. in the City of New York, NYC, NY; [4]Lab. of Cognitive Neurorehabilitation, Fac. of Med., Univ. of Geneva, Geneve, Switzerland; [5]CENIR - Ctr. de Neuro-Imagerie de Recherche, Paris Brain Institute, ICM, Hôpital de la Pitié-Salpêtrière, Paris, France; [6]AP-HP, Pitié-Salpêtrière Hospital, Epilepsy Unit, Paris, France

Abstract: Competing theories disagree on the involvement of fronto-parietal brain networks in consciousness. We recorded neural activity from 727 intracerebral contacts in 13 epileptic patients, while they detected near-threshold targets preceded by attentional cues. Unsupervised clustering revealed three patterns: (1) Attention-enhanced conscious report accompanied sustained right-hemisphere fronto-temporal activity, in networks connected by the superior longitudinal fasciculus (SLF) II-III, and late accumulation in bilateral dorso-prefrontal and righthemisphere orbitofrontal cortex (SLF I-III). (2) Attentional reorienting affected conscious report through early, sustained activity in a right-hemisphere network (SLF III). (3) Conscious report accompanied left-hemisphere dorsolateral-prefrontal activity. Task modeling with recurrent neural networks supported the causal contribution of these networks to conscious perception, elucidating their interactions. Thus, distinct, hemisphere-asymmetric fronto-parietal networks support attentional gain and reorienting interactions with conscious report.

Disclosures: J. Liu: None. **D. Bayle:** None. **A. Spagna:** None. **J.D. Sitt:** None. **A. Bourgeois:** None. **K. Lehongre:** None. **S. Fernandez-Vidal:** None. **C. Adam:** None. **V. Lambrecq:** None. **V. Navarro:** None. **T. Seidel Malkinson:** None. **P. Bartolomeo:** None.

Poster

074. Executive Functions of the Brain Network Activity

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 074.01

Topic: H.04. Executive Functions

Support: HL040962
HL1089850

Title: Similarity in evoked responses does not imply similarity in macroscopic network states across tasks

Authors: *J. RASERO DAPARTE[1], R. BETZEL[2], A. I. SENTIS[1], T. E. KRAYNAK[3], P. J. GIANAROS[3], T. VERSTYNEN[1];
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Abstract: There is an ongoing debate as to whether cognitive processes arise from a group of functionally specialized brain modules (modularism) or as the result of a distributed nonlinear process (dynamical systems theory). The former predicts that tasks that recruit similar brain areas should have an equivalent degree of similarity in their connectivity. The latter allows for differential connectivity, even when the areas recruited are largely the same. Here we evaluated both views by comparing activation and connectivity patterns from fMRI data in a large sample of healthy subjects (N=242) that performed two executive control tasks, color-word Stroop task and Multi-Source Interference Task (MSIT), known to recruit similar brain areas. By temporally unwrapping static functional connectivity matrices, we first computed edge time series, a novel measure of scanner's frame-wise instantaneous connectivity. These edge time series were then used as response variables within a general linear model framework to estimate task-related network profiles, represented as connectivity changes between incongruent and congruent information task conditions. Topological differences across tasks prominently expressed in the dorsolateral prefrontal and posterior parietal cortex, both responsible for executive function, as well as in the posterior cingulate cortex, that is strongly implicated during control processes, and the primary visual cortex. As a consequence of these differences, the degree of similarity of network profiles at the group level between both tasks (Spearman's $\rho = 0.64$ between un-thresholded t-stat maps; and Dice Similarity Coefficient *(DSC)* = 0.43 between binarized maps) was substantially smaller than their overlapping activation responses ($\rho = 0.87$, $DSC = 0.86$). These findings were replicated when employing a generalized Psychophysiological Interaction (gPPI) model, a standard method for defining task-related connectivity ($\rho = 0.53$, $DSC = 0.35$); and using instead subject-level estimations, which showed a significantly greater average between-tasks similarity in brain activation (M=0.30, SD=0.17) than in network profiles (M=0.04, SD=0.06); Cohen's $d = 2.07$, $p < 0.001$. Our results then demonstrate that tasks with highly similar patterns of evoked activity may rely on fundamentally different functional network topologies. This is consistent with a perspective of the brain as a dynamical system, suggesting that task representations should be understood at both node and edge (connectivity) levels.

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Poster

074. Executive Functions of the Brain Network Activity

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 074.02

Topic: H.04. Executive Functions

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Title: A three-dimensional functional investigation of the avian visual cortex - Do birds share a canonical forebrain circuit?

Authors: *R. PUSCH[1], C. S. SEVINCIK[1], D. RODERS[2], W. CLARK[2], O. GUNTURKUN[1], J. ROSE[2];
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Abstract: The visual system of mammals and birds are highly evolved and specialized. In fact, many avian species surpass most mammals in spatial accuracy, temporal resolution and color sensitivity. Pigeons are further able to learn discriminations between hundreds of images, and to categorize visual stimuli as was famously demonstrated with images of humans, paintings or histological images. These visual abilities are fundamental for higher cognitive functions and enable visually guided adaptive behaviors. With the exception of humans, higher cognitive functions are developed to a comparable degree in birds and mammals. Despite such similarity in sensory adaptation and behavioral output, the forebrains of both taxa are radically different in their gross anatomy. Mammalian vision relies on highly structured visual cortices, consisting of distinct layers with functional and anatomical columns. Using this structured, repetitive bauplan, the visual environment is centrally represented in a strict topographic manner. In contrast, the avian visual forebrain is organized in multiple clusters that resemble, at the first glance, the histological pattern known from the basal ganglia of both taxa. However, a recent anatomical study has shown that the cyto-architectonic organization of the sensory aspects of the avian forebrain show astonishing similarities to its mammalian counterpart. This raises the question, if the neuronal computations in the different aspects of the avian visual forebrain also match this similarity. In an electrophysiological study using multi-site silicon probes we recorded neuronal responses in the visual forebrain of pigeons. We simultaneously sampled responses of neurons in the receiving center of the visual foerebrain, the entopallium, and the visual associative region mesopallium ventrolaterale as well as the intermediate nidopallium. These regions are deemed to be part of the visual hierarchy and show distinct response pattern. By using visual stimuli as grating pattern, basic forms and random-dot pattern, our three-dimensional, simultaneous recordings allow for a detailed analysis of the neuronal responses within and between each region, opening the opportunity for a direct comparison of avian and mammalian computations in the visual forebrain.

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Poster

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University of Toronto, Faculty of Medicine, Graduate Studies Endowment Fund
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Title: Trail Making Test performance using a tablet: behavioural kinematics and electroencephalography

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Abstract: Introduction: The Trail Making Test (TMT) is widely used to assess frontal lobe function and assist in diagnosis of brain disease. The test assesses cognitive processes such as visual search and visuomotor control. Each of the two parts (A and B) of the test involves linking a total of 25 items in ascending order: TMT-A involves linking numbers (1-2-3); TMT-B involves linking numbers alternating with letters (1-A-2-B), which is more challenging. Each part is scored by the completion time. Despite its wide use, the brain activity supporting TMT performance remains poorly understood. Via scalp electrodes, electroencephalogram (EEG) records voltage fluctuations from the ionic currents produced by neural activity, with excellent temporal resolution (~ms). The goal of the present study is to compare the differences in behavioural performance and EEG signal content between TMT parts, and different aspects of performance during each TMT part. Methods: Sixteen healthy young adults (8 female, age 19-27) participated. Behavioural TMT performance was recorded using a digitizing tablet to capture enhanced metrics such as seconds per link (SPL), average link speed, and linking and non-linking periods (LP and NLP). The two periods were derived as estimates of the time spent executing and preparing movements, respectively. The EEG data were recorded using a 32-channel system (actiCHamp, BrainVision) at a sampling rate of 5 kHz, from which the total time-frequency power was computed for task partial least squares analysis. Results: Longer SPL, LP, and NLP were evident in TMT-B compared to TMT-A. As for brain activity, compared to LP, NLP demonstrated widespread power increases in delta, theta and alpha bands, as well as frontally localized increases in beta and gamma power with a slight right lateralization. In addition, TMT-B exhibited reduced left-lateralized delta band power and posterior midline theta band power compared to TMT-A. Conclusions: The effect of TMT parts in both behavioural and EEG data indicated an increased cognitive demand in TMT-B than TMT-A, as expected. Consistent with task-related synchronization, increased working memory involvement and performance accuracy maintenance were implicated in the EEG signal power during NLP, compared LP. Furthermore, EEG signal power changes for TMT-B compared to TMT-A were consistent with elevated memory and attentional demands in the former condition. Overall, the results are sufficiently strong to consider evaluating tablet TMT performance and brain activity in other pertinent cohorts, such as elderly patients with age-related brain dysfunction. Eye tracking technology can also be incorporated in the future.

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Poster

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Program #/Poster #: 074.04

Topic: H.04. Executive Functions

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Title: Influence of sleep/wake state on functional networks in the infant brain

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Abstract: The adult brain is organized into functional networks that support different cognitive and perceptual processes. The developmental trajectory of these networks may thus place important constraints on early cognitive abilities. The maturity of functional networks in infancy is typically assessed using fMRI by comparing their resting-state functional connectivity to adults. However, how these resting-state data are acquired differs between infants and adults: infants are almost universally scanned during sleep, while adults are typically scanned while awake. Thus, it is unclear whether reported (im)maturity of infant functional networks results from the refinement of these networks over development or from these differing levels of consciousness. Here we address this question in a subset of infants who provided both sleeping rest and awake movie-watching fMRI data. Data were collected from 9 unique infants ranging from 3.6 to 24.9 months of age, with a total of 11 sleep runs and 14 movie runs. For comparison, we collected data from 12 adults during awake rest and while watching the same movies. After preprocessing and regressing out motion parameters, parcelwise functional connectivity matrices were created for each participant by calculating the Pearson correlation between the average timeseries across voxels for all pairs of parcels from the Schaefer atlas. Replicating prior work, adult functional connectivity matrices were more similar within-state (rest or movie) than across these states. This was also true of infants, demonstrating that movie watching elicits a different functional connectivity profile than typical infant sleep acquisitions. Interestingly, infant functional connectivity during both sleeping rest and movie watching were equally similar overall to adult resting-state functional connectivity, driven mostly by visual and somatosensory networks. However, some of the connections responsible for adult network similarity differed by infant level of consciousness: between-network connections contributed more to similarity during infant sleep and within-network connections in salience and control networks contributed more to similarity during infant movie watching. Similar results were found when comparing infant sleep and movie watching to adult movie watching, but infants in both states were more similar to adults watching movies than adults during awake rest. Together these results show that infant functional brain networks are similar yet dynamic across sleep/wake states, with potential implications for drawing conclusions about infant network maturity from sleeping data alone.

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Poster

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Program #/Poster #: 074.05

Topic: H.04. Executive Functions

Title: Cognitive Performance in Healthy Young Adults Relates to Stable Multi-Network Connectivity Dynamics at Rest

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Abstract: Resting brain activity wanders through a dynamic repertoire of functional network states, and previous work shows that variability in functional network dynamics influences individual differences in cognitive ability. Here, we investigate how the prominence and stability of dynamic brain states relates to individual differences in cognitive performance. In a large (N=297) sample of resting state connectivity data in healthy young adults, we examine the relationship between brain network activity and general intelligence using leading eigenvector decomposition of dynamic functional connectivity. After detecting network patterns in dynamic functional connectivity, we examine: (1) whether individual profiles of dynamic functional connectivity are a unique and meaningful index of intelligence; (2) whether the frequency and temporal stability of dynamic states reliably explain individual differences in intelligence; and (3) whether statistical learning (via iterative random forests) can identify generalizable properties of brain dynamics that reliably predict individual differences in intelligence. Our results demonstrate that dynamic functional connectivity is a unique and meaningful biomarker of general intelligence within subjects. We observe that segregated, disconnected network states are reliably associated with lower intelligence individuals. Further, we observe that higher intelligence is reliably associated with dynamic functional interaction in a multi-network state that combines frontoparietal, default mode, and limbic network regions, and with the temporal stability of the task-positive network. Predictive modeling demonstrates that low intelligence is reliably indexed by segregated functional network dynamics. These findings demonstrate that dynamic profiles of resting state connectivity are a unique and reliably predictive biomarker for individual differences in intelligence.

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Poster

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Topic: H.04. Executive Functions

Support: NIH Grant 5R01MH116268-03

Title: Increased functional connectivity between superior lateral occipital cortex regions and frontal regions during the probe compared to the encoding phase of a spatial working memory task

Authors: *T. KELLEY, H. TRUONG, J. DIAZ, S. K. LOO, A. LENARTOWICZ;
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Abstract: Spatial working memory (SWM) involves encoding and maintaining visual information about the location of objects in space. We may utilize information from SWM to determine if an object is in the same location it was previously in, perhaps by comparing our maintained representation of that object's location to its current location. In the current study, 44 participants completed a stimulus localizer task (stimloc) and a spatial working memory task while in the MRI scanner. In the stimloc task, participants saw an array of dots ranging from one to five dots and were instructed to count the number of times they saw a red cross appear on the screen. In the SWM task, participants saw an array of dots ranging from one to five dots (encoding), saw a fixation cross for three seconds (maintenance) and were then presented with a probe dot. Participants indicated if this probe dot was in the same location as any of the dots seen during the encoding phase or if it was in a different location. Psychophysiological interaction (PPI) analyses utilized the left and right superior lateral occipital cortex (LOC) as seed regions to establish functional connectivity during these tasks. Both the precentral and postcentral gyri featured significantly more functional connectivity with the left and right superior LOC regions during the stimloc task compared to the encoding phase of the SWM task. There were no significant differences in functional connectivity when comparing the stimloc task to the probe phase of the SWM task. However, when comparing the encoding to the probe phase of the SWM task, there was significantly more functional connectivity between the left and right superior LOC regions and frontal pole, superior frontal gyrus, frontal orbital cortex, and anterior cingulate cortex. These results suggest that there is similar functional connectivity and likely similar cognitive processes when viewing a stimulus during a localizer task, encoding the same type of stimulus during a SWM task, and determining if a probe stimulus is in the same or different location as a just viewed set of items. The further increased functional connectivity with frontal regions during the probe phase suggests different processing during this phase such as evidence accumulation utilizing the representation maintained by the LOC.

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Poster

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Topic: H.04. Executive Functions

Support: NIH 5R01NS100849-05

Title: Basal Ganglia Network Alterations in Parkinson's Disease

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Abstract: Changes to functional connectivity have been evidenced in Parkinson's disease (PD), but the precise mechanisms and effects that lead to cognitive dysfunction have yet to be fully elucidated. We set out to understand the specific alterations to the brain's functional architecture that result in cognitive impairment, with a focus on the basal ganglia network (BGN). The basal ganglia can control movements, but this group of structures also has important contributions to cognition. We hypothesize that there will be differences in the functional connectivity of the BGN in patients with PD when compared to healthy older adults. We also anticipate that this will relate to cognition. Neuroimaging data were used from the Parkinson's Progression Marker Initiative (PPMI) dataset (N = 102). FMRI data were processed and analyzed using the CONN toolbox in MATLAB, where seed-to-voxel measures were computed for within-network connectivity. Using Pearson's correlation coefficients as the measure of functional connectivity, we compared functional connectivity strength between three groups: healthy older adults (n = 20), patients with PD and normal cognition (PD-Norm; n = 67), and patients with PD and mild cognitive impairment (PD-MCI; n = 15). A linear mixed effects model was used to evaluate the effect of group on functional connectivity. Our first model showed a significant main effect of group on BGN functional connectivity, with a post-hoc comparison correcting for multiple comparisons revealing that PD-MCI patients have less functional connectivity within the BGN than older healthy adults. As an exploratory analysis, we evaluated inter-network functional connectivity between the BGN and frontoparietal network (FPN), given executive dysfunction is hallmark to PD and the FPN plays a major role in cognitive control. Consistent with our first model, we found that PD-MCI patients had less functional connectivity between the BGN-FPN than older healthy adults. These data provide evidence that functional connectivity is lowered within the BGN and between the BGN and FPN in PD patients who have cognitive impairment. Our findings suggest that impaired fronto-striatal networks contribute to cognitive impairments in PD.

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Poster

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Title: Individual variation in the size of large-scale functional networks and its role in cognition

Authors: *S. KOIRALA[1], R. HERMOSILLO[1], E. FECZKO[1], O. MIRANDA-DOMINGUEZ[1], A. PERRONE[1], N. BYINGTON[1], A. RUETER[1], O. MAYO[1], T. D. SATTERTHWAITE[2], J. ELISON[1], D. FAIR[1];
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Abstract: The human cerebral cortex is organized into a tightly interconnected set of large-scale functional networks. Work using resting-state functional connectivity MRI (fcMRI) has established a canonical network architecture that is broadly shared across the population. While the use of population average network topographies has yielded important discoveries, it obscures individual differences in brain network organization and its possible behavioral relevance. Recent work has revealed individual differences in the size of functional networks however it is not clear how such variation relates to behavior. In this study, we investigated whether individual variation in functional network size predicts individual differences in cognition. By leveraging a discovery sample (N = 2932) and a matched replication sample (N = 3037) from the Adolescent Brain and Cognitive Development (ABCD) study, we delineated person-specific functional networks using a template matching procedure and calculated cortical surface area for each individual's networks. We hypothesized that the cortical surface area of networks could predict various domains of cognition (general ability, executive functioning (EF), and learning/memory scores). Controlling for gender, parental education, and site, preliminary regression analyses showed that the surface area of FP, DMN, DAN, CO, and Tpole networks positively predicted general ability scores (p_{Bonf} <0.05). We also found that greater surface area of the CO network predicted better EF and learning/memory scores (p_{Bonf} <0.05). All findings were successfully replicated in the replication sample. These results suggest that larger cortical representation of specific large-scale functional networks appears to reflect differences in cognitive ability. Furthermore, our work emphasizes the relevance of individualized functional network topography in understanding behavioral variability and sets the stage to better understand individual variation in functional neuroanatomy in psychopathology.

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Poster

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Title: Overlapping functional networks in the mouse brain studied using simultaneous whole-brain functional MRI and cortex-wide Ca^{2+} imaging data

Authors: *H. VAFAII[1], G. DESROSIERS-GRÉGOIRE[2], D. O'CONNOR[3], X. GE[6], F. MANDINO[7], X. SHEN[7], P. HERMAN[7], X. PAPADEMETRIS[4], M. CHAKRAVARTY[2], M. C. CRAIR[5], R. T. CONSTABLE[8], E. M. R. LAKE[7], L. PESSOA[9];
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Abstract: Functional connectivity studies have revealed rich information about the community structure of the brain. Most commonly, disjoint clustering methods have been explored where each brain region is allowed to belong to one and only one community. However, there is growing evidence that some regions may participate in more than one community. To address this question, we apply an overlapping community detection algorithm to a simultaneous Ca^{2+}/fMRI dataset of lightly anesthetized mice. We find that the resulting communities are highly similar across data modalities, and bandpassing Ca^{2+} data using an infraslow band further increases this similarity. However, there were some notable differences most visibly in the Retrosplenial area. For both modalities, we find a robust community structure with substantial overlap. Analyzing the distribution of membership values revealed that more than half of brain regions belonged to two or more communities. To explore this further, we defined an "overlap score" for communities as the number of overlapping nodes of a community divided by its total size. We found that most have high overlap scores in the range of 60-100%, except for the Visual and Latero-Cortical networks (slightly below 40% and 50%, respectively). Next, we quantified membership diversity by applying Shannon entropy to each region's membership probability vector. We found that unimodal regions including visual and somatomotor areas exhibit low membership diversity, as anticipated. In contrast, most regions in the Default Network have high membership diversity, indicating that they participate in multiple communities. Finally, we combine our membership diversity score (a measure of how diversely connected brain regions are) with degree centrality (a measure of how functionally connected these regions are) to achieve a cartographic visualization of the mouse brain. In sum, our data suggests that an overlapping framework captures the intrinsic organization of the mouse brain more faithfully compared to disjoint alternatives. The majority of brain regions belong to multiple communities; therefore, enforcing strict disjoint organization leads to loss of valuable information about the varying strength of their affiliation across multiple communities.

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Poster

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Title: Biophysical neural model of thalamocortical circuits underlying sleep spindles

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Abstract: Sleep spindles are brief oscillations indicative of non-REM sleep. They originate within the thalamocortical loop that consists of the inhibitory thalamic reticular nucleus, thalamus and cortex. Anatomical and physiological evidence supports the presence of two distinct thalamocortical loops: matrix and core. The matrix networks consist of calbindin-positive thalamocortical relay neurons that have widespread projections to the superficial layers of the cortex and are implicated in modulatory processing and memory consolidation. Core networks consist of parvalbumin-positive thalamocortical relay neurons that project in an orderly fashion to the middle layers of the cortex and are associated with sensory processing. While the mechanisms underlying spindle generation are well defined, their functional consequences are still not fully understood. To address the gap, we developed a detailed biophysical neural model of the matrix and core circuits in isolation and combination. In the model, we differentiated the matrix and core based on the distinct channel distributions, both voltage- and ligand-gated, as well as the laminar distributions of focal and widespread projections. Our model provides a base platform to analyze changes in spindle patterns, synchronization and recruitment in the thalamocortical loops as a function of their mixing. With regards to the isolated circuits, the matrix has a tendency for synchronized spindles and higher degrees of neural recruitment, while the core has periods of asynchronized spindles and higher degrees of localized neural activity. Continuing our exploration into integrated matrix-core activity, our model acts as a tool to infer the components crucial for i) neurotypical spindle activity and ii) creating a list of changes that have a higher probability of leading to spindle deficits observed in schizophrenia and autism spectrum disorder. Our findings can guide future experiments to understand potential involvement and disruption of the matrix-core thalamocortical loops and targeted interventions for the treatment of disruptions in schizophrenia and autism spectrum disorder.

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Poster

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Topic: H.04. Executive Functions

Title: Transdiagnostic prediction of cognition in a developmental sample using connectome-based predictive modeling

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Abstract: Prediction of inter-individual behavioral phenotypes remains a challenge. Stable prediction performance across a wide spectrum of clinical presentations offers enhanced practical utility. This study used connectome-based predictive modeling (CPM) for transdiagnostic prediction of cognition across multiple cognitive domains in two large-scale independent datasets. First, resting-state (n=691) and N-back task (n=738) fMRI data from the Philadelphia Neurodevelopmental Cohort (PNC) were used to construct 268x268 connectomes for young individuals aged 8-21 years. Participants were distributed throughout typical and atypical psychopathology as assessed by the Kiddie Schedule for Affective Disorders and Schizophrenia (K-SADS). Models were trained with 10-fold cross-validation and evaluated with Pearson's r. We predicted measures from the Complex Cognition (Verbal Reasoning, Matrix Reasoning, and Line Orientation) and Executive (Conditional Exclusion, Continuous Performance, and Letter N-Back) scales of the Computerized Neurocognitive Test Battery (CNB). CPM successfully predicted measures of cognitive performance transdiagnostically ($r's>0.16$, $p's<0.05$). Sex differences in model performance were not observed. Models trained with task fMRI data outperformed those trained with resting-state fMRI data on average by 21%. On average, measures from the Complex Cognition scale were predicted nearly twice as accurately as measures from the Executive scale. Next, a second transdiagnostic dataset (n=652, ages 5-21) from the Healthy Brain Network was used to construct independent models. CPM similarly predicted measures of complex cognition (Weschler Verbal Reasoning, Matrix Reasoning, and Block Design, $r's>0.11$, $p's<0.05$) which outperformed measures from the NIH Toolbox (Card Sorting, Flanker Task, and Pattern Comparison, $r's<0.04$) with the exception of List Sorting ($r=0.12$). These models were generated and internally validated in two large, behaviorally diverse cohorts, suggesting measures of complex cognition as targets for reliable assessment of neurodevelopmental status. Future directions include generation of a single 'latent' complex cognition measure derived from multiple cognitive scales and its validation across datasets.

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Title: Do sensorimotor beta oscillations help or hinder task switching?

Authors: *P. L. DANIEL[1], A. KIYONAGA[2];
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Abstract: Switching between different task goals or rules is critical for adaptive living in humans. This is known as task switching, and it is considered a core component of cognitive control. Beta-band oscillations (13-30 Hz) have been implicated in movement and cognition, but it remains an open question what precisely beta activity reflects in the switching domain. A well-known 'status quo' framework (Engel & Fries, 2010) purports that beta should maintain prior task-set rules, and therefore its engagement should hinder switching to using a different task-set rule. However, a newer 'clear-out' framework (Schmidt et al., 2019) instead purports that beta clears out prior task-set rules, and therefore its presence should support switching. Our study tests these competing accounts. While these frameworks describe the role of beta activity in general, we make the critical assumption that task-set rules are held in sensorimotor working memory, and that beta activity in sensorimotor cortex can either maintain or clear out task-set rules to impact switching performance. Here, human subjects complete a standard task switching paradigm while we record scalp electroencephalography (EEG). They are instructed to respond to number stimuli according to one of two rules: e.g., if the number is red, use an 'odd/even' rule (respond L if odd, R if even), but if the number is blue, use a 'high/low' rule (L if < 5, R if > 5). Our analyses take advantage of the established increase in sensorimotor beta power that occurs after a movement, known as the post-movement beta rebound (PMBR). We relate PMBR power following the button-press response on the previous trial to performance on the current trial. If trials that were preceded by more PMBR power have worse switching performance, that would support the status quo framework. If trials that were preceded by more PMBR power have better switching performance, that would support the clear-out framework. Preliminary analyses show that switch trials with faster response times (RTs) are preceded on average by more PMBR power than switch trials with slower RTs. We do not see similar difference for non-switch trials. This supports the interpretation that sensorimotor beta activity serves a clear-out role during task switching. Further support for this interpretation could come from seeing increased beta elicited at the time of a switch cue, plausibly in prefrontal cortex, as a top-down control process to clear out irrelevant task-set rules. This work will help clarify the elusive role of beta oscillations in

complex cognitive processing and in clinical dysfunction, such as in Parkinson's disease patients who have switching deficits and pathological beta.

Disclosures: P.L. Daniel: None. **A. Kiyonaga:** None.

Poster

074. Executive Functions of the Brain Network Activity

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 074.13

Topic: H.04. Executive Functions

Support: NIH P01 HL040962
NIH R01 HL 1089850

Title: Task demand alters cortical network states ensuing integration and modularization

Authors: *K. NESTOR[1], J. RASERO[1], R. BETZEL[2], P. J. GIANAROS[3], T. D. VERSTYNEN[1];
[1]Psychology, Carnegie Mellon Univ., Pittsburgh, PA; [2]Dept. of Psychological and Brain Sci., Indiana Univ., Bloomington, IN; [3]Psychology, Univ. Pittsburgh, Pittsburgh, PA

Abstract: Brains naturally shift states of activity, moving from largely integrated (i.e. many separate networks talking together) to largely segregated (i.e. little cross-talk between separable networks) depending on the information demands of the environment. Specifically, greater information processing demands should be associated with more integrative brain networks, as this is required to coalesce cross network input (Shine, 2021). To examine these network dynamics we used dynamic connectivity, from fMRI (N = 181 participants), during an adaptive Stroop task to assess incongruent (conflicting) and congruent (no conflict) task effects on brain networks. Task blocks were interleaved for a duration of 60s followed by a 10s delay with crosshair fixation. Task and resting state data were generated by the Pittsburgh Imaging Project (PIP) with the goal of determining a brain phenotype that could reliably predict changes in blood pressure reactivity due to stressors (Gianaros et al., 2017). The data analysis method of edge time series (Zamani Esfahlani et al., 2021), with k = 268 parcels of functional regions (Shen et al., 2013) was used to examine network connectivity patterns. We obtained modularity values of the networks at each point in the time series and used an autoregressive linear model to obtain the following resultants for tasks blocks: incongruent (beta = 0.0020; 95% CI = 0.001, 0.003), congruent (beta = 0.0034; 95% CI = 0.002, 0.005). Thus, the incongruent blocks had lower modularity than congruent task blocks. A one sample t-test for significance using resting state as the null determined the effect of condition (t(df) = 2.48, p = 0.014). The results from this study indicate that during high stress and cognitively demanding tasks, networks exhibit lower modularity (high integration), while during lower stress and mildly strenuous tasks networks exhibit higher modularity (low integration).

Disclosures: **K. Nestor:** None. **J. Rasero:** None. **R. Betzel:** None. **P.J. Gianaros:** None. **T.D. Verstynen:** None.

Poster

074. Executive Functions of the Brain Network Activity

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 074.14

Topic: H.04. Executive Functions

Support: PNI Innovation Fund

Title: Computation through coherence: how oscillations control and organize neural dynamics

Authors: *A. LIBBY[1], T. J. BUSCHMAN[2];
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Abstract: Cognition relies on controlling and organizing dynamics within and between brain regions. The brain is highly recurrent; brain regions are connected through both feedforward and feedback connections, which suggests that multi-region dynamics arise when their activity is coupled. Meanwhile, synchronous oscillations have been proposed as a mechanism through which regions communicate. We propose that coherent oscillations are not just providing channels of communication, but rather couple the dynamics across regions to engage multi-region dynamical systems. Flexibly coupling different regions creates different dynamical systems, allowing for selection of different, complex, computations. To understand how synchrony might couple the dynamics of multiple regions, we used a firing rate model to show how oscillations in inhibition can turn on and off different dynamics. Specifically, coherent oscillations couple and uncouple the stabilizing dynamics in one region with the transitional dynamics of another region to either perform pattern completion or make predictive sequences. Oscillations controlled the network's overall dynamics by changing each region's overall balance of excitation and inhibition (E/I balance). Our model shows the phasic relationship among regions is a mechanism knob, which can be turned to control multi-region dynamical systems. Moreover, because oscillations are rhythmic, they provide a biologically plausible and predictable mechanism to sample various computations. Thus, this model may explain why coupled oscillations have been observed across cognitive domains that require multiple regions to engage and disengage their dynamics.

Disclosures: **A. Libby:** None. **T.J. Buschman:** None.

Poster

074. Executive Functions of the Brain Network Activity

Location: SDCC Halls B-H

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Program #/Poster #: 074.15

Topic: H.04. Executive Functions

Support: Simons SFARI Pilot Award
NJ ACTS Fellowship TL1TR003019
NIH Grant RO1 MH126022

Title: Brain-wide interactions are organized into multiplexed subspace networks

Authors: *C. J. MACDOWELL[1], S. TAFAZOLI[2], E. S. PAPADOYANNIS[3], C. I. JAHN[2], A. G. LIBBY[2], T. BUSCHMAN[3];
[2]Princeton Neurosci. Inst., [1]Princeton Univ., Princeton, NJ; [3]Princeton Neurosci. Inst., Princeton, NJ

Abstract: Brain-wide recordings have shown that neural representations are widely distributed and dynamic. Different networks of regions are engaged on a moment-by-moment basis, both during spontaneous behavior and in a task-dependent manner, to support different cognitive computations. The distributed and flexible nature of these networks are thought to support flexible cognition. Yet, the dynamic organization of brain wide networks and the mechanisms underlying this flexibility remain unclear. To begin to address these questions, we leveraged simultaneous cortex-wide calcium imaging and multi-region Neuropixels recordings of ~9500 neurons in awake mice engaging in spontaneous behaviors. We then used reduced rank regression modeling to uncover the functional interactions between neural populations distributed across the brain. We found that neural activity in each brain region could be partitioned into multi-dimensional subspaces that were correlated with unique cortex-wide 'subspace networks'. For example, the first subspace dimension of most brain regions was correlated with a broad network that spanned the majority of the dorsal cortex. In contrast, subsequent subspace dimensions engaged more localized networks of regions; some of which corresponded to known functional boundaries while others captured novel networks that bridged traditionally identified borders. These subspace networks were multiplexed, which enabled individual regions to flexibly interact with multiple independent, yet overlapping, networks of brain regions. In addition, we found that aligning neural representations with different subspaces within a brain region engaged specific brain-wide networks. Indeed, how well the neural response within a region aligned with a subspace predicted moment-by-moment changes in the way neural activity propagated across cortical areas. Altogether, our results suggest that brain-wide interactions amongst neural population are organized into dynamic and multiplexed subspace networks. These networks may provide a basis set of possible ways in which neural representations can flow across neural populations and changing the geometry of neural representation within a brain region to align to a specific subspace network may be a mechanism by which neural activity can be selectively routed across the brain.

Disclosures: C.J. MacDowell: None. **S. Tafazoli:** None. **E.S. Papadoyannis:** None. **C.I. Jahn:** None. **A.G. Libby:** None. **T. Buschman:** None.

Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.01

Topic: H.07. Long-Term Memory

Support: R01MH107886 awarded to K.M.F.
F31MH118782 to K.S.G.

Title: Sex differences in training-induced protein degradation in the dorsal hippocampus of male and female mice

Authors: *S. B. BEAMISH, K. S. GROSS, M. A. ANDERSON, F. J. HELMSTETTER, K. M. FRICK;
Univ. of Wisconsin-Milwaukee, Milwaukee, WI

Abstract: The ubiquitin proteasome system (UPS) targets substrate proteins to the 26S proteasome for degradation by tagging them with ubiquitin. UPS activity in the dorsal hippocampus (DH) is necessary for multiple types of memory, including object memory, in male rodents. However, sex differences in DH UPS activation after fear conditioning suggest that other forms of learning may also differentially regulate DH UPS activity in males and females. Here, we examined markers of UPS activity in the synaptic and cytoplasmic fractions of dorsal hippocampus (DH) tissue collected 1 h following object training in adult male and female mice. Mice were first handled for 30 s/d for 3 d. Mice were then habituated in an empty testing arena for 5 min/d for 2 d. During training, mice accumulated 30 s of exploration with two identical objects and DH tissue was collected 1 h after completion of training. In males, training increased phosphorylation of proteasomal subunit Rpt6, 20S proteasome activity, and amount of postsynaptic protein PSD-95 in the DH synaptic fraction. In females, training did not affect measures of UPS or synaptic activity the DH synaptic fraction, but instead increased Rpt6 phosphorylation in the DH cytoplasmic fraction. Levels of K48 polyubiquitination were not affected by training in either sex, nor were levels of phosphorylated CaMKII and PKA, both of which regulate proteasome activity. Overall, training-induced UPS activity was greater in males than females and greater in synaptic fractions than in cytosol. These data suggest that object training drives sex-specific alterations in UPS activity across subcellular compartments in the DH.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.02

Topic: H.07. Long-Term Memory

Support: NSF PIRE IIA1504157
USDA NIFA2021-6704-34999

Title: Role of DNA repair in long-term memory consolidation and decision-making on honey bees

Authors: Y. ORTIZ ALVARO[1], A. MEHMET[1], T. GIRAY[2], J. L. AGOSTO[4], A. N. DELGADO[3], ***F. NOEL**[3];
[1]Univ. of Puerto Rico, Rio Piedras, San Juan, PR; [2]Dept. of Biol., [3]Univ. of Puerto Rico, San Juan, PR; [4]Biol., Univ. of Puerto Rico, Rio Piedras Campus, San Juan, PR

Abstract: Living organisms depend on memory to survive and evolve within the environment. For the past decades, psychologists and neuroscientists have discussed that the memory developed from short-term memory (STM), working memory (WM) to long-term memory (LTM). But the process from STM to LTM has been controversial by many of these scientists about the idea that it involves synaptic plasticity and cellular and molecular mechanisms. For example, honeybees use their cognitive capacity to purchase the best food and their memory to be more effective. Here, we asked if the DNA drives the memory consolidation on honeybees. In this research, we used drugs to block honeybees' DNA repair and recombination mechanism in a conditioning experiment associated with proboscis extension response (PER). Recent studies showed that inhibiting DNA transcription and repair affect LTM. Therefore, we hypothesized that if the DNA recombination is essential in LTM formation and recombination, blocking DNA repair and recombination would block the LTM consolidation from STM to LTM without affecting the learning process. The results of the STM within 20 minutes after completing the conditioning and the LTM 24 hours after showed no significant differences. However, we found a significant difference between the groups of treated and untreated bees in the LTM recall and no LTM call. We discuss these results based on the role of DNA as a long-life molecule within living organisms.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 075.03

Topic: H.07. Long-Term Memory

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Title: Exercise parameters that open a 'molecular memory window' for cognitive enhancement shine light on key memory mechanism in the adult, aging, and Alzheimer's Disease brain

Authors: *A. A. KEISER[1], T. DONG[4], E. KRAMÁR[1], C. BUTLER[2], D. P. MATHEOS[1], J. BEARDWOOD[1], A. AL-SHAMMARI[1], S. CHEN[3], V. ALIZO VERA[1], L. TONG[2], N. C. BERCHTOLD[2], M. SAMAD[3], C. MAGNAN[3], S. SHANUR[1], P. BALDI[3], C. W. COTMAN[2], M. A. WOOD[1];
[1]Dept. of Neurobio. and Behavior, [2]Dept. of Neurol., [3]Inst. for Genomics and Bioinformatics, Sch. of Information and Computer Sci., Univ. of California, Irvine, Irvine, CA; [4]Nash Family Dept. of Neurosci., Icahn Sch. of Med. at Mount Sinai, Irvine, CA

Abstract: The ability to learn, consolidate and retrieve information is critical for everyday survival and this ability begins to decline with normal aging and is severely exacerbated by Alzheimer's Disease (AD). Basic research and clinical trials have universally demonstrated the benefits of exercise for cognitive function including enhancements in long-term memory formation and synaptic plasticity in addition to alleviating cognitive impairments associated with normal aging and AD, however, the mechanism by which exercise leads to cognitive enhancement is unclear. Here, we apply exercise as an approach to unlock a novel understanding of the molecular mechanisms that drive memory consolidation by utilizing specific exercise parameters that enhance cognitive benefits. Employing an intermittent exercise protocol, we demonstrate that 14 days of voluntary wheel-running facilitates hippocampus-dependent memory and synaptic plasticity in adult mice, effects which can be maintained and re-engaged with brief 2-day re-introduction to exercise following a sedentary delay. To identify novel mechanisms that drive memory consolidation, we utilized an unbiased RNA-sequencing approach to uncover genes in the dorsal hippocampus that are differentially expressed under conditions where exercise benefits are maintained throughout sedentary delay periods and enable the formation of long-term memory and synaptic plasticity. We identify a gene coding for a type 1 receptor for the TGF-β family of signaling molecules, *Acvr1c* as one of few genes (including *Bdnf*) showing up-regulation in the hippocampus under exercise conditions that enable the formation of long-term memory and synaptic plasticity. Utilizing viral manipulations in the adult hippocampus to disrupt and over-express *Acvr1c*, we identify *Acvr1c* as a key bi-directional regulator of hippocampus-dependent long-term memory and synaptic plasticity. We find *Acvr1c* levels decrease in the hippocampus with age in C57Bl/6J and 5xFAD female and male mice and demonstrate that *Acvr1c* over-expression ameliorates age and AD-associated impairments in memory and synaptic plasticity. These data suggest that promoting ACVR1C through exercise or pharmacological intervention may protect against age and AD-associated cognitive impairment, providing a potentially powerful and novel disease modifying treatment strategy for AD.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.04

Topic: H.07. Long-Term Memory

Support: NSERC

Title: Acetylcholine in perirhinal cortex mediates destabilization and updating of resistant object memories in a CaMKII dependent manner.

Authors: *C. E. WIDEMAN, B. D. WINTERS;
Univ. of Guelph, Univ. of Guelph, Guelph, ON, Canada

Abstract: The content of long-term memory is neither fixed nor permanent. Reminder cues can destabilize consolidated memories, rendering them amendable to change before being reconsolidated. Reactivation-induced memory destabilization is thought to reflect a mechanism through which long-term memories can be adaptively maintained over time. However, not all memories destabilize following reactivation. Characteristics of a memory, such as its age or strength, cause them to become resistant to the process of destabilization. Previously, we demonstrated that presentation of salient novel information at the time of reactivation can readily destabilize resistant object memories in rats, and this form of novelty-induced destabilization depends on acetylcholine (ACh) activity at muscarinic receptors (mAChRs) in perirhinal cortex (PRh). In the present study, we sought to identify the link between M_1 mAChRs and the molecular events required at the synapse to destabilize and update object memories. We found evidence that activation of CaMKII downstream of M_1 mAChRs in PRh is necessary for initiating novelty-induced object memory destabilization in male rats. Using our post-reactivation object memory modification (PROMM) task, we then demonstrated that resistant object memories can be updated with new contextual information. Updating of resistant object memories was bidirectionally regulated by M_1 mAChRs, with their inhibition in PRh preventing memory updating and their pharmacological activation sufficient to promote memory updating in the absence of novelty at the time of reactivation. Consistent with its role in novelty-induced object memory destabilization, we also found that CaMKII activation downstream of M_1 mAChRs is necessary for updating resistant object memories. These results demonstrate that CaMKII is a critical component of the mAChR pathway through which ACh mediates the destabilization and updating of resistant object memories, and further highlight the importance of ACh in accurately maintaining the content of long-term memories over time.

Disclosures: C.E. Wideman: None. **B.D. Winters:** None.

Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 075.05

Topic: H.07. Long-Term Memory

Support: NIA AG050787
NIA AG051807
NIA AG054349
NIA AG054349

Title: Phosphorylation state of histone deacetylase 3 (HDAC3) modulates long-term memory formation and synaptic plasticity

Authors: *A. RODRIGUEZ[1], A. A. KEISER[1], E. A. KRAMAR[3], T. DONG[4], J. L. KWAPIS[5], D. P. MATHEOS[2], M. A. WOOD[6];
[2]Neurobio. & Behavior, [1]Univ. of California, Irvine, Irvine, CA; [3]Neurobio. and Behavior, Univerisity of California, Irvine, CA; [4]Icahn Sch. of Med. at Mount Sinai, New York, New York, NY; [5]Dept. of Biology, Ctr. for Mol. Investigation of Neurolog. Disord, Penn State Univ., University Park, PA; [6]Neurobiol & Behavior, Univ. of California Irvine, Irvine, CA

Abstract: Long-term memory formation is negatively regulated by histone deacetylase 3 (HDAC3), a transcriptional repressor. Emerging data suggests that the phosphorylation state of HDAC3 may be crucial for its functional activity in regulating transcription. However, it's unclear if the phosphorylation state of HDAC3 directly determines the ability of HDAC3 to modulate long-term memory formation. Unpublished data from the Wood lab revealed that aging (18-month-old) mice exhibited elevated levels of phosphorylated HDAC3 in hippocampal neurons compared to young mice, which correlated with impaired performance on an object location memory task. This finding led us to hypothesize that the phosphorylation state of HDAC3 is a mechanism that regulates HDAC3 activity, and thereby, regulates long-term memory formation in the hippocampus. To test this, we developed viruses to express HDAC3 phospho-mimetic mutations for either constitutively active deacetylase activity (phospho-mimic) or constitutively inactive deacetylase activity (phospho-null). We ran two separate cohorts of mice to compare the expression of both HDAC3 mutant viruses against controls. In one cohort, mice received viral infusions of either the phospho-mimic virus or an empty vector virus for control into the dorsal hippocampus. In the second cohort, we infused either the phospho-null virus or an empty vector virus. We tested mice on an object location memory (OLM) task before measuring synaptic plasticity with slice electrophysiology in CA1. We found that mice expressing the phospho-mimic virus in dorsal hippocampus exhibited *impaired* OLM performance and impaired synaptic plasticity in CA1 compared to mice expressing the empty vector virus. However, mice expressing the phospho-null virus exhibited *enhanced* OLM performance and synaptic plasticity compared to controls. Overall, our findings indicate that the phosphorylation state of HDAC3 is critical for hippocampal-dependent memory formation and synaptic plasticity. Hyper-phosphorylation or maintained phosphorylation of HDAC3 in the aging brain may contribute to age-related cognitive and synaptic plasticity dysfunction.

Disclosures: A. Rodriguez: None. **A.A. Keiser:** None. **E.A. Kramar:** None. **T. Dong:** None. **J.L. Kwapis:** None. **D.P. Matheos:** None. **M.A. Wood:** None.

Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.06

Topic: H.07. Long-Term Memory

Support: CNPq Universal 2018

Title: Memory updating through deconditioning as an effective and long-lasting way to hinder aversive memory

Authors: *L. D. ALVARES[1], B. POPIK[2];
[1]Federal Univ. Rio Grande do Sul, Federal Univ. Rio Grande do Sul, Porto Alegre, Brazil;
[2]UFRGS, Porto Alegre, Brazil

Abstract: Ample evidence suggests that consolidated memories can enter a labile period and become susceptible to modulations. It is proposed that this labialization allows the update of the fear memory trace by the mechanisms of reconsolidation. Here, we demonstrate a new approach that eliminates aversive memory in an effective and permanent way, transforming it into an innocuous memory. This method consists of "deconditioning" rats which have previously been trained to associate a sound with a strong foot-shock, where the shock information is updated and replaced by an extremely low aversive/neutral stimulus during retrieval. We also show the molecular mechanisms underpinning the deconditioning effect. The effectiveness and long-lasting suggest a possible therapeutic potential of this approach to eliminate pathological memories in humans.

Disclosures: L.D. Alvares: None. **B. Popik:** None.

Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 075.07

Topic: H.07. Long-Term Memory

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C Irvine Institute for Clinical and Translational Sciences Pilot Award
Robert Wood Johnson Foundation Amos Medical Faculty Development Program (awarded to A.S.I.)

Title: Early life exercise primes the neural epigenome to facilitate gene expression and hippocampal memory consolidation.

Authors: *A. RAUS[1], N. E. NELSON[2], T. D. FULLER[3], S. A. VALIENTES[3], A. BAYAT[3], A. S. IVY[4];
[1]Physiol. and Biophysics, [2]Physiol. & Biophysics, [4]Pediatrics, [3]Univ. of California, Irvine, Irvine, CA

Abstract: Exercise in adults modulates hippocampal function and synaptic plasticity to influence learning and memory. Our lab previously found that exercise during adolescence (P21-41; EX) enables hippocampal memory such that EX mice form long-term memory for a typically insufficient learning stimulus. Given that epigenetic mechanisms are engaged by early-life experiences and may mediate lasting changes in neuronal function and behavior, our lab considers the possibility that epigenetic mechanisms are engaged by EX to influence hippocampal maturation and function. Here, we test the hypothesis that EX alters occupancy of specific histone posttranslational modifications to influence networks of gene expression underlying hippocampal memory. Transgenic mice expressing the NuTRAP (Nuclear tagging and Translating Ribosome Affinity Purification) cassette were crossed with the *Emx1*-cre mice, allowing for neuronal specificity. Emx1-NuTRAP mice (n=4/group) were given access to a running wheel (EX) or not (SED) during postnatal days 21-41. On P42 they were exposed to a 3-min (subthreshold) or 10-min (threshold) training period in an object location memory task and sacrificed 60 min later to evaluate transcription during memory consolidation. Controls were not allowed access to a running wheel and were not exposed to OLM training. We combined nuclear isolation using Isolation of Nuclei TAgged in specific Cell Types (INTACT) with Translating Ribosome Affinity Purification (TRAP) methods (“SIT”, Simultaneous INTACT and TRAP) to determine neuron-specific epigenetic modifications influencing mRNA expression. Translating mRNA and nuclear chromatin were isolated from Emx1-expressing hippocampal neurons in order to pair EX-induced changes in gene expression (RNA-Seq) with epigenetic modifications (CUT&RUN-seq). RNA-seq revealed EX-induced transcriptional networks of neuroplasticity genes, as well as novel genes and upstream regulators not previously associated with exercise. These correlated with new associated H4K8ac (permissive) and H3K27me (repressive) peaks as determined by CUT&RUN-seq. We link EX-induced histone modifications with altered transcription after exposure to OLM subthreshold and threshold learning events, and identify gene networks in 3min trained EX animals whose expression is regulated in the same direction as those genes induced by a learning event of sufficient time (10min) in sedentary mice. This suggests EX “primes” transcriptional responsiveness to hippocampal learning. We conclude that EX induces a primed epigenetic state that is associated with altered transcription to support enabled hippocampal memory.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.08

Topic: H.07. Long-Term Memory

Support: NIH P20GM103423
Pitt-Hopkins Research Foundation
The Orphan Disease Center
The Sherman Fairchild Foundation

Title: Tet enzyme inhibition modulates dna methylation and enhances memory function in mice

Authors: *R. I. CHUGHTAI, S. G. FAASEN, K. ZENGELER, H. SMITH, A. J. KENNEDY; Bates Col., Lewiston, ME

Abstract: Active DNA methylation is required for the formation and maintenance of long-term memories. TET enzymes are a family of nuclear oxidases that initiate the removal of DNA methylation and their activity has been linked to memory extinction and loss. Here, we test the hypothesis that TET gene knockout and enzymatic inhibition of the gene product, using novel small molecule inhibitors, is sufficient to enhance long-term memory fidelity in mice. Additionally, the effects of TET enzyme knockout and inhibition on DNA methylation levels in neurons were investigated using whole genome sequencing approaches. Our results suggest that the TET enzymes are sufficient targets to enhance memory function. Lastly, TET enzyme inhibition was evaluated as a potential therapeutic in a mouse model of the rare monogenic learning and memory disorder Pitt-Hopkins Syndrome, caused by the haploinsufficiency of the Tcf4 gene.

Disclosures: R.I. Chughtai: None. **S.G. Faasen:** None. **K. Zengeler:** None. **H. Smith:** None. **A.J. Kennedy:** None.

Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 075.09

Topic: H.07. Long-Term Memory

Support: NIH P20GM103423

Title: Tet2 mediated demethylation of DNA after experiential learning destabilizes memory

Authors: A. L. KIRSCHNER, M. CARON, K. ZENGELER, H. SMITH, *A. KENNEDY; Bates Col., Lewiston, ME

Abstract: Active DNA methylation in neurons that encode experience is required for long-term memory formation. TET enzymes oxidize and lead to the removal of DNA cytosine methylation. Here we demonstrate that the conditional knockout of Tet2 in glutamatergic neurons induces enhanced long-term memory retention in mice. We found enhanced DNA methylation patterns in the promoter of experience-regulated genes due to Tet2 deletion in these neurons, which corresponded with altered gene expression. Additionally, we demonstrate that deleting Tet2 from recently activated neurons, induced by learning, selectively enhanced the recall of individual memories. These data suggest that Tet2 is a negative regulator of memory retention in neurons following learning and a target for memory enhancement. Lastly, Tet2 inhibitors were administered to mice either 2 or 14 months of age to determine whether Tet2 inhibition is sufficient to enhance memory in young and old mice.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.10

Topic: H.07. Long-Term Memory

Support: BrainMatrix NFR

Title: An updated suite of viral vectors for in-vivo calcium imaging using local and retro-orbital injections

Authors: *S. GRØDEM[1], I. NYMOEN[2], G. H. VATNE[1], V. BJÖRNSDOTTIR[1], D. G. HILDEBRAND[3], L. THOMAS[4], F. E. THEUNISSEN[5], K. K. LENSJØ[1], M. FYHN[1]; [1]Univ. of Oslo, Oslo, Norway; [2]Univ. of Oslo, OSLO, Norway; [3]The Rockefeller Univ., New York, NY; [4]Biophysics, UC Berkeley, Berkeley, CA; [5]Psychology and HWNI, California Clin. Trials, Berkeley, CA

Abstract: Calcium imaging using genetically encoded Ca2+ indicators (GECIs) is a widely adopted method to measure neural activity in modern neuroscience. Here, we explore the use of systemically administered viral vectors for brain-wide expression of GECIs, and adapt novel GECIs to optimize signal-to-noise. We show that systemic injections of PHP.eB or CAP-B10 serotype AAVs to express GECIs, especially jGCaMP8, is a highly promising technique for imaging neural activity and circumvents the need for transgenic GECI-expressing mouse lines. We also establish the use of soma-targeted GECI constructs where we combine the most advanced soma-targeting peptides with the recently developed jGCaMP8 GECIs, providing unparalleled signal to noise ratios paired with the fastest GECI kinetics. Additionally, our most promising soma-targeted constructs have been successfully tested in marmosets and zebra

finches in a dual-AAV Tet-off system, and are currently being tested in a novel single-AAV Tet-off system.

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Poster

075. Long-Term Memory: Consolidation and Reconsolidation: Molecular Mechanisms

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 075.11

Topic: H.07. Long-Term Memory

Support: RCN Grant No. 250259
RCN Grant No. 248828
University of Oslo

Title: Perturbation of inhibitory activity interferes with memory reactivation and attenuates learning in visual association cortex

Authors: K. K. LENSJØ[1], **I. NYMOEN**[2], F. ROGGE[3], A. U. SUGDEN[4], I. SHURNAYTE[5], S. GRØDEM[1], M. L. ANDERMANN[6], *M. FYHN[7];
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Abstract: Consolidation of salient experiences to form memories rely on precise coupling of high frequency neural network events between hippocampus and neocortex. Within these events, reactivation of subpopulations of neurons is believed to strengthen connections within and between networks. We have previously shown that reactivation occurs in visual association cortex during learning of a visual association task. Moreover, recent work suggests a role for parvalbumin-expressing (PV) inhibitory interneurons in consolidation by synchronizing hippocampal and cortical activity. We therefore hypothesize that PV interneurons ensure the precise timing between hippocampus and cortex to drive successful reactivations necessary for learning.To investigate this, we trained mice in a visual association task and silenced PV interneurons in visual association cortex after training by chemogenetics on alternating days. In ongoing experiments, we perform two-photon imaging of cell ensembles in visual association cortex, during and after daily training. Activity is estimated by RiboL1-jGCaMP8s fluorescence, and PV+ cells identified (and inhibited) by expressing DIO-DREADD-mScarlet, or DIO-Ruby in control mice. In a separate group we perform simultaneous electrophysiological recordings in visual association cortex and hippocampus area CA1 after daily training.Our preliminary results indicate that local silencing of PV interneurons strongly attenuates memory consolidation. Notably, the animals' performance improved following training days with saline injections, and

reverted to chance levels following training days with PV silencing. Early results from imaging suggest that reactivations are present after chemogenetic silencing, but less structured compared to controls. Together our preliminary data indicate that PV silencing during consolidation prevents learning, possibly by interfering with memory reactivations.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 076.01

Topic: H.07. Long-Term Memory

Support: NIH T32 GM139807
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Title: In vivo PET imaging to assess class IIa HDACs of long-term extinction fear memory in female and male rodents

Authors: *M. M. GLOVER[1,2], X. LU[3], F. REZAZADEH[4], N. T. VIOLA[4], S. E. BOWEN[1], S. A. PERRINE[2];
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Abstract: Fear conditioning studies in humans and animals (primarily rodents) have elucidated the neurobiological and behavioral bases of extinction learning. Valuable insights have been gained into this part of the learning process, informing novel treatment options for the healthy extinguishing of fear-related memories. The development of memories, including those throughout extinction learning, requires neuroplasticity in encoding and retrieval. When investigating extinction learning of conditioned fear behavior, neuroimaging and critical behavioral analyses provide an essential understanding on the approaches to mediate the detrimental effects of fear memories and restore the healthy extinction of fear behavior. To illuminate the neurobiological mechanism(s) of extinction learning of acquired long-term fear memories, a group of rats (n = 6) was subjected to contextual fear conditioning (day 1: habituation; day 2: acquisition, five 10-second tones, co-terminating with a 1-second 1mA footshock). After a 21-day rest period (to model a long-term memory), rats were subjected to extinction learning (thirty 10-sec tones). All animals will be assessed for changes in class IIa histone deacetylase (HDAC) activity via positron emission tomography (PET) imaging. At baseline (i.e., 9-12 days before fear conditioning) and immediately after extinction learning, the novel substrate-based PET-ligand [^{18}F]TFAHA was used to measure class IIa HDAC expression-activity *in vivo*. To date, preliminary data reveal that males successfully acquire and extinguish

long-term fear memory. This study remains ongoing, and PET imaging analysis is underway to assess the impact of class IIa HDACs in the hippocampus after extinction learning.

Disclosures: M.M. Glover: None. **X. Lu:** None. **F. Rezazadeh:** None. **N.T. Viola:** None. **S.E. Bowen:** None. **S.A. Perrine:** None.

Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 076.02

Topic: H.07. Long-Term Memory

Support: CONACyT grant 856256
CONACyT grant FOINS 474
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Title: Editing Memories: Updating contextual recognition memory by catecholaminergic terminals in the dorsal hippocampus from the locus coeruleus

Authors: *D. GÁLVEZ-MARQUEZ[1], M. SALGADO-MÉNEZ[1], P. MORENO-CASTILLA[1], L. RODRÍGUEZ-DURÁN[1], M. ESCOBAR-RODRÍGUEZ[2], F. TECUAPETLA-AGUILAR[1], F. BERMÚDEZ-RATTONI[1];
[1]IFC, Coyoacán, CDMX, Mexico; [2]Facultad de Psicología, Coyoacán, CDMX, Mexico

Abstract: Detecting contextual novelty is critical to consolidating declarative memories, such as spatial recognition memory. It has been shown that stored memories, when retrieved, are susceptible to modification incorporating new information through an updating process. Previous studies have shown that a catecholaminergic release in the hippocampal CA1 consolidates a spatial object location memory. This work hypothesized that updating spatial location memory could be changed by decreasing the catecholaminergic release in the hippocampal CA1 terminals from the Locus Coeruleus (LC). In a mouse model expressing Cre-recombinase under the control of the tyrosine hydroxylase promoter, it was observed that memory updating was impaired by photo-inhibition of the CA1 catecholaminergic terminals from the LC (LC-CA1) but not from the Ventral Tegmental Area (VTA-CA1). By performing an *in vivo* microdialysis, it was confirmed that the extracellular concentration of both dopamine (DA) and noradrenaline (NA) decreased after photo-inhibition of the LC-CA1 terminals (but not VTA-CA1) during OLM memory retrieval. Furthermore, it was observed that DA D1/D5 and beta-adrenergic receptor antagonists disrupt memory retrieval, but only the former impair memory updating. Finally, photo-inhibition of LC-CA1 terminals was observed to suppress the long-term potentiation induction in Shaffer's collateral as a plausible mechanism for updating memory. These data will help understand the underpinning mechanisms of DA and NA in contextual memory updating. Modulating the extracellular concentration of catecholamines in the hippocampus could modify

contextual maladaptive memories, such as aversive memories, drug addiction, and phobias, through memory updating

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

Location: SDCC Halls B-H

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Program #/Poster #: 076.03

Topic: H.07. Long-Term Memory

Support: NIH R01-DA042057
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Title: Neurotoxic lesioning of norepinephrine improves memory consolidation in male but not female rats

Authors: *A. GHEIDI[1], C. J. DAVIDSON[1], S. C. SIMPSON[1], M. A. YAHYA[1], N. SADIK[1], S. A. PERRINE[2];
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Abstract: The role of the Locus Coeruleus (LC) and norepinephrine (NE) in modulating memory processes remains elusive. Notably, their contribution to different phases of memory, such as acquisition, consolidation, and updating, remains unknown. In two separate, but complementary, experiments we manipulate NE levels in the brain of rats using the specific noradrenergic neurotoxin N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4). Published studies show that this neurotoxin crosses the blood-brain barrier and depletes pre-synaptic NE stores. In both experiments of this study, before being trained on a Barnes Maze memory apparatus, female and male Wistar rats received DSP-4 (50 mg/kg/i.p.) or saline-control administration and were housed for ten days to allow for NE depletion. For experiment 1, rats (n=16/sex) were then trained (3 trials per day, 5 days) to perform the Barnes Maze spatial memory task, which involved finding a hidden platform. Regardless of DSP-4 treatment, all animals found the location with equivalent latency following training. Decreased NE in the hippocampus, striatum, and medial prefrontal cortex (mPFC) for the DSP-4 group was confirmed via HPLC analysis. In experiment 2, we replicated and extended these findings. In addition to the undergoing acquisition as in the first experiment, rats (n=16/sex) were kept in the home cage for an additional 2 days unhandled, and then they were subjected to a recall session where the platform location (memory consolidation) was covered. The next day, the location of the hidden platform was shifted 90 degrees (reversal-learning task), and rats were again trained for 2 days to enter the new hidden location (memory updating). When rats were required to recall the location of the hidden platform, male rats administered DSP-4 performed better than male controls

suggesting greater memory consolidation. During the reversal-learning task (memory updating), males treated with DSP-4 continued to visit the previously hidden platform significantly more despite learning the new hidden location comparable to their control counterparts. No differences existed among the female rats in the consolidation or updating tasks. These results collectively suggest that NE plays a specific role in regulating memory consolidation, specifically in males, rather than affecting general acquisition or memory updating. In conclusion, our results indicate that this regulation by the LC may be sex-specific. Further investigations are needed to validate and extend the current findings, including determining if the loss of the NE in the hippocampus, specifically while leaving other regions undisturbed, yields the same results.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Ludwig Family Foundation Grant
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Office of Naval Research
Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Characterizing the cellular and circuit-level mechanisms of therapeutic psychedelics

Authors: *C. DELGADO SALLENT, R. A. SENNE, S. A. AHMED, J. GOMEZ, B. B. SCOTT, S. RAMIREZ;
Boston Univ., Boston Univ., Boston, MA

Abstract: Recently, the use of psychedelics, such as ketamine, have proven to be effective in treating stress-related maladaptive states, including post-traumatic stress disorder (PTSD) and major depressive disorder (MDD), often producing a sustained effect after a single dose. The therapeutic effects of these psychedelics have been related with their ability to robustly promote structural and neural plasticity. Moreover, psychedelics can increase long-term cognitive flexibility which may lead to a reduction of negative bias and rumination in depressed patients. However, these psychedelic drug interventions produce brain-wide, non-specific effects, and the neural mechanisms underlying these effects are poorly understood. Here, we study the therapeutic effects of psychedelics and their corresponding neural correlates. In this study we

focus on the psychedelic ketamine, an NMDA antagonist. We characterize its therapeutic properties by studying the brain-wide mapping of drug-responsive populations by using activity-dependent gene expression. First, to detect and map ketamine active cells we measured endogenous c-fos expression after ketamine administration. Subsequently, we performed unbiased brain-wide analyses of all cells active after ketamine administration in cleared brains to identify activation of active cell populations. We find that ketamine administration (30 mg/kg) produces an increased c-fos expression in several areas, and in particular thalamic areas, which suggests that the thalamus may be a key site of action for ketamine's effects on the brain. Our future experiments include targeted reactivation of cell populations active during ketamine administration to recapitulate ketamine's behavioral effects and to characterize these effects on a decision-making task. Taken together, our work aims to reveal the neural and behavioral correlates underlying the therapeutic doses of ketamine.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Topic: H.07. Long-Term Memory

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Office of Naval Research
Boston University Center for Systems Neuroscience

Title: Visualization and Modulation of Hippocampal Engram-driven Networks

Authors: *K. E. DORST[1], R. A. SENNE[2], A. DIEP[3], S. RAMIREZ[2];
[1]Boston Univ., Boston Univ. Grad. Program For Neurosci., Boston, MA; [2]Boston Univ., [3]Boston Univ., Boston, MA

Abstract: Animals utilize a repertoire of defensive behaviors to avoid predators and other noxious stimuli. Successful implementation of these behaviors depends on both the external environment and the internal state of the animal as the brain makes a series of computations to integrate and use such information. Importantly, cognitive dysfunction can disrupt this integration and generate maladaptive defensive behaviors. Memory systems, in particular, play a highly influential role in mediating defensive strategies based on previous experiences. Yet, how cells that drive memory expression modulate downstream neural systems to properly gate

defensive behaviors is unknown. To address this, we use activity-dependent labeling strategies to leverage optical control over populations of cells (i.e., an engram) within the dorsal dentate gyrus (dDG) that encode information of a fearful experience. Here, we optogenetically reactivate a dDG fear engram under varying environmental sizes to test for its capacity to mediate defensive behaviors in a manner contingent on spatial demands placed on the animal. We found an inverse relationship between the size of the environment and the amount of light-induced freezing: mice significantly froze during fear engram reactivation in the smallest arena, but not in the largest arena. This suggests that a dDG engram is not fixed to generate one behavioral output, but can be integrated into brain-wide pathways to elicit adaptive defensive behaviors. Our current work uses network analyses to 1) examine correlations in endogenous cFos across brain regions to identify putative hub regions that mediate these state-dependent alterations in defensive strategies during fear engram reactivation, and 2) delete these regions in silico and in vivo to test for their causal contributions to light-induced memory expression. By identifying and manipulating areas supporting memory, our work seeks to resolve systems-level biological mechanisms mediating memory's capacity to modulate maladaptive behavioral states.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Topic: H.07. Long-Term Memory

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Office of Naval Research
Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Astrocytic responses to artificial fear-memory reactivation

Authors: *R. A. SENNE[1], R. L. SUTHARD[1], M. D. BUZHARSKY[2], S. RAMIREZ[3];
[1]Grad. Program for Neurosci., Boston Univ. Grad. Program For Neurosci., Boston, MA; [2]Boston Univ. Undergraduate Program In Neurosci., Boston, MA; [3]Boston Univ., Boston, MA

Abstract: Historically, glial cells have been often thought of as support cells of the brain, but increasing evidence suggests they take an active role in cognition and their dysfunction is often a hallmark of many pathologies. In animal models of PTSD or anxiety for instance, astrocytes often are reduced in density, have aberrant activity, and display maladaptive changes in

morphology, which correlate with a battery of behavioral impairments corresponding to disordered states of the brain. One open question is how do astrocytes respond during the reactivation of a fearful experience, and does any ensuing activity reflect internal states of an agent? To that end, we combined optogenetics and fiber photometry to tag fear engrams in the dentate gyrus (DG) while recording from neuronal and astrocytic populations in the ventral CA1 (vCA1) regions of the hippocampus. The goal of these experiments is to record these interlinked cell types and characterize their dynamics during encoding, recall, and optogenetic reactivation of a fear ensemble. In our first experiment mice co-expressed ChR2 in fear encoding DG ensembles and jRGECO1a and GCaMP6f in vCA1 neurons and astrocytes, respectively. During fear conditioning, we observed highly time-locked transients to shocks in both populations, as shown in concurrent work in our lab. One day later, when subjected to natural recall, we observed robust transients in both cell types of varying magnitudes during the duration of the session. Finally, when subjected to artificial reactivation of DG-mediated fear memory in a neutral environment, calcium dynamics in vCA1 mimicked natural recall in experimental (ChR2) and not control (eYFP) mice. Our future analyses will incorporate generalized linear models to test if the calcium signal is reliably predicted by kinematics, freezing behavior, and optogenetic stimulation. With this analysis we hope to test the hypothesis that post fear-conditioning, astrocytes respond coincidentally with the termination of freezing bouts, possibly suggesting a suppression of fear-related and/or anxious internal states.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Center for Systems Neuroscience and Neurophotonics at Boston University

Title: The role of positive and negative engrams in memory guided decision-making.

Authors: *A. CABAN[1], M. SURETS[2], M. KAUFMANN[2], R. A. SENNE[1], K. E. DORST[1], C. DELGADO SALLENT[2], S. RAMIREZ[1];
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Abstract: The ability to make flexible memory-guided decisions is fundamental to our daily lives. Such decision-making often requires us to strategically balance motivational drives including the pursuit of reward and avoidance of danger-which become impaired in addiction and anxiety disorders respectively. Here, we aim to understand the underlying mechanisms that support flexibility during memory-guided decision-making. To this end, we developed a novel decision-making task that allows animals to strategically combine the drive to approach a reward and avoid a footshock without compromising either goal. During training, mice learn that a 30s light cue indicates the availability of reward at a nose port (i.e. sucrose water), and a 30s auditory cue predicts the onset of a footshock which they can avoid by stepping into a platform. After animals learn the reward and punishment contingencies, the light and auditory cues are delivered at the same time, creating a conflict between reward-seeking and punishment avoidance. Over the course of 7 days, we find that mice initially utilize unique sex-specific strategies that then converge over 7 days on the same resolution to this conflict. Initially, female mice are more risk-taking and opt to get the maximal reward at the expense of footshock. However, by day 7 all mice learn to balance both drives; seeking reward early during the simultaneous cue presentations while then stepping on the platform towards the end of the cue presentations, thus avoiding the footshock without omitting reward seeking. To shed light into the brain regions recruited during this flexible decision-making process, we combined whole-brain inmuno-staining of the activity-dependent immediate early gene cFos with the theoretical framework of graph theory. By applying topological measures of centrality to correlation networks created from cFos expression we identified key regions (Hubs) in our networks such as the prelimbic cortex (PL) and the basolateral amygdala (BLA). Our ongoing experiments aim to elucidate the real-time dynamics of both PL and BLA during flexible memory-guided decision-making through population calcium imaging and to consequently bias decision-making through optogenetic reactivation of valence-specific engrams. Together our data will further our understanding of how memories guide decision-making and may point to key behavioral and population-level signatures that underlie their impairments in neuropsychiatric disorders.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Cellular and Brain-Wide Network Effects of Acute Sleep Deprivation on Hippocampal-Dependent Memories

Authors: *H. LEBLANC[1], R. H. COLE[2], S. RAMIREZ[1];
[1]Boston Univ., Boston, MA; [2]Ctr. for Neurosci., Univ. of Pittsburgh, Pittsburgh, PA

Abstract: Sleep and decreased states of arousal are evolutionarily conserved behaviors that serve an important yet relatively unknown purpose throughout an organism's lifespan. Despite its generally elusive function, there is an emerging consensus that sleep serves a profound role in the regulation of cognitive processes, especially those linked with memory (Abel et al., 2013). The hippocampus, a structure commonly associated with its roles in learning and memory, has been shown to be distinctly affected by sleep and its structural and functional integrity is particularly susceptible to sleep deprivation. Here, we sought to characterize the effects of acute sleep deprivation on the cellular and brain-wide network dynamics activated by two hippocampal-dependent memory tasks previously shown to be susceptible to sleep deprivation-induced deficits. Following contextual fear conditioning or object location memory training, male C57BL/6J mice underwent 6 hours of acute sleep deprivation by the gentle handling method. Contrary to findings reported in previous literature, acute sleep deprivation did not yield deficits in the recall or context-specificity of a contextual fear memory at 24 hours or two weeks following training. These results demonstrate notable variability in the effects of sleep deprivation on contextual fear memories and demand further study in the future. In contrast, and consistent with previous studies, we observed object location memory deficits at 24 hours following acute sleep deprivation. Finally, our current work employs brain-wide *cfos* expression quantification strategies to characterize the cellular and global activation of neuronal ensembles during object location memory recall. Together, our work provides cellular insight to the effects of sleep deprivation on memory and behavior.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Office of Naval Research
Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Erasable hippocampal neural signatures predict memory discrimination

Authors: *E. RUESCH[1], N. R. KINSKY[2], D. ORLIN[3], S. RAMIREZ[1];
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Abstract: Despite the extensive use of contextual fear conditioning (CFC) to study episodic memory, there is a knowledge gap pertaining to how a neural code evolves during the consolidation of short-term memory into long-term memory. Previous studies have demonstrated that CFC causes a reorganization, or remapping, of hippocampal place fields. This phenomenon suggests that the hippocampal neural code may provide a substrate for the retention and recall of CFC memories. Here, we leveraged the spatial and temporal resolution of single-photon calcium imaging in conjunction with CFC and a protein synthesis inhibitor to characterize the heterogeneity of hippocampal remapping over long-time scales in freely moving mice. We exposed mice to two different arenas for 7 days to test the specificity of the CFC memory. Moreover, we tracked freezing and non-freezing behavior in the days before, during, and after shock to examine the long-term stability of the CFC memory. A separate cohort of mice received anisomycin, a protein synthesis inhibitor that disrupts long-lasting synaptic plasticity, immediately after shock to block consolidation. In these experiments, we hypothesized that anisomycin would temporarily arrest learning-related changes in the event rate of neurons and turnover of active neurons between sessions, and would prevent remapping of their place field locations. As expected, we found that freezing returned to pre-shock levels in the days following CFC for anisomycin mice but not for control mice corroborating previous findings. Surprisingly, we also observed that anisomycin temporarily accelerated the turnover of active neurons. This increased cell turnover was concomitant with a general shutdown of neural activity up to two days later. Therefore, we posit the amnestic effects of anisomycin could stem from the disruption of constitutive processes in conjunction with blocking learning-related plasticity. Additionally, the degree to which mice discriminated between arenas correlated with the amount of cell turnover between the arenas. This finding supports the idea that the hippocampal neural code provides a substrate for differentiating between arenas. Future analyses will investigate whether cell turnover between arenas relates to cell turnover across days and how freezing-related neural responses evolve through the course of consolidation and amnesia. Taken together, our data provide physiological insight into how hippocampal cell populations are differentially contributing to the processes underlying memory consolidation and recall.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Center for Systems Neuroscience and Neurophotonics Center at Boston University

Title: Ventral hippocampal engrams in healthy and disease states

Authors: *M. SHPOKAYTE[1], R. SUTHARD[2], A. JELLINGER[1], R. A. SENNE[1], S. LIU[3], S. RAMIREZ[1];
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Abstract: Memories can be imbued with positive and negative emotions. The ventral hippocampus (vHPC) is known for its ability to process this positive and negative information and to relay it to its downstream targets. By combining activity-dependent tagging strategies to visualize components of memory's physical manifestation (i.e. a memory engram) within the vHPC, here we find that the vHPC parses differentially valenced information into two discrete populations of cells. Additionally these cells have different transcriptomic and methylation patterns, different terminal projection patterns, and drive behavior differentially. Moreover, we identified that a subpopulation of genes associated with neurodegeneration, such as APP and APOE, were specifically upregulated and downregulated in negative and positive engrams, respectively. Interestingly, we found that genes associated with neuroprotection, such as BDNF and Pin1, were upregulated and downregulated in positive vs negative engrams respectively. Our ongoing work tests whether or not chronic stimulation of an aversive engram in an aged mouse is sufficient to induce neurodegenerative or tau-like pathology. We found that these mice had numerous behavioral deficits and strong physiological changes through histological changes in p-tau, neurofibrillary tangle expression, and changes in microglia and astrocyte quantity and morphology. Together, we demonstrate that the vHPC is embedded with both positive and negative engrams, and specifically, when these negative engrams are in a hyperactive state they have the ability to induce systems-wide changes that are detrimental for overall brain and behavioral health.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

Location: SDCC Halls B-H

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NIH Transformative R01 Award
Young Investigator Grant from the Brain and Behavior Research Foundation
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Title: Characterizing amygdalar and hippocampal neuron-astrocyte calcium dynamics across fear learning and extinction.

Authors: *R. L. SUTHARD[1], R. A. SENNE[1], M. D. BUZHARSKY[2], R. H. COLE[3], S. RAMIREZ[4];
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Abstract: Post-traumatic stress disorder (PTSD) can result from witnessing or experiencing an acute traumatic event. Many individuals with PTSD will undergo exposure therapy-the intentional re-experiencing of the context, thoughts and emotions associated with the traumatic experience, to suppress or 'extinguish' the original fear memory and reduce avoidance behaviors. However, the reduction in fear may be temporary for some individuals and return with the passage of time, which is known as 'spontaneous recovery'. While the field of neuroscience has focused primarily on the role that neurons play in complex brain processes, there have been a plethora of studies elucidating the role that astrocytes and other glial cells play in the circuits involved in learning and memory, including the amygdala and hippocampus. Here, we record astrocytic and neuronal calcium dynamics to gain real-time insight into population-level activity during the acquisition, recall and extinction of fear, as well as the subsequent spontaneous recovery of this extinguished memory. In our first experiment, we utilized the genetically-encoded calcium indicator, GCaMP6f, in astrocytes within the basolateral amygdala (BLA) to record calcium dynamics across fear learning using in vivo fiber photometry. Mice were subjected to contextual fear conditioning, recall and three extinction sessions to dissociate contextual- vs. shock-driven activity. We found that astrocytes display robust, high amplitude, short duration calcium events in response to 1.5mA footshock and have reliable transients across recall and all days of extinction. Moreover, our current work assesses event metrics across sessions: peak height, area under the curve (AUC), full width half max (FWHM), and frequency (peaks/min), to compare shock and no shock conditions across extinction. Additionally, we will correlate astrocytic calcium events with the onset or termination of freezing bouts. Finally, to further investigate the role of canonical fear learning 'hubs', we will record both neurons (jRGECO1a) and astrocytes (GCaMP6f) simultaneously in ventral CA1 of the hippocampus (vCA1) across fear conditioning, recall, extinction and spontaneous recovery after the passage of time. Together, our dual-color recordings will enable us to analyze coherence between signals

and parse out unique contributions of each cell type to the maintenance or extinction of learned fear.

Disclosures: R.L. Suthard: None. **R.A. Senne:** None. **M.D. Buzharsky:** None. **R.H. Cole:** None. **S. Ramirez:** None.

Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Topic: H.07. Long-Term Memory

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Title: The phase of the cortical slow oscillation determines the efficacy of hippocampal sharp-wave ripples as an index for systems consolidation

Authors: *R. GOLDEN[1], O. C. GONZALEZ[3], M. TATSUNO[4], B. L. MCNAUGHTON[5], M. BAZHENOV[2];
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Abstract: Systems Consolidation Theory posits that the hippocampus encodes new information for later cortical consolidation during non-REM sleep. Specifically, phase locking of cortical slow oscillations (SOs) and hippocampal sharp-wave/ripples (SWRs) is thought to allow the hippocampus to replay recent memories and to *index* corresponding cortical memory traces to be replayed and learned for long-term storage. Despite the success of Systems Consolidation Theory in explaining many experimental findings regarding how cortex and hippocampus coordinate memory consolidation, many of its central predictions remain untested. To understand the details of this coupling, we analyzed single-unit activity from the medial prefrontal cortex (mPFC) and CA1 of the hippocampus from rats trained to run a spatial sequence memory task and developed a biophysically-realistic thalamocortical network model implementing SWR input and SOs. First, we analyzed the distribution of SWR arrival times and observed that SWRs *in vivo* exhibit a tendency to occur immediately after the down-to-up transition (DUt) of SOs. To investigate the function of this phase preference on the efficacy of hippocampal indexing, we developed a biophysically-realistic thalamocortical network model based on Hodgkin-Huxley neurons capable of transitioning between awake and sleep states, and equipped with a spike-timing-dependent plasticity on synapses between pyramidal cells. Using this model, we systematically applied artificial indexing cues at various phases of the SO. This allowed us to

assess the relationship between the phase of the SO when indexing occurs and the extent of synaptic consolidation for the indexed memory. We found that the phase interval which facilitated robust synaptic consolidation was contained within the preferred phase distribution for SWR arrival times. Next, we focused on characterizing the unique properties of this preferred phase. We found that the von Neumann entropy of cortical population activity is a suitable proxy to measure transient sensitivity to external perturbations, while the PC dimension can capture persisting effects of the perturbation throughout a SO. Using these measures, we found evidence that SWRs can transiently bias cortical activity during any phase of the cortical SO, but there is an optimal phase during which SWRs can maximally bias persistent activity and robustly drive consolidation. Thus, our findings provide a functional and computational account for SO-SWR phase-locking - maximizing consolidation by ensuring SWRs arrive during a phase of SO in which the cortex is most responsive to incoming signals.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Title: Sleep prevents catastrophic forgetting in spiking neural networks by forming a joint synaptic weight representation

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Abstract: Artificial neural networks overwrite previously learned tasks when trained sequentially, a phenomenon known as catastrophic forgetting. In contrast, the brain learns continuously, and typically learns best when new training is interleaved with periods of sleep for memory consolidation. Here we investigated the role of sleep in preventing catastrophic forgetting by utilizing a three layer network composed of spiking neurons that are subjugated to Hebbian learning rules and homeostatic mechanisms in a reinforcement learning paradigm. The network could be trained to learn a complex foraging task but exhibited catastrophic forgetting when trained sequentially on several different tasks. Synaptic weight analysis revealed the

presence of solution manifolds corresponding to the multiple weight configurations which correspond to and solve individual specific tasks. New task training moved the synaptic weight configuration away from the manifold representing old tasks and towards the new task manifold, leading to forgetting. Interleaving new task training with periods of off-line reactivation, mimicking biological sleep, circumvented catastrophic forgetting. Periods of sleep constrained the network weight state to the original task manifold while short periods of new task training allowed the synaptic weight configuration to slide towards the intersection of old and new manifolds. Through interleaving training and sleep, the resulting weight state that the network achieved enabled it to perform on multiple tasks simultaneously, successfully maintaining performance on the original task while introducing a new task all without the explicit replay of previously learned data (a common approach in artificial intelligence). This study reveals a possible strategy of synaptic weights dynamics the brain applies during sleep to prevent forgetting and optimize learning.

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Poster

076. Long-Term Memory: Consolidation and Reconsolidation: Neural Mechanisms

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Title: Geometry of memory manifolds in synaptic weight space

Authors: O. C. GONZALEZ[1], E. DELANOIS[2], R. GOLDEN[3], ***M. BAZHENOV**[4];
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Abstract: Accumulating evidence points to the important role of sleep in consolidation of recent memories. In this new study, we explored how selective memory replay during slow-wave sleep allows formation of new memory traces without catastrophic forgetting of the old memories. We developed a concept of a “memory manifold” - a subspace of the synaptic weight space that describes a set of synaptic weights which strongly represent specific memories, and we discuss sleep-induced dynamics of a neural system on these manifolds. We used a computational model of the thalamocortical network capable of transitioning between periods of wake and slow-wave (N3) sleep and implementing spike-time dependent plasticity (STDP). Training the network on a single memory sequence during awake state resulted in selective increase of synaptic weights and performance for the trained task. By testing multiple networks with random initialization of

synaptic weights, we found that synaptic weight space includes a subspace, we call it memory manifold, where different configurations of weights all allow for a given task to perform well. Sleep following training moved the system further along the memory specific manifold to regions characterized by the highest performance on a sequence completion task. Subsequent training a second memory, which competes for synaptic resources of the previously trained memory, moved the system away from the identified memory manifold towards the new task manifold, resulting in "catastrophic forgetting" of the old task. If, however, sleep was implemented before old task was completely forgotten, the system trajectory approached a region in synaptic weight space where two memory manifolds intersect and display high performance on both tasks. Reaching this state, however, was sensitive to the amount of new training. Implementing of hippocampal indexing solved this problem - indexing of a new memory moved the system along the old memory manifold towards the intersection region circumventing the risk of forgetting old task. In summary, our new study presents a novel theory for the role of sleep in consolidation of memories, and predicts the utility of hippocampal indexing in preventing catastrophic forgetting and driving robust memory consolidation.

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Poster

077. Oscillations, Synchrony, and Memory

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Title: Traveling waves regulate neuronal spiking activity across space and time

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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. To answer this question, we used high-density electrode microarrays to record spiking and local field potential (LFP) responses in the dorsolateral prefrontal cortex (dlPFC) and parietal area 7A of behaving monkeys. In a visually guided saccade task, monkeys were trained to develop expectations of probabilistic rewards, cued by visual stimuli. In each area LFP oscillations in the 10-35 Hz range formed TW that propagated in two approximately opposite directions across the microarray with typical speeds of 0.1-0.3 cm per second. Moreover, the majority of neurons in both areas were sensitive to the phase and direction of the TW, showing higher firing rates at the peak phase of a TW cycle or for one

direction of TW propagation. Finally, while spiking responses in both areas encoded cue location and expected and experienced rewards, the strength of TW was exclusively correlated with the prior trial reward. We speculate that TWs in frontal and parietal areas convey contextual information about a recent reward and modulate the areas' outputs, which are conveyed by spiking responses and signal current rewards.

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Poster

077. Oscillations, Synchrony, and Memory

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Title: Thalamically-initiated Brain-wide Spindles Alleviate Associative Memory Dysfunction in Aging Animals

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Abstract: The decline of memory consolidation capacity due to aging can severely affect the quality of life. Impairment of thalamo-cortical spindle oscillatory activities has been proposed as a mechanism underlying such memory dysfunction. However, it remains unknown whether targeted neuromodulation of spindle activities can arrest the decline of memory consolidation functions in aging brains. Previously, through combining optogenetic stimulation, functional magnetic resonance imaging (fMRI), and visual-somatosensory associative fear conditioning tests in normal rats, we revealed the facilitating effects of thalamically-initiated brain-wide spindle propagation on memory consolidation in normal brains. In this study, we employed this integrative approach to investigate the therapeutic effects of optogenetically-evoked spindle activities on memory consolidation dysfunction in a rat aging model. We injected AAV5-CaMKIIα::ChR2(H134R)-mCherry or saline to somatosensory-specific ventroposteromedial thalamus of 6 weeks Sprague Dawley rats in the optogenetic (OG) or Sham group, respectively. D-galactose (50mg/kg, subcutaneous) was injected daily for eight weeks to generate the model of

accelerated aging. We find that optogenetically-evoked thalamic spindle activities in aging animals (n=7) evoke robust fMRI activations across brain-wide regions as in normal animals (n=10). Twenty-four hours after visual-somatosensory associative fear memory acquisition and consolidation, Sham-Aging animals (n=7) show significantly poorer general memory recall performance than Sham-Normal animals (n=8). Notably, evoking brain-wide spindle activities in OG-Aging animals (n=7) during memory consolidation rescues such decreased memory performance to a level similar to Sham-Normal animals. Parallel visual fMRI experiments find that OG-aging animals (n=10) show significantly increased visual cortical, thalamic and brainstem activations after optogenetic stimulation compared to baseline and displays additional activations in the amygdala, insular area and various somatosensory, motor, frontal and retrosplenial cortical regions. Our study demonstrates that optogenetically-evoked brain-wide spindle propagation from the somatosensory thalamus alleviates visual-somatosensory associative memory consolidation decline in accelerated aging animals, specifically through response potentiation in brain-wide sensorimotor and limbic regions. Our findings identify targeted spindle manipulation as a potential therapeutic paradigm to rescue age-related memory consolidation dysfunction.

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Poster

077. Oscillations, Synchrony, and Memory

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Topic: H.08. Learning and Memory

Support: NIH T32 GM136573
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Title: Cortical beta oscillations underlie verbal working memory function

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Abstract: Due to their proposed role in inhibitory multiregional communication, we hypothesized that cortical beta oscillations (13-30 Hz) would play a critical function in the higher-order cognitive processing required in verbal working memory. Here, we use intracranial electrocorticography (ECoG) in treatment-resistant epilepsy patients to test whether changes in cortical beta are associated with successful completion of a verbal working memory task. The

data analyzed were previously reported in Cogan et al., 2017. Briefly, subdural ECoG recordings were obtained from five patients with epilepsy (n=5, mean age=30.0 yrs, 4 females) under approval from the NYU Grossman School of Medicine IRB. Each trial consisted of presentation of a rule ("match" or "mismatch"), a 1.5 s delay, one of two auditory cues ("kig" or "pob"), a variable delay (1.5 to 2 s), and a go cue ("speak"). In the match condition, a response was correct if the participant reported the cue, whereas in the mismatch condition, the correct response was to report the cue that was not presented. Spectral estimates of neural activity were obtained using the multitaper method (250 ms window, 10 Hz smoothing, 20 ms steps). We analyzed data from 1,332 trials, of which 66.2% were performed correctly and quickly (<1 s response time). In fast-correct trials, the normalized beta power between match and mismatch conditions significantly differed (two-sided permutation test, n=10,000, $p<0.0005$, mean diff>0.2) in the 500 ms surrounding rule presentation in 22 (10.6%) of 207 total electrodes. Beta power was significantly higher in the mismatch condition in 81.8% (18/22) of those electrodes, suggesting that higher levels of cortical beta are associated with higher-order cognitive processing. Next, we investigated whether poor task performance was correlated with lower levels of cortical beta power. In 8 (44.4%) of 18 electrodes which had higher beta power in fast-correct mismatch trials, beta power at rule presentation (+/- 250 ms) was significantly lower in mismatch trials with incorrect and slow-correct responses compared to fast-correct responses ($p<0.0005$). These 8 electrodes were distributed across 3/5 patients and were localized to left superior temporal gyrus, right sensorimotor cortex, and right lateral prefrontal cortex. In none of the 18 electrodes was a significant increase in beta power found at rule presentation in the mismatch condition for incorrect and slow-correct trials compared to fast-correct trials. Low levels of cortical beta at rule presentation are associated with worse performance, suggesting increasing beta power with electrical stimulation could enhance verbal working memory function.

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Poster

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Topic: H.08. Learning and Memory

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Title: Neurophysiology features of different meditations in Master and beginner of Meditator

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Abstract: Mental health disorder is a global health epidemic linked to more than 23 million deaths worldwide each year. Chronic mental stress is associated with cognitive impairments with pathological adaptations such as increased neuroinflammation in the hippocampal region of the brain. Research in recent years has shown increasing evidence of beneficial effects of meditation practice in alleviating mental stress and improving cognitive function in individuals with mental disorders. However, the neural mechanisms underlying different meditation methods, training experience, and the related health benefit are not well understood. The goal of the study was to investigate brain activities recorded from a master meditator with >40 years of daily practice and a beginner meditator with ~2 years of meditation experience during an active (transcendental meditation [TM]) and passive (listening to meditation music [MM]) practice session. Brain activities were recorded using a digital 64-channel scalp EEG device (ActiCap, Brain Products Inc., Germany), based on the international 10-20 system. EEG was acquired from the two individuals during the following conditions (tasks) in a sitting position and eyes closed: 1) a 5-min rest, 2) TM for 10 min, 3) MM for 10 min, and 4) a 5-min rest. *EEG Preprocessing*. Using the EEGLAB toolbox in MATLAB, EEG data were denoised with 1-50 Hz bandpass filter and a 60 Hz notch filter. *EEG Analysis*. At current stage, the following EEG parameters, delta, theta, alpha, and beta band power was extracted. *Results.* The EEG frequency power data derived from the master and beginner meditators showed that 1) an alpha-peak (8-13Hz) was observed during both TM and MM in both meditators, but the absolute alpha-band power was higher during TM compared to MM in both meditators; 2) EEG scalp topographies corresponding to two types of meditations (TM vs. MM) and two meditators showed that the parietal-occipital activity was much stronger during TM vs. MM in both meditators; and 3) in general, the power was higher for the beginner compared to the master meditator. *Conclusion & discussion.* Both TM and MM are associated with increased EEG alpha power with a greater increase for TM vs. MM, suggesting perhaps stronger brain activities during active compared to passive meditations. Weaker EEG power activities across delta, theta and alpha bands for the master meditator indicate perhaps that the mind of the master meditator is in deeper tranquil state compared to the beginner meditator. These data would be helpful in guiding design of meditation interventions for people with mental disorders and interpretation of clinical outcomes related to meditation therapy.

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Poster

077. Oscillations, Synchrony, and Memory

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Title: Novel alpha oscillatory events in rodents during complex discrimination

Authors: *V. ESTELA-PRO[1], S. G. TRETTEL[2], R. D. BURWELL[2];
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Abstract: Alpha rhythms (8–12 Hz) are the most predominant oscillations in the human brain, but their functional role remains controversial, with contradictory reports about their proposed function prominent. Historically, alpha was thought of as a correlate of a “ground state” or “cortical idling” that occurs during the absence of sensory input (Haegens et al., 2011; Pfurtscheller et al., 1996). According to this view, alpha oscillations are a passive mechanism that emerge when the brain is disengaged, whereas during sensorimotor or cognitive challenges they lose amplitude and are replaced by other neural mechanisms. More recently, however, alpha has been shown to be an active mechanism, becoming stronger when sensory inputs are actively ignored, leading to a proposed top-down inhibitory role for alpha rhythms in attention (Klimesch et al., 1998; Worden et al., 2000; Jenson & Mazaheri, 2010; Jones et al., 2010; Sacchet et al., 2015; Sadaghiani & Kleinschmidt, 2016; Kizuk & Mathewson, 2017). Currently, oscillation bands described for the cerebral cortex of rodents include a very broad theta band of 4-12 Hz and do not include a distinct range of frequencies for the alpha band. In humans, theta is generally defined as 4-8 Hz and alpha is defined as 8-12 Hz (Uhlhaas & Singer, 2010). Here, we describe a phenomenon in the rodent brain recorded in the hippocampus and postrhinal cortex, termed Alpha Oscillatory Events (AOEs), that exhibit characteristics similar to alpha oscillations in the human brain. These AOEs are generally 2-5 second episodes of increased power in the 9-12 Hz frequency band, peaking at ~10 Hz. They are markedly different from Type 1 theta in that they occur only when the rat is nearly motionless, whereas Type 1 theta power positively correlates with running speed. Behaviorally, AOEs occurred during three parts of a bi-conditional task trial. They are most robust immediately after stimulus onset and after object selection, but they can also occur between trials. AOEs appear to emerge as the animal moves from simple strategies in early trials to complex strategies in later trials. Together, these data suggest the need to update the defined oscillation bands in the rodent to include this novel oscillation that is analogous to attentional alpha in the human brain.

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Poster

077. Oscillations, Synchrony, and Memory

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Topic: H.08. Learning and Memory

Support: P50 MH109429

Title: Sharp Wave Ripple Dynamics in the Human Hippocampus around Event Boundaries during Movie Watching

Authors: G. TOSTAEVA[1], A. MISHRA[3], M. NENTWICH[4], M. LESZCZYNSKI[6], E. ESPINAL[2], L. C. PARRA[5], A. D. MEHTA[7], *S. BICKEL[8];
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Abstract: Naturalistic human experience is continuously segmented into discrete units with transitions referred to as event boundaries that organize memory into meaningful episodes. However, the neural mechanisms underlying such segmentation are not yet fully understood. Previous studies have indicated hippocampal activity as potentially involved in episodic memory. In particular, hippocampal sharp wave ripples (SWRs) - synchronous bursts of excitatory activity - have been implicated in memory function and consolidation and thus may contribute to event boundary formation. Here, we recorded intracranial EEG data from 6 patients undergoing presurgical evaluation for treatment of drug-refractory epilepsy while they passively watched narrative video clips. A separate dataset was obtained on the same movie clips to delineate event boundaries based on responses from 66 online (normally functioning) subjects instructed to mark transitions between movie scenes as boundaries. To investigate the SWR activity around detected event boundaries, we identified electrodes in the hippocampus, minimized common noise by subtracting the signal from a nearby white-matter contact, and used an established detection algorithm in the 70-180 Hz ripple band. Candidate SWRs were then manually distinguished from interictal epileptiform discharge events commonly observed in epilepsy patients. Across all patients, we found an increase in the peri-boundary ripple activity starting prior to the detected boundaries. These unique temporal dynamics of ripples around event boundaries are in line with the previously shown involvement of SWRs in memory consolidation, which has been proposed to occur at event boundaries. Our results build on previous studies interrogating the role of SWRs in memory encoding and highlight a potential new avenue for investigating how episodes are constructed in human memory.

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Poster

077. Oscillations, Synchrony, and Memory

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Title: Online learning of spike timing patterns by combining STDP and phasor-associative memories

Authors: M. GRAETZ[1], A. RENNER[1], P. E. FRADY[2], ***F. T. SOMMER**[3];
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Abstract: Although associative memory and temporal encoding have both been attributed to the mammal hippocampus, there are only few models of associative memory for storing and retrieving spike-timing patterns. A recent model, a spiking implementation of so-called threshold phasor associative memories (TPAM), fills this gap (Frady & Sommer, PNAS 2019). Phasor associative memories (PAM) are a generalization of Hopfield attractor networks to complex-valued synapses and neural states. While in PAM the fixed point attractors are complex-valued phasor vectors, in TPAM the stored complex patterns can additionally be sparse, i.e. with a large fraction of vector components being zero, which significantly increases the memory capacity over traditional PAM. In the spiking implementation of TPAM, each complex fixed point attractor is mapped onto a limit cycle of the spiking activity with an a priori defined fixed cycle time. The corresponding memory network consists of spiking neurons connected by synapses whose weights and delays are calculated from the patterns to be stored in an offline outer-product learning scheme. The network dynamics will settle into one of the stored cyclic spike-timing patterns.
Here, we investigate whether learning in such spike-timing associative memory networks can be implemented in an online biologically plausible manner. Specifically, we propose online learning in TPAM by employing a local spike timing-dependent plasticity (STDP) rule in a network of integrate-and-fire neurons. To enable proper shaping of synaptic delays, the network has multiple synaptic connections between the same pair of neurons with different fixed delay values. In the simplest form, each neuron pair in the model is connected by four excitatory synapses with relative delays of 1, 2, 3 and 4 quarters of the cycle period (and a random absolute delay). Via a damped cosine-shaped STDP kernel, external spike patterns potentiate and depress these synapses, which approximates the delays resulting from outer-product learning of individual patterns.
The novel online TPAM network achieves qualitatively similar results to the offline model, however, the memory capacity is about 40% lower because the approximation of the original learning scheme by the proposed online-learning mechanism is imperfect. The resulting network is able to perform pattern denoising and pattern completion of spike timing patterns, thus providing a novel computational model for hippocampal memory networks, potentially contributing to theories of hippocampal function and learning. In addition, our model has technical applications for online learning and error correction in neuromorphic computing.

Disclosures: M. Graetz: None. **A. Renner:** None. **P.E. Frady:** None. **F.T. Sommer:** None.

Poster

077. Oscillations, Synchrony, and Memory

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 077.08

Topic: H.08. Learning and Memory

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Title: Functionally distinct hippocampal rhythms and circuits predict valence of the subsequent locomotion

Authors: *S. MIKULOVIC, P. MOCELLIN, P. BAUER, S. REMY;
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Abstract: Theta oscillations (4-12 Hz) is one of the most extensively investigated rhythms of the brain, closely linked to locomotion, diverse types of learning and memory, and controlled by the medial septum circuitry. Previous studies (Whishaw et al, 1973; Bland et al, 2006) have reported that theta frequency predicts different heights in a jump avoidance test. The widely accepted view, in accordance to the "sensorimotor integration model" is that theta frequency predicts the subsequent movement. However, the jump avoidance tests involve a strong fear component, leading to an elusive conclusion whether theta activity predicts solely movement or fear of the subsequent jump. To address this conundrum, we designed an experiment in which we performed 2 Photon imaging in combination with oscillations recordings and pharmacological medial septum inhibition, in mice running on a treadmill. To investigate the predictive coding of a motor- and fear-related stimuli, we first introduced a brake, and subsequently an air puff stimulus at specific locations. Analysis of oscillatory activity revealed that theta frequency was largely unaffected by the learned position of the brake, while a strong relationship between the increased frequency and the location of the air puff stimulus was observed. Calcium imaging data revealed that brake- and puff- related cells form largely non-overlapping populations. Finally, we discuss the effect of the medial septum inhibition on both types of coding. These results indicate that distinct hippocampal rhythms and circuits predict valence of the subsequent locomotion rather than movement per se.

Disclosures: S. Mikulovic: None. **P. Mocellin:** None. **P. Bauer:** None. **S. Remy:** None.

Poster

077. Oscillations, Synchrony, and Memory

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Program #/Poster #: 077.09

Topic: H.08. Learning and Memory

Title: Neural signatures of odor cueing of declarative memories during sleep

Authors: *A. SANCHEZ CORZO[1], D. M. BAUM[2], J. G. KLINZING[3], R. SITARAM[1];
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Abstract: Neuronal assemblies activated during the encoding of declarative memories are reactivated during Slow Wave Sleep (SWS) (Ji & Wilson, 2007). This reactivation relies on specific neural oscillations such as Slow Oscillations (SO), Sleep Spindles (SS), and their temporal coordination (phase coupling (Klinzing et al., 2019)). Reactivations occur spontaneously during sleep but can also be induced by sensory cues (e.g., odors or sounds). This technique, sometimes called Targeted Memory Reactivation (TMR) has been shown to improve memories that are associated with the cues (Rasch et al., 2007). Here, we analyze EEG recordings during odor TMR in detail. Our results may shed light on the underlying neural mechanisms of odor TMR.
We recorded high-density EEG data from 23 participants on two experimental nights. Participants performed a motor task without a learning component in the presence of an odor (Odor M) and a declarative memory task in the presence of another odor (Odor D). Later, while participants were in SWS, we performed odor stimulation either with Odor D or Odor M.
We found that the two odors elicited different neural activation patterns, depending on their association with either task. During the first half of the stimulation period, the spindle rate was increased over centro-parietal areas for Odor D compared to Odor M. Spindle duration in turn was increased over frontal areas. SO's downstate amplitude was larger over parietal electrodes. In terms of temporal coupling of SOs and spindles, coupling strength decreased over central areas for the entire stimulation period We report further exploratory results that may be specific for uninterrupted trains of odor stimulation.
Further analyses need to be done in order to identify reliable electrophysiological patterns that correlate with behavior.
Ji, D., and Wilson, M. A. (2007). Coordinated memory replay in the visual cortex and hippocampus during sleep. In Nature Neuroscience (Vol. 10, Issue 1, pp. 100-107). https://doi.org/10.1038/nn1825
Klinzing, J. G., Niethard, N., and Born, J. (2019). Mechanisms of systems memory consolidation during sleep. Nature Neuroscience, 22(10), 1598-1610.
Rasch, B., Büchel, C., Gais, S., and Born, J. (2007). Odor cues during slow-wave sleep prompt declarative memory consolidation. Science, 315(5817), 1426-1429.

Disclosures: A. Sanchez Corzo: None. **D.M. Baum:** None. **J.G. Klinzing:** A. Employment/Salary (full or part-time):; Bit&Brain Technologies SL (Bitbrain), 50008 Zaragoza, Spain. **R. Sitaram:** None.

Poster

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Program #/Poster #: 077.10

Topic: H.08. Learning and Memory

Support: NIH/NIEHS Intramural Research Program

Title: Behavioral, learning, and theta ratio dynamics during trace fear conditioning

Authors: E. THOMSON[1], K. STEVANOVIC[2], Z. GU[3], J. YAKEL[3], J. CUSHMAN[2]; [1]Integrative Bioinformatics, [2]Neurobehavioral Core, [3]Ion Channel Physiol. Group, Natl. Inst. of Envrn. Hlth. Sci., Durham, NC

Abstract: Theta oscillations (4-12Hz) in the hippocampal local field potential (LFP) have been extensively studied for their role in the establishment and maintenance of place cells during locomotion. Theta has been much less studied in fear learning where the primary behavioral output is freezing, characterized by a distinct lack of locomotion. Hence, we trained mice in a hippocampus-dependent trace fear conditioning task while recording LFPs from dorsal CA1 of the hippocampus. During acquisition training, a 20s tone was followed by 20s trace period, followed by a 2s foot shock (repeated five times). The animals' freezing response increased significantly over the course of training. CA1 displayed significant theta oscillations during trace conditioning. However, the peak theta frequencies were not constant, but clearly fluctuated over time: sometimes showing strong low-frequency modes (especially during freezing), and other times distinct higher-frequency modes (especially during movement). We processed the LFP spectrograms to extract the peak frequency in the theta range over time, and used kernel density estimators to generate robust estimates of the cutoff point between the high and low theta frequency modes during the task. We then used this estimate to calculate a theta *ratio* for each animal. This theta ratio, which varies between -1.0 and 1.0, is a measure of the relative power in high and low theta, and provides a moment-by-moment readout of whether high theta or low theta (or neither) is dominating at a given time. We examined the correlation between theta ratio and multiple factors during trace conditioning, including animal motion, acceleration, and freezing. Also, we calculated the mean theta ratio triggered on multiple events during the task, finding clear theta-ratio fluctuations dependent on behavioral parameters on fine temporal scales. Overall, the extraction of the theta ratio parameters for each animal using kernel density estimators, provides a simple, sensitive, and powerful way to extract fine-grained information about the substructure of theta oscillations in real time.

Disclosures: E. Thomson: None. **K. Stevanovic:** None. **Z. Gu:** None. **J. Yakel:** None. **J. Cushman:** None.

Poster

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Program #/Poster #: 077.11

Topic: H.08. Learning and Memory

Support: NIMH R01NS115233

Title: Disruption of Sharp-Wave Ripples Impairs Object-Place Recognition

Authors: *S. DUTTA[1], K. G. VOKT[1], A. HO[2], J. ZHANG[1], C. T. KEMERE[1,3];
[1]Electrical and Computer Engin., [2]Neurosci., Rice Univ., Houston, TX; [3]Neurosci., Baylor Col. Of Med., Houston, TX

Abstract: Rodents ethologically have an innate curiosity to explore novelty whether in the form of contexts or objects resulting in them spending more time with novel or displaced objects or even novel locations as opposed to familiar ones. Extensive lesion studies have demonstrated this novelty preference to be hippocampally dependent via a variety of object recognition memory (ORM) paradigms. More recent work has correlated hippocampal CA1 LFP signatures, such as fast-gamma and beta-band oscillations, to be of importance in object-place recognition memories and has demonstrated predictable changes in other transient hippocampal events, sharp-wave ripples (SWRs). Specifically, SWRs have been shown to increase after encoding of novelty in the testing paradigm; however, the concomitant spiking activity has not been correlated to object-place pairings prompting the question of the role of these events in this curiosity driven task. To illuminate the role of SWRs in potentially driving the curiosity towards novelty, we selectively modulate SWR activity using our previously engineered open-source, closed-loop SWR detection system during a ORM displaced object paradigm. Our results indicate that suppression of SWR activity during object encoding and post-encoding sleep sessions significantly (p-value 0.00573) impairs object-place recognition memory of familiar objects in novel locations. Analysis of recorded CA1 LFP shows changes in SWR rates when comparing disruption to control groups while overall exploration metrics remain similar across groups. We go on to demonstrate correlations between SWR durations changes between pre-encoding and post-encoding sleep sessions and discrimination measures which suggest ripple duration distributions skewed towards longer durations lead to higher novelty preference scores. Similarly, preliminary exploration of pre-encoding sleep sessions and post-test sleep sessions comparing control stimulation and SWR disruption experimental groups show variations in different SWR measures (inter-event interval, center frequency, duration distributions). Additionally, LFP activity during behavior in the test session comparing disruption to control groups shows changes in the spectral space within the theta and gamma bands. Altogether, the preliminary findings suggest SWRs (specifically, longer duration ripples) play a deeper role in curiosity driven object-place recognition memory. We look to further understand the role of SWRs along with its co-occurring neural activity and potential causal changes that SWRs transiently cause within the hippocampal circuit that may lead to such a deficit in recognition memory.

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Poster

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Program #/Poster #: 077.12

Topic: H.08. Learning and Memory

Support: R01GM134363

Title: Asymmetries in the human inspiration/exhalation cycle relate to the nonsinusoidal waveforms of medial temporal lobe oscillations

Authors: *E. KOSIK[1], B. VOYTEK[2];
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Abstract: The breath cycle is a periodic rhythm consisting of asymmetric inhalation and exhalation phases. There is emerging evidence that these peripheral respiratory rhythms entrain neural oscillations in the central nervous system. Given that oscillations are believed to play key roles in cognition and disease, this suggests that respiration might be causally influencing these processes via its influence on neural oscillations.
To examine this, we begin with the fact that both neural oscillations and respiration have a nonsinusoidal waveform shape. These nonsinusoidal features of neural oscillations have been hypothesized to represent the underlying neural physiology, and they have been shown to relate to disease states and cognitive performance (Cole, 2017). Here, we test the hypothesis that the nonsinusoidal waveform shape of respiration may be driving the nonsinusoidal waveform shapes of neural oscillations. Although it has been established that respiration-entrained neural rhythms exist in humans, we are investigating if the nonsinusoidalities of respiration on a breath-by-breath basis are related to the nonsinusoidalities of subcortical rhythms on a cycle-by-cycle basis. To test this hypothesis, we extracted the non-sinusoidal waveform shape features from resting human intracranial EEG and simultaneous respiration recordings using the Python bycycle toolbox (Cole, 2019). We found significant correlations between nonsinusoidal respiration features and neural oscillations. Specifically, the asymmetric inspiration/expiration cycle is related to neural oscillation asymmetry and frequency. The quantification of this relationship provides a more detailed understanding of breathing's influence on the brain.

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

Poster

077. Oscillations, Synchrony, and Memory

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Topic: H.08. Learning and Memory

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Title: A classification-based generative approach to selective targeting of global slow oscillations during sleep

Authors: S.-C. SEOK[1], S. C. MEDNICK[2], ***P. MALERBA**[3];
[1]The Res. Inst. at Nationwide Children's Hosp., Columbus, OH; [2]Dept. of Cognitive Sci., Univ. of California, Irvine, Irvine, CA; [3]The Ohio State Univ. Sch. of Med., Columbus, OH

Abstract: Interacting with sleep slow oscillations (SOs, 0.5-1.5 Hz) is essential to understanding the mechanisms underlying their role in sleep functions, including homeostasis, synaptic reorganization, and memory consolidation. Techniques that deliver electrical waveforms non-invasively at times close to the SO trough have been shown to affect memory outcomes, and have great translational potential in populations that show differentiation in their sleep dynamics (e.g., hypersomnia, ADHD, autism spectrum disorder). However, current approaches to SO-based non-invasive closed-loop alternate current stimulation (cl-tACs) focus on timing, and do not differentiate among oscillations based on their space-time profiles.
The relevance of targeting SO space-time profiles selectively is evinced by their differentiation as Global, Local, and Frontal on the scalp. In particular, Global SOs, which appear simultaneously on large portions of the scalp, have different biophysical properties, stronger coordination with sleep spindles (10-16Hz), and facilitate communication across long-range cortical distances. These Global SO properties predicted sleep-dependent improvement in a memory task. Combined, these findings underscore a potential functional relevance of Global SOs.
In this study, we developed a machine-learning driven approach to shape a cl-tACs intervention enabling causal manipulation of Global SOs as detected on the EEG. Using the sleep night of 22 volunteers acquired in the lab with high-density EEG (175 electrodes), we classified the SOs as Global/Local/Frontal. First, we used available software (Brainstorm) to estimate the current density of a Global SO. We then trained multiple machine learning algorithms to distinguish between Global SOs and other SO types, and probe variance of Global/non-Global SO profiles within and across subjects. Ensemble subspace methods reached highest accuracy (98.5%). Feature selection identified cortico-thalamo-hippocampal currents at the trough of the SO as the most relevant for Global SO differentiation in stage 2 sleep, and cortico-thalamic-striatal networks for slow wave sleep. This analysis enabled the construction of a ‘standard Global SO’ profile. Lastly, we associated a parametric representation of a generic cl-tACs montage and waveform to its current-density profile in the brain with existing software (ROAST), and applied a genetic algorithm to this output, using the standard Global SO profile as goal function.
This data-driven method builds on natural sleep data to shape a stimulation protocol that imitates Global SO dynamics, and is generalizable to other SO types.

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Poster

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Title: Single and complex spikes relay distinct frequency-dependent circuit information in the hippocampus

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[2]Boston Univ., [1]Boston Univ., Boston, MA; [3]Boston Univ. Ctr. For Memory and Brain, Boston Univ. CILSE, Boston, MA; [4]Univ. of Illinois at Urbana-Champaign, Univ. of Illinois, Urbana, IL

Abstract: Hippocampal CA1 neurons produce single spikes (SS) and bursts of spikes, known as complex spikes (CS). While these distinct spiking modes are well studied in the cerebellum, it is largely unclear how hippocampal SS and CS participate in network information processing during behavior. To probe the functional role of SS and CS, we performed membrane voltage imaging of individual hippocampal CA1 neurons expressing the genetically encoded voltage indicator SomArchon, in conjunction with simultaneous local field recordings in CA1 in mice during voluntary locomotion. We found that within single neurons, CS and SS occur dynamically, with CS preferentially phase locked to subthreshold membrane voltage theta oscillations (3-12Hz) while SS phase locked to subthreshold gamma oscillations (30-100Hz). Theta-frequency rhythmic optogenetic CoChR stimulation selectively resulted in entrainment of CS, whereas gamma-frequency stimulation resulted in the entrainment of SS, demonstrating that CS and SS are distinct neuronal output modes dependent on specific cellular membrane voltage rhythmicity. Parallel to our voltage imaging results, CS and SS recorded with tetrodes were more strongly phase locked to LFP theta and gamma oscillations respectively in rats during a spatial navigation task, and CS and SS encode different spatial information. Finally, a biophysical CA1 bursting model reproduced the distinct relationship of CS and SS to theta and gamma frequency inputs. Together, our findings demonstrate that individual CA1 neurons dynamically switch between CS and SS spiking modes, and these distinct firing patterns are regulated by membrane voltage theta and gamma rhythmicity at the single neuron level. Since different input pathways to CA1 have been linked to theta and gamma oscillations, our results further demonstrate that individual neurons can multiplex circuit specific dynamics to relay distinct frequency-dependent information in the hippocampus during learning and memory.

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Poster

077. Oscillations, Synchrony, and Memory

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KAKENHI(19H01131, 21H05296, 19K12768)

Title: Elucidation of the effects of theta rhythm on the performance of hippocampus-dependent and independent tasks

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Abstract: It is well known that the theta rhythms in the local field potential is involved in spatial navigation. Recent studies have reported that optogenetic manipulation of medial septum (MS) paces or abolishes hippocampal theta rhythms and impairs spatial working memory, resulting in lower scores on maze tests in rodents. However, a question of whether the hippocampal theta rhythms only link to the hippocampal-dependent task remains unanswered. Here, we trained mice to perform the hippocampus-dependent spatial alternation (SA) task and the hippocampus-independent visual discrimination (VD) task alternately in a maze which combines two T-shaped mazes using the reconfigurable maze, in which the parts including paths, movable walls, treadmills, and dispensers can freely combine to form any existing mazes. During the task performance, the mice made a decision under the SA rule at a junction of the maze and the VD rule at another junction to get a reward. After training over the course of a week, the rate of correct answers in well-trained mice was significantly higher than chance level in both the SA task and VD task (binomial test, $p<0.05$). To investigate whether the hippocampal theta rhythm affects choice accuracy on not only the hippocampus-dependent SA task but also the hippocampus-independent VD task, we injected AAV-DIO-ChrimsonR to the MS of parvalbumin (PV)-Cre mice and implanted an optical fiber to the MS. We report the task performance during 8 Hz pulse and white noise stimulations and discuss whether the theta rhythm is involved in more than just hippocampal-dependent spatial working memory by comparing choice accuracy at both junction points of the maze in response with the optogenetic pacing.

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Poster

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Title: Behavioral variability in pattern completion and separation is associated with infraslow phase and event related potential amplitudes

Authors: *L. GRANGER[1], K. CANTRELL[1], E. BERGER-WOLF[1], M. B. DASH[2];
[1]Neurosci., [2]Psychology, Middlebury Col., Middlebury, VT

Abstract: Memory is an integrative process involving pattern separation and completion. Pattern separation is the ability to distinguish between memories while pattern completion involves generalizing memories from partial cues. Under different circumstances, memory retrieval may benefit from increased specificity or increased generalization. Given that the spontaneous infraslow (<0.1 Hz) oscillation (ISO) has previously been shown to modulate a range of behaviors and patterns of neuronal communication, we investigated whether this spontaneous brain activity could likewise affect memory. To do so, we noninvasively recorded neuronal activity via a 32-channel electroencephalograph (EEG). Over the course of an hour, the participants completed three trials of a memory task in which they 1) distinguished learned photos apart from novel photos, and 2) performed similar discriminations when the same photos were presented at various completion levels (100%, 35%, 21%, 5%). The 35 participants between the ages 18 and 22 included 24 females, 10 males, and one non-binary individual. Our preliminary behavioral results showed an inverse relationship between errors and masking level, indicating that pattern completion varied as a function of the amount of cues present during recall. While our participants showed proficiency on the pattern separation and completion tasks (92.45% +/- 0.93 accuracy across 240 trials for each participant), they nevertheless made errors. These errors included both false alarms when incorrectly identifying new photos (a measure of poor pattern separation) and errors in identifying novel photos as previously seen (a measure of incorrect pattern completion). Strikingly, the timing of these errors was significantly modulated by the phase of the ISO. Moreover, when either error was made, we observed a smaller event-related potential (ERP) amplitude (P300 within occipital electrodes) as compared to either correct response. Collectively, these results indicate that ongoing ISO activity may alter responses within the brain and therefore bias memory. Thus, spontaneous brain activity may produce periods of time when generalizability is favored and other times when specificity is favored. This flexibility may provide memory systems with the ability to best apply previous knowledge to current circumstances.

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Poster

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Topic: H.08. Learning and Memory

Support: Intel Labs

Title: Cross-frequency coupling increases memory capacity in oscillatory neural networks

Authors: *C. BYBEE[1], A. BELSTEN[2], F. T. SOMMER[3];
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Abstract: A debated problem in neuroscience is the functional role of neural oscillations, for example, in perception, attention, or memory. Cross-frequency coupling (CFC), i.e., the phase or amplitude correlation between neural oscillations of different frequencies, is associated with information integration across populations of neurons (Canolty et al., 2010). Impaired CFC has been linked to neurological disease (Zhang et al 2016) but is still unclear what role CFC has in information processing. We construct a model of CFC which predicts a computational role for observed theta-gamma couplings in the hippocampus and cortex (Nandi et al., 2019). Our model predicts that the complex dynamics of coupled oscillators in recurrent and feedforward networks perform robust information storage and pattern retrieval. Complex phasor associative memories (PAM) (Noest, 1987) are interesting models for neuroscience because they can be mapped to networks of spiking neurons (Frady, 2019) that essentially form oscillator neural networks (ONN) (Hoppensteadt et al., 1998). Here we present a novel ONN model that includes harmonic phase locking (Nishikawa et al., 2004). We show that the presence of CFC increases the memory capacity of a population of neurons connected by plastic synapses. CFC enables error-free pattern retrieval whereas pattern retrieval fails without CFC. Interestingly, our model reproduces some of the experimental observations of CFC. In particular, for frequency ratios that correspond to the ratio of theta and gamma frequencies observed in the hippocampus and cortex, the memory capacity exceeds the capacity of previous models. Further, our model makes specific predictions. First, it defines specific functional roles of phase-phase vs. phase-amplitude coupling in ONNs. Second, the model enables us to explore the quantitative trade-offs between sparse connectivity, capacity, and information per connection.

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Poster

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Support: R21 – MH117687

Title: Harnessing prefrontal-hippocampal theta synchrony to enhance memory

Authors: *J. STOUT[1], A. GEORGE[2], A. L. GRIFFIN[3];
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Abstract: Local field potentials (LFPs) reflect transmembrane fluctuations amongst neuronal ensembles. When LFPs between two brain regions synchronize, it may open a window for the temporal coordination of spike timing. It has therefore been hypothesized that brain regions communicate when their neural oscillations are coherent (Fries 2005). For decades, it has been demonstrated that prefrontal-hippocampal theta (4-12Hz) phase coherence is positively correlated with spatial working memory task performance (Jones and Wilson, 2005; Benchenane et al., 2010; Hallock et al., 2016). However, whether this form of long-range neural synchrony is causal to successful decision making remains unknown. Addressing this question is central to our understanding of the mechanisms by which neural communication occurs. To test the communication through coherence hypothesis, we developed a novel brain-machine interface (BMI) capable of detecting states of high and low magnitude theta coherence and presenting coherence-contingent choices. On an automated T-maze, our BMI was active during the delay period in between spatial choices on a delayed alternation task. Thresholds for high and low magnitude theta coherence were unique to each rat, defined according to a distribution of prefrontal-hippocampal coherence estimates. Within a given session, rats experienced coherence-contingent choices, but also various control trials to account for delay duration confounds. By presenting spatial choices during states of high magnitude theta coherence, we enhanced spatial memory (high theta coherence trials vs delay matched control trials; N = 8 rats; t(7) = 3.38, ci = [5.5 to 31], $p_{adj(x2)}$ = 0.023). Compared to low coherence states, high coherence states were associated with increased hippocampal-to-prefrontal theta directionality (N = 8 rats; t(7) = 3.2, ci = [0.1 to 0.67], $p_{adj(x3)}$ = 0.046; Granger prediction) and increased prefrontal phase-locking to the local theta oscillation (N = sessions; t(20) = 3.5, $p_{adj(x3)}$ = 0.008; spike-field coherence). In summary, our novel BMI allowed us to test the communication through coherence hypothesis in rats. We report for the first time that high magnitude prefrontal-hippocampal theta coherence is sufficient to enhance memory. Mechanistically, these data indicate that theta phase synchrony opens a window for the temporal coordination of neural activity.

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Poster

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Program #/Poster #: 077.19

Topic: H.08. Learning and Memory

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Title: Optogenetic Theta Stimulation of the Medial Septum Facilitates Spatial Working Memory

Authors: *Z. M. GEMZIK[1], A. L. GRIFFIN[2];
[1]Psychological and Brain Sci., Univ. of Delaware, Newark, DE; [2]Psychological and Brain Sci., Univ. Delaware, Newark, DE

Abstract: Spatial working memory (SWM) is the ability to process and maintain spatially-relevant, goal-directed information over a temporal gap, and relies on an intact hippocampus (HPC). The medial septum (MS) is necessary for the generation of theta, (4-12 Hz) oscillations in the HPC and SWM (Mizumori et al., 1990). A recent study from our lab showed that optogenetic suppression of MS delivered during the delay period of a delayed non-matched to position task disrupted choice accuracy, suggesting that MS activity is necessary for the maintenance of spatial information during SWM tasks (Gemzik et al., 2020). Based on these previous findings, we hypothesized that MS-generated theta facilitates the maintenance of spatial information to enable optimal SWM task performance. To test this hypothesis, we examined the effects of optogenetic MS theta stimulation delivered during the delay period of a SWM-dependent task. We first trained rats (N=9) to perform a delayed alternation task with a 10s delay. After rats reached asymptotic performance, we injected an excitatory optogenetic viral vector encoding a blue light-activated cation channel, channelrhopsin-2 (AAV5-hSyn-hChR2-EYFP) into the MS and implanted an optogenetic fiber above the injection site. We also implanted two of these rats with LFP electrodes aimed at the MS and HPC to verify the effectiveness of our stimulation procedures in inducing theta oscillations. We challenged SWM demand by adding a long (30s) delay period in addition to the short (10s) delay. We predicted that MS theta stimulation would facilitate choice accuracy especially for the longer delay. Each testing session consisted of 10 trials each of 4 different conditions that were pseudo-randomly interleaved within the session: red (638nm/control) or blue (470nm/excitatory) laser stimulation at theta frequency (6Hz) delivered during a short (10s) or long (30s) delay period. As predicted, for the control stimulation, choice accuracy was significantly lower on the 30s delay trials vs. the 10s delay trials, verifying that longer delay periods pose a significant challenge to SWM. This delay-dependent decrease in choice accuracy was eliminated by delivering MS theta stimulation during the delay period (rmANOVA: laser color x delay length, $F(1,8) = 12.84$, $p = 0.007$; post-hoc Tukey test, red/control 10 vs. 30s, $p = 0.005$; 10s red/control vs. blue/excitatory, $p = 0.919$; 30s red/control vs. blue/excitatory, $p = 0.004$). These findings support the hypothesis that MS theta stimulation facilitates SWM and suggest that MS may equip HPC neurons with a temporal framework upon which to process and organize task-relevant information on a theta frequency timescale.

Disclosures: Z.M. Gemzik: None. **A.L. Griffin:** None.

Poster

077. Oscillations, Synchrony, and Memory

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 077.20

Topic: H.08. Learning and Memory

Support: OEAW - DOC fellowship
FWF W1277

Title: Sleep's effects on beta oscillations predict the adaptation of motor behavior.

Authors: *M. AMEEN, K. HOEDLMOSER;
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Abstract: The remarkable plasticity of the sensorimotor cortex enables the brain to update its knowledge and adjust movements to meet the demands of the surrounding environment, a process known as motor adaptation. In this study, we aimed at investigating the role of sleep in the adaptation of an automatic fine-motor skill. We recruited experts on touch-typing on the regular keyboard (N=33, all males) and trained them to type on a mirrored keyboard, then tested their performance after a retention interval of either overnight sleep with polysomnography (N=16), or daytime wakefulness (N=17). During training and testing, participants had to type 5-letter; German words on regular and mirrored keyboards, respectively, while we measured their brain activity via electroencephalography (EEG). We show that sleep benefits adaptive performance. Typing performance, i.e. a measure that accounts for both typing accuracy and speed, on the mirrored keyboard improved significantly after sleep but not after wakefulness. Adaptive performance-gain after sleep correlated positively with the change in the slope of the aperiodic brain activity (3-55Hz) measured over all leads. The improved adaptive performance after sleep was accompanied by a significant decrease in the power of beta oscillations (13-30Hz). However, the decrease in beta band activity after sleep occurred for both the regular and the mirrored keyboards suggesting a general increase in adaptive drive. We then trained a classifier to differentiate between regular and mirrored typing and we show that the classifier's performance dropped significantly after sleep but not wakefulness, corroborating the role of sleep in consolidating the adaptation task and suggesting a role for sleep in inducing interference between the two tasks. Therefore, we checked whether the typing performance on the regular keyboard declined for the words that were trained on the mirrored keyboard (List 1) as compared to words that were typed only on the regular keyboard (List 3) before sleep. Indeed, we found that only after a period of sleep but not wakefulness, the typing performance on List 1 decreased significantly, while that on List 3 showed a significant increase. Our results suggest that sleep promotes the consolidation of all forms of motor behavior indiscriminately, possibly during different phases of sleep. We speculate that bursts of beta oscillations during tonic episodes of rapid eye movement (REM) sleep might reflect the consolidation of the motor aspects of adaptive behavior.

Disclosures: M. Ameen: None. **K. Hoedlmoser:** None.

Poster

077. Oscillations, Synchrony, and Memory

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Program #/Poster #: 077.21

Topic: H.08. Learning and Memory

Support: NIH Grant R21NS095094-01A1

Title: Drifting Spectral Similarity Represents a Shifting Temporal Context in Primacy Items

Authors: *N. KULKARNI, B. C. LEGA;
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Abstract: The tendency to remember the beginning of an ordered set is a highly conserved effect across memory tasks. Theories of episodic memory suggest items early in a sequence can serve as the temporal context for subsequent memories. With each subsequent item, the reinstatement of context information serves to cue recall of adjacent items. Alternatively, primacy encoding may result from direct item-item associations with boundary information that does not rely on the representation of temporal context. In this experiment, we studied the electrophysiological correlates of context recovery for primacy versus non primacy items to examine how these findings may support updated conceptions of temporal context models. We studied 140 patients with intracranial EEG electrodes implanted for drug-resistant epilepsy within the left anterior temporal lobe and medial temporal lobe regions (hippocampus, entorhinal cortex, and parahippocampal). The subjects performed a delayed free recall task over multiple sessions consisting of 12-word lists. Importantly, only lists where patients recalled 3 or more items were analyzed to differentiate between primacy and other memory effects. For data analysis, a morlet wavelet transform was used to obtain spectral power (3-100hz) of each item for 1000ms after encoding. Each item's time-frequency representation was decomposed into a unidimensional vector which was compared with the adjacent items using Spearman's rank correlation coefficient. For primacy, middle, and late items, adjacent item similarity was grouped by lagged positional distance (-1, +1, +2, etc.). We found successfully encoded items immediately adjacent to primacy items to have the highest spectral similarity between any list two items. Primacy items also demonstrated a graded decline in similarity with increasing list distance. When recalled primacy items were compared to unrecalled adjacent items, spectral similarity was significantly lower and mirrored that of events adjacent to middle and late list items. Our findings suggest the spectral characteristics of primacy encoding are distinct from non—primacy items across regions in the temporal lobe. During encoding, the rate of drift for temporal context of primacy items differs significantly from that of later list items. These results mirror the differences in temporal information provided by populations of time-sensitive cells. Specific drift rates for primacy versus non primacy items may inform novel conceptions of temporal context models.

Disclosures: N. Kulkarni: None. **B.C. Lega:** None.

Poster

078. Memory, Genes, and Molecules

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Program #/Poster #: 078.01

Topic: H.08. Learning and Memory

Support: NHMRC Grant GTN2011334

Title: Psilocybin facilitates learning in female rats across multiple operant testing conditions

Authors: *K. A. CONN[1], L. K. MILTON[2], B. J. OLDFIELD[2], C. J. FOLDI[2];
[2]Physiol., [1]Monash Univ., Melbourne, Australia

Abstract: The therapeutic potential of psilocybin for the treatment of neuropsychiatric disorders such as anorexia nervosa (AN) is currently being assessed in clinical trials, and is proposed to act by alleviating the cognitive inflexibility that characterises AN. Our previously unpublished preliminary data examined cognitive flexibility with a reversal learning paradigm using rodent home cage operant testing devices. Rats (N=31) were trained to nose-poke into one of two operant ports (i.e. the active port), using fixed ratio schedules of reinforcement, in order to obtain a sucrose reward. A single, clinically relevant dose, of psilocybin (1.5mg/kg) or saline was administered 24h prior to reversal of the reward-paired port (i.e. the previously inactive port became active). Psilocybin improved reversal learning ($F_{(1,29)}$ =5.128, p=.031), suggesting a specific improvement in flexible behaviour. However, the precise behavioural mechanisms through which psilocybin may improve this remain unknown. Here, we have further examined the effects of psilocybin on specific aspects of learning to understand the bases of improved cognitive flexibility. Female Sprague-Dawley rats (8-10 weeks old; N=45) were trained in operant tasks assessing instrumental learning as well as extinction and reinstatement. In order to deconstruct this improvement in flexibility, a cohort of rats (n=23) were assessed on their ability to learn to respond into the active port (i.e., instrumental learning), 24h after psilocybin administration, using variable ratio reinforcement schedules. Psilocybin increased response vigour in sessions where reinforcement was highly variable and unpredictable but did not alter the ability to extinguish responding during the extinction phase (conducted 8 days post-administration). Response behaviour trended towards a shift to being exploratory in nature. Extinction and reinstatement of responding for sucrose rewards were also examined more acutely, i.e., 24h after treatment, in a separate cohort of rats (n=22), with no direct effects of psilocybin on extinction ($F_{(1,20)}$ =0.097, p=.758) or reinstatement ($F_{(1,20)}$ =0.081, p=.779) seen. Taken together, these findings provide initial support for the therapeutic potential of psilocybin for treating cognitive inflexibility in AN, via mechanisms that may include increased response vigour and exploratory behaviour, that result in an improved ability to adapt behaviour in the face of changing outcomes.

Disclosures: K.A. Conn: None. **L.K. Milton:** None. **B.J. Oldfield:** None. **C.J. Foldi:** None.

Poster

078. Memory, Genes, and Molecules

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Program #/Poster #: 078.02

Topic: H.08. Learning and Memory

Support: UW System Regent Scholar Award
2R15GM118304-02
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Title: Effects of a novel estrogen receptor beta agonist and APOE genotype on memory, vasomotor, and anxiety outcomes in an Alzheimer's mouse model

Authors: *A. FLEISCHER[1], M. SCHWABE[1], R. KUEHN[1], S. CHAUDBURY[2], W. A. DONALDSON[2], D. S. SEM[3], J. M. YORK[4], M. LADU[4], K. M. FRICK[1];
[1]Psychology, Univ. of Wisconsin - Milwaukee, Milwaukee, WI; [2]Chem., Marquette Univ., Milwaukee, WI; [3]Pharmaceut. Sciences, Ctr. for Structure-Based Drug Design and Develop., Concordia Univ. Wisconsin, Mequon, WI; [4]Anat. and Cell Biol., Univ. of Illinois Chicago, Chicago, IL

Abstract: Rationale: Alzheimer's disease (AD) prevalence and severity are associated with increased age, female sex, and apolipoprotein E (*APOE*) genotype. Menopausal women who carry two *Apoe4* alleles are at greatest risk of developing AD. Although estrogen-based treatments effectively reduce symptoms of menopause and seem to reduce AD risk, there is a pressing need for treatments that are more selective for estrogen receptor β (ERβ) than ERα, as ERα activation is linked with increased breast and uterine cancer. Objective: Here, we aimed to test the memory-, hot flash-, and anxiety-related effects of long-term oral treatment with a novel, highly selective ERβ agonist, EGX358, in a female mouse model of AD. Methods: Transgenic female mice expressing 5 familial AD mutations (5xFAD-Tg) and homozygous for human Apoe3 (E3FAD) or heterozygous for Apoe3 and Apoe4 (E3/4FAD) were ovariectomized at 5 months of age. Following recovery, mice were treated orally with either vehicle (DMSO) or EGX358 (10 mg/kg/day) via hydrogel for 8 weeks. Mice were weighed weekly and tested for spatial and object memory in the object placement (OP) and object recognition (OR) tasks, respectively. In addition, anxiety-like behaviors were tested in the open field (OF) and elevated plus maze (EPM), as were hot flash-like symptoms (change in tail skin temperature) following injection of the tachykinin receptor 3 agonist, senktide (0.5 mg/kg). Results: Treatment with EGX358 enhanced object recognition memory in both E3FAD and E3/4FAD mice, but did not affect spatial memory. EGX358 treatment also caused a modest, but not significant, reduction in tail temperature changes following senktide administration. EGX358 treatment did not affect anxiety-like behaviors or weight gain. However, genotype effects were observed, such that compared to the E3FAD mice, E3/4FADs spent more time in the center of the OF, had greater sensitivity to the tail temperature effects of senktide administration, and gained more weight following ovariectomy. Conclusions: Our data suggest that the highly selective ERβ agonist, EGX358, can facilitate object recognition memory in a mouse model of AD, similar to results seen in wild-type mice. However, genotype appears to play a significant role in other regards, such that Apoe4 carriers had lower anxiety-like behaviors, but greater sensitivity to neurokinin B-related hot flash-like symptoms and ovariectomy-induced weight gain compared to Apoe3

homozygotes. Therefore, careful consideration of genotype and drug type is necessary for development of future AD treatments.

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Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.03

Topic: H.08. Learning and Memory

Support: 5T34GM136467-03

Title: Effect of 5-HT1A receptor agonist treatment on behavioral flexibility in the BTBR mouse model of autism spectrum disorder.

Authors: C. CAVAZOS, C. MORALES, S. LOPEZ, A. GUTIERREZ, P. FLORES, E. TORRES-LOZANO, *D. AMODEO;
California State Univ. San Bernardino, San Bernardino, CA

Abstract: Repetitive behaviors are a prevailing symptom across several neuropsychiatric and neurodevelopmental disorders. To date, there is a lack of effective treatments for the attenuation of repetitive behaviors with restricted interests (RRB) in autism spectrum disorder (ASD). Reversal learning is often utilized to gage behavioral inflexibility, a measure of insistence on sameness in ASD. The current experiments aim to better determine how the 5-HT1A receptor agonist OH-DPAT attenuates and behavioral inflexibility and repetitive grooming in the BTBR mouse model of ASD and control C57BL/6J strain. These studies also examine the potential sex

differences. Mice received an injection of vehicle, 0, 1 or 5 mg/kg OH-DPAT before probabilistic reversal learning. Before the reversal phase, mice were injected with OH-DPAT and the reinforcement contingencies were reversed to examine how many trials were required for mice to reach learning criterion. Learning criterion was met when mice made six consecutive correct choices. As previously demonstrated, all groups performed similarly on acquisition of the initial spatial discrimination. Current results demonstrate that 5-HT1A receptor activation attenuates behavioral flexibility in BTBR mice, while the lower dose of 1 mg/kg did not produce similar increase. These findings demonstrate the potential therapeutic effects of 5-HT1A receptor agonists for the attenuation of behavioral flexibility in ASD.

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Poster

078. Memory, Genes, and Molecules

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Program #/Poster #: 078.04

Topic: H.08. Learning and Memory

Support: PAPITT-DGAPA IN209822

Title: Class I Histone deacetylase inhibition reverses memory impairment induced by acute stress in mice

Authors: H. MARTÍNEZ-PACHECO[1], R. C. ZEPEDA[2], ***G. L. QUIRARTE**[1], G. ROLDÁN-ROLDÁN[3];
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Abstract: Multiple studies in animals and humans have shown that chronic stress induces learning and memory impairments, while the effects of acute stress critically depend on their temporal relationship, facilitating or preventing memory consolidation depending on whether the stress occurs during the learning event or before it, respectively. On the other hand, it has been shown that histone acetylation regulates the persistence of long-term memory. The aim of this study was to evaluate the effect of two inhibitors of class I histone deacetylase (HDAC), 4-phenylbutyrate (PB) and IN14, in mice exposed to a single 15-min acute stress session of forced swimming, 30 min before training. The study comprised three factors: 1) pharmacological treatment: Vehicle, IN14, or PB (100 mg/kg, i.p. for 2 days); 2) acute stress: present or absent; 3) memory test: present or absent. Three memory tasks were performed 1 h after the last drug injection: Novel object recognition test (NORT), Elevated T Maze (ETM), and Buried food location test (BFLT). After completion of behavioral testing, mice were sacrificed, the

hippocampus removed, and samples collected to perform ELISA assays for HDAC2 expression. Acute stress induced an increase of hippocampal HDAC2 content, as well as plasma corticosterone levels, along with a poor performance in NORT, ETM, and BFLT tests. Moreover, PB and IN14 treatment prevented memory loss in stressed mice. These findings suggested that HDAC2 is involved in acute stress-induced cognitive impairment. Yet, it is worth mentioning that none of the drugs improved memory in non-stressed animals, indicating that HDACs inhibitors are not cognitive boosters, but rather potentially useful drugs for mitigating memory deficits in cognitively compromised patients. The protective effects of HDACs inhibitors on acute stress-induced amnesia observed here open the possibility of the potential use of these drugs to prevent the cumulative effects of stressful events on cognitive function.
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Poster

078. Memory, Genes, and Molecules

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Topic: H.08. Learning and Memory

Support: NIH grant GM118801

Title: Dose-dependent suppression of hippocampal contextual memory by (R)-CPP, a potent NMDAR antagonist, is recapitulated in the modulation of place cell formation and spatial engram stability

Authors: *M. ZHU, R. A. PEARCE;
Univ. of Wisconsin, Madison, Univ. of Wisconsin, Madison, Madison, WI

Abstract: N-methyl-D-aspartate receptors (NMDARs) mediate excitatory transmission throughout the central nervous system (CNS) and are strongly implicated in higher cognitive functions such as memory and learning. We recently reported a mismatch between the dose and brain concentration of CPP that suppresses contextual fear conditioning (CFC; IC50 = 2.3 mg/kg -- 53 nm) versus the concentration that blocks NMDARs and LTP in hippocampal brain slices (IC50 = 361 - 464 nm; Laha et al., Neuropharmacology 2022). Here we tested one possible explanation for the mismatch - that the hippocampus is relatively resistant to CPP compared to other brain structures engaged in CFC. We used the context pre-exposure facilitation effect (CPFE) paradigm to isolate the hippocampal component of contextual learning, and in-vivo calcium imaging of place cells and spatial engrams to assess the effects of CPP on hippocampal

spatial coding. CPFE experiments took place over 3 days: on day1 (preexposure), mice (n = 8/group) freely explored a novel arena for 10min; on day2 (contextual fear conditioning), they explored the same arena and received a foot shock after 15sec; on day3 (recall), their freezing behavior in that arena was measured. (R)-CPP (1, 3, or 10mg/kg) was administered on either day1 or day2. For Ca^{2+}-imaging experiments, a mini-endoscopic camera (Inscopix nVoke) was used to capture the activity of CA1 pyramidal neurons expressing GCaMP6f in freely exploring mice (n = 4). Experiments were conducted in two phases: on day1, mice were exposed to a novel context after injection of saline or (R)-CPP (1, 3, or 10mg/kg); on day2, mice were reintroduced to the same context. In CPFE experiments, (R)-CPP administered on either day1 or day2 dose-dependently reduced freezing, with IC50s of 3.1 mg/kg and 4.4 mg/kg respectively. In Ca^{2+}-imaging experiments, (R)-CPP dose-dependently reduced the proportion of place cells (defined by mutual information) with IC50 = 2.3 mg/kg. Spatial engrams, quantified by rate-map (RM) and population-vector (PV) correlations, were also significantly reduced (linear mixed effects model, $p < 0.0001$ at 3 & 10mg/kg) in a dose-dependent manner. We conclude that low doses of (R)-CPP modulate hippocampal contextual memory by interfering with hippocampal function, even at concentrations to that do not suppress NMDARs on pyramidal neurons or LTP. We speculate that CPP induces amnesia by targeting NMDARs at sites that are not engaged in vitro in the same manner that they are in vivo, such as those on interneurons.

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Poster

078. Memory, Genes, and Molecules

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Program #/Poster #: 078.06

Topic: H.08. Learning and Memory

Support: MRC DTP

Title: Role of mu and delta opioid receptors in the memory enhancing effects of the atypical antidepressant tianeptine

Authors: *M. TRIGO[1], T. KNOTT[2], H. SPOONER[1], R. LANGSTON[1], S. J. MARTIN[1], J. J. LAMBERT[1];
[1]The Univ. of Dundee, Dundee, United Kingdom; [2]The Univ. of St Andrews, St Andrews, United Kingdom

Abstract: According to the World Health Organisation, depression affects an estimated 5% of the global adult population. Most current pharmacological therapies target monoamine neurotransmitter levels. Such drugs have a relatively slow onset of therapeutic effects, requiring weeks of treatment, and achieve modest remission rates. Tianeptine is an atypical antidepressant with no direct effect upon monoamine transporters and enzymes, but it acts as an agonist of μ- and δ- opioid receptors (ORs). Recent studies reveal that the anti-anhedonic and “antidepressant”

effects of tianeptine are blunted in mice engineered to lack expression of the μ-OR in somatostatin-positive GABAergic interneurons (Han et al., Neuropsychopharmacology, 47: 1387-1397). The ability to disinhibit hippocampal pyramidal cells is shared by several other atypical antidepressants, including ketamine. Additionally, tianeptine enhances hippocampus-dependent memory after acute administration. Whereas the μ-OR is critical for tianeptine's "antidepressant" actions in rodent models, the mechanism underpinning memory facilitation is unknown. We are investigating the role of μ- and δ-ORs in tianeptine's effects on locomotor activity, spatial memory, and hippocampal local-field-potential (LFP) activity. Consistent with previous work, the increase in locomotor activity following tianeptine administration (30 mg/kg, s.c.) in wild-type mice is abolished in μ-OR$^{-/-}$ but not δ-OR$^{-/-}$ mice. Tianeptine (10 mg/kg, s.c.) also enhances allocentric spatial memory in a food-rewarded cross-maze task; we are investigating the μ- or δ-OR-dependence of this effect. Hippocampal oscillatory activity in the beta- and gamma-frequency ranges is associated with memory encoding and retrieval and has been proposed as a biomarker for antidepressant efficacy. Preliminary recordings of LFP activity from hippocampal area CA1 in freely moving wild-type, μ-OR$^{-/-}$ and δ-OR$^{-/-}$ mice reveal that tianeptine increases spectral power in the 20-30-Hz range in a μ-OR-dependent manner. Our investigation of tianeptine's novel OR-dependent actions highlights a potential strategy for developing fast-acting pharmaceuticals that target both the 'classic' and the cognitive symptoms of depressive illness.

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Poster

078. Memory, Genes, and Molecules

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Program #/Poster #: 078.07

Topic: H.08. Learning and Memory

Support: Extraordinary support VIEP-BUAP-2021

Title: Evaluation of the sub-chronic oral administration of Cannabidiol on spatial memory in rats lesioned with β-amyloid 25-35

Authors: *A. PATRICIO-MARTÍNEZ[1,2], L. GUTIÉRREZ-PRÁXEDIS[1], D. MARCELO-PÉREZ[1], F. PATRICIO[1], I. MARTÍNEZ-GARCÍA[3], I. D. LIMÓN[1];
[1]Lab. de Neurofarmacología, [2]Facultad de Ciencias Biológicas, [3]Lab. de Neuroquímica, Benemérita Univ. Autónoma de Puebla, Puebla, Mexico

Abstract: Cannabidiol (CBD) is the main non-psychotropic component of marijuana, the first reports of the properties of cannabidiol proposed it as an anticonvulsant agent, later many pharmacological effects were reported, including sedation, hypnosis, anxiolytic, antipsychotic, anti-inflammatory and neuroprotective. Recently, its antiemetic effect has been shown and it has

been reported as an antioxidant compound with antineoplastic and chemo-preventive properties and as a possible medicine for rheumatoid arthritis. This variety of effects caused by CBD is due to its pleiotropic action since the actions are mediated by cannabinoid 1 (CB1) and 2 (CB2) receptors, also involving receptors 18 and 55 coupled to G protein (GPR18 and GPR55), the receptor for transient potential vanilloid subtype 1 (TRPV1) and peroxisome proliferator-activated receptors alpha and gamma (PPARα and PPARγ). In recent years, attention has focused on the study of CBD as a therapeutic candidate for neurodegenerative diseases such as Alzheimer's disease. For this reason, the aim of this study was to evaluate the effect of sub-chronic oral administration of CBD on spatial memory in rats lesioned with β-amyloid 25-35 ($A\beta_{25-35}$). Male Wistar rats were used, eight days before stereotaxic surgery, spatial learning test was performed in the Morris water maze. Four experimental groups were formed: the SSI + vehicle group; SSI + CBD group, $A\beta_{25-35}$ + Vehicle and, the $A\beta_{25-35}$ + CBD group. All groups underwent oral administration of CBD (10mg/Kg) or vehicle (short-chain triglyceride oil) every 24 hours for 14 days. The first memory test was performed on day 6 post administration of $A\beta_{25-35}$ (10 µg/10 µL) or SSI icv. The second memory test was performed on day 21 after subacute administration of CBD to see if this compound improves the process of recalling information over a longer period. The navigation trajectory, the latency to find the platform, and the number of crossings through the quadrant where the platform is located were evaluated. The results show that the escape latency increases in the $A\beta_{25-35}$+vehicle group. However, the group administered with $A\beta_{25-35}$+CBD decreased escape latency. Finally, the number of visits in the white quadrant decreases in the $A\beta_{25-35}$+vehicle group, while a better stay is shown when $A\beta_{25-35}$ plus CBD is administered. These results suggest that the cannabidiol decreases neurotoxicity induced by $A\beta_{25-35}$ peptide and improves spatial memory process.

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Poster

078. Memory, Genes, and Molecules

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Topic: H.08. Learning and Memory

Support: PAPIIT-DGAPA IN209122
CONACYT 1085633
Vera-Rivera, G.
Rangel-Hernández, J. A.

Title: Differential role of the lateral habenula during sugar aversion conditioning and latent inhibition after high familiarization

Authors: *J. LOMELI-CASTILLO[1], M. I. MIRANDA[2];
[1]Inst. De Neurobiología, UNAM, Queretaro, Mexico; [2]Inst. Neurobiologia-UNAM, Queretaro, Qro., Mexico

Abstract: Conditioning taste aversion (CTA) is associative learning, where a novel taste and gastric discomfort are associated, for which the animals learn to avoid this taste, and latent inhibition (LI) is a model to study the robustness of this kind of associative learning, depending on the degree of stimuli familiarity. Therefore, LI refers to the reduced ability to learn the relevance of a stimulus paired with an aversive or positive condition through classic conditioning if there has been previous exposure to the stimulus in a neutral context. In this regard, the lateral habenula (LHb) connects with some structures involved with aversive memory formation and reward associations; for example, electrolyte injury to LHb has been shown to attenuate CTA to saccharin and ethanol administration. Furthermore, the evidence suggests that LHb processes signals of negative motivational value (absence of a reward or presence of a punishment). However, the intervention of this structure in associative learning and its impact on subsequent learning, such as a latent inhibition of CTA (LI-CTA), is still unknown. In this way, we decided to evaluate the function of the LHb during aversive memory formation of novel sweet flavor and after its high familiarization (LI-CTA). Male Wistar rats (initial weight of 270-310 g) were deprived and trained to consume liquid in a single daily presentation for 20 min, and 30 min before CTA acquisition, they were injected bilaterally in the LHb with a cytotoxic dose of NMDA (10 μg/μl). Sugar consumption (10% solution) was measured during the acquisition, recall, and three extinction memory sessions. For LI-CTA, rats were permanently sugar exposed for 21 days and then trained with the previously mentioned CTA procedure. The results showed that NMDA cytotoxic lesions in LHb before CTA acquisition induced a decreased taste aversive learning and accelerated memory extinction. On the other hand, the same NMDA lesions in LHb, after 21 days of exposure to sugar, do not alter the LI-CTA. Thus, LHb lesions impair sugar-taste aversive learning and the extinction of aversive memory when the taste is novel; however, LHB lesions do not alter the expression of latent inhibition, that is, when the taste is highly familiar. Our results suggest that LHb has a crucial role during aversive learning but not during highly familiar taste memory updating processes.

Disclosures: J. Lomeli-Castillo: None. **M.I. Miranda:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.09

Topic: H.08. Learning and Memory

Title: Chronic Oral Administration of Midazolam and Spatial Memory in Rats

Authors: *H. M. MURPHY, C. H. WIDEMAN;
Neurosci. Program, John Carroll Univ., University Heights, OH

Abstract: Twelve Long-Evans female rats were placed in individual cages with a running wheel connected to a computer that recorded daily activity. Rats were divided into control and experimental groups and a 12hr/12hr light/dark cycle was maintained with room temperature held at 21-23°C. Spatial memory was tested using the Morris water maze (MWM) which was a circular pool divided into eight segments with three towels, serving as cues, placed over the side in a permanent location. The towels helped the rats find the platform which was submerged below the surface of the water and moved randomly every day forcing the rats to use their spatial memory instead of memorizing a permanent location of the platform. Rats were habituated to their environment for one week. At the beginning of the dark cycle each day, all rats received a 250μL "treat" of condensed milk in a glass dish. During the 3-week experimental period, the experimental group received midazolam diluted in distilled water at a dose of 9 mg/kg. The control group was given a placebo in the condensed milk. Animals consumed the drug or placebo within five minutes. The MWM protocol was as follows: the first trial (sample) allowed the rat to discover the location of the platform by trial and error while being timed. If the rat took more than 90 seconds to find the platform, it was guided to it. The rat was then allowed to rest on the platform for 15 seconds. The second trial (test) occurred after a 15-second break. The rat was placed at the same starting location as the first trial. If the rat recalled the location of the platform, it swam faster to it. The starting position of the rat was changed every day. There was an interaction between runs (sample and test) and experimental weeks for the control group. There was a significant difference between sample and test runs for the control group during weeks 1 and 2 with sample runs taking a longer time than test runs. There also was a significant difference between sample runs of weeks 2 and 3 with the sample run of week 2 taking a longer time. For the experimental group, there was no interaction between runs and experimental weeks. The pattern of the sample run and the test run was similar over the three weeks. However, there was a significant difference between weeks 1and 2 sample runs with the sample run in week 1 taking a longer time than the sample run of week 2. There was no significant difference between sample runs of weeks 2 and 3. Concerning the pattern of activity, in control rats, the first peak of activity was higher than the second peak during the dark cycle. In experimental rats, however, the first peak of activity, following drug administration, was lower than the second peak which developed later in the dark cycle.

Disclosures: H.M. Murphy: None. **C.H. Wideman:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.10

Topic: H.08. Learning and Memory

Support: Kjell and Märta Beijers foundation, The Beijer laboratory

Title: Inhibition of insulin-regulated aminopeptidase (IRAP) restores cell viability after induced ROS damage in primary hippocampal cells

Authors: *F. STAM[1], S. FLORÉN LIND[1], A. SCHROFF[1], S. ZELLEROTH[1], E. NYLANDER[1], J. GISING[2], A. GRÖNBLADH[1], M. LARHED[2], M. HALLBERG[1];
[1]Dept. of Pharmaceut. Biosci., [2]Dept. of Medicinal Chem., Uppsala Univ., Uppsala, Sweden

Abstract: Abstract
Insulin-regulated aminopeptidase (IRAP) is expressed throughout different tissues, including neurons of the hippocampus and neocortex, and is believed to have a role in cognitive functions like memory and learning. It is known to be the receptor of Angiotensin IV (Ang IV), a metabolite of Angiotensin II. The bioactive Ang IV inhibits IRAP and has previously been shown to improve performance in memory tasks *in vivo*. HA08 is a macrocyclic IRAP inhibitor that is significantly more stable than the endogenous compound Ang IV. In the present study the restorative effects of HA08 in rat primary hippocampal cells, along with the cognitive effects in rats have been investigated. The primary cells were damaged with hydrogen peroxide before treated with HA08. The restoring effects of the compound were investigated by measuring mitochondrial activity and membrane integrity, as assessed by the tetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assay, respectively. In addition to the in vitro studies, rats were injected intracerebroventricularly with a single dose of HA08 before performing a novel object recognition test in order to elucidate the cognitive effects. Overall the results demonstrate that the mitochondrial activity was significantly increased by HA08 in the primary hippocampal cells, whereas the LDH release was unaffected. The rats' cognitive performance in the novel object recognition test was however not affected by HA08. To conclude, these results indicate that inhibition of IRAP can restore cells subjected to hydrogen peroxide damage but that the inhibitor, under these specific conditions, does not seem to have an effect on memory.

Disclosures: F. Stam: None. **S. Florén Lind:** None. **A. Schroff:** None. **S. Zelleroth:** None. **E. Nylander:** None. **J. Gising:** None. **A. Grönbladh:** None. **M. Larhed:** None. **M. Hallberg:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.11

Topic: H.08. Learning and Memory

Support: NIAAA R01AA026347
NIAAA F31AA029305

Title: Adolescent vs. Adult Binge Ethanol: Impacts on Memory, Ethanol Metabolism, and Protein Expression

Authors: *M. A. M. BENT[1], I. BETANCOURT[2], J. T. WOLSTENHOLME[3];
[1]VCU, richmond, VA; [2]VCU, Richmond, VA; [3]Pharm/Tox, Virginia Commonwealth Univ., Richmond, VA

Abstract: Ethanol is the most consumed drug among adolescents, which can cause long-term effects on protein expression, drug dependence, and memory formation. While both adolescents and adults engage in binge drinking, the neurological and behavioral effects differ. Indeed, adolescents show fewer adverse physiological effects to ethanol than adults, meanwhile presenting greater memory deficits, which may be due to differences in ethanol metabolism. Additionally, proteins critically involved in memory formation, cAMP response element-binding protein (CREB) and CREB binding protein (CBP), found in the hippocampus (HI) and prefrontal cortex (PFC), can be decreased following ethanol exposure and when disrupted, result in memory deficits. This study assessed the impact of age, sex, and ethanol on memory, ethanol metabolism, and CREB-related protein expression. Female and male DBA/2J mice were given 4g/kg of ethanol or water via oral gavage intermittently from either PND 29-42, for adolescent mice, or PND 64-77, for adults. Three weeks later mice began Barnes Maze testing, while another cohort was used for Novel Object Recognition (NOR). A behavioral naïve cohort was used for tissue collection (HI & PFC) for western blot analysis. A behavioral and ethanol naïve cohort was used to obtain blood ethanol concentrations (BEC) at 0.5, 1, 2, and 4 hours. At PND 35 (adolescents) or PND 65 (adults) mice were given 4g/kg of ethanol via gavage. Mice given ethanol in adolescence showed decreased spatial memory, compared to controls, but no difference in cognitive flexibility. Adult treated mice showed no differences from controls in either spatial memory or cognitive flexibility. Adolescent treated mice showed deficits in NOR while adult ethanol exposed mice did not. Plasma ethanol levels differed due to a timepoint X age interaction, with adolescent animals regardless of sex showing a consistent decline in ethanol concentration over time. Meanwhile, adult mice showed an initial rise in ethanol concentration during the first hour, followed by a decline. Additionally, area under the curve for BEC was significantly smaller in adolescent mice regardless of sex when compared to adult mice. We hypothesize CREB expression will remain unchanged in all groups following ethanol treatment, but CBP will be decreased following adolescent but not adult ethanol treatment. Together this data suggests ethanol metabolism differs in adolescent and adult mice but, despite higher BEC in adult mice, memory performance is unaffected. This further suggests the adolescent developing brain is more severely affected by the long-lasting impacts of ethanol.

Disclosures: M.A.M. Bent: None. **I. Betancourt:** None. **J.T. Wolstenholme:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.12

Topic: H.08. Learning and Memory

Support: AG062166

Title: Activation of Hippocampal CA1 Adiponectin Receptors Rescues Age- and AD-related Impairment of Cognitive Function and Long-term Synaptic Plasticity

Authors: *Y. CHEN[1], Y. BAI[1], W. WANG[2], H. ZHANG[1], K. DENNEY[1], J. NOUGAISSE[1], Y. LEI[1], X.-Y. LU[1];
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Abstract: While adiponectin is well known for maintaining metabolic homeostasis, evidence implicates its role in the pathogenesis and treatment of Alzheimer's disease (AD). Adiponectin can cross the blood-brain barrier to exert biological effects through binding to two receptor subtypes, AdipoR1 and AdipoR2, in the brain. In the current study, we determined the effects of adiponectin receptor activation on age- and AD-related cognitive impairment and synaptic deficit using the AdipoR agonist AdipoRon. Circulating adiponectin levels decreased with aging. Infusion of AdipoRon into the hippocampal CA1 region rescued cognitive deficits in aged mice and in 5XFAD mice. Moreover, long-term potentiation (LTP) was measured in the Schaffer Collateral-CA1 region. AdipoRon treatment restored impaired LTP induced by aging and in 5XFAD mice. AdipoR1 knockout abolished the effects of AdipoRon on LTP. These findings suggest that AdipoR1 signaling in the hippocampal CA1 is critical for cognitive function and synaptic plasticity.

Disclosures: Y. Chen: None. **Y. Bai:** None. **W. Wang:** None. **H. Zhang:** None. **K. Denney:** None. **J. Nougaisse:** None. **Y. Lei:** None. **X. Lu:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.13

Topic: H.08. Learning and Memory

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NIH Grant 2R15GM118304-02
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UWM Office of Undergraduate Research
UWM College of Letters and Science
NIH Grant R01AG056472
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Title: Effects of a novel estrogen receptor beta agonist and APOE genotype on synaptic markers of memory in a mouse model of Alzheimer's disease

Authors: *M. R. SCHWABE[1], A. W. FLEISCHER[1], R. K. KUEHN[1], H. A. BEATY[1], E. M. MILKIE[1], A. L. SCHNITZLER[1], S. CHAUDHURY[2], W. A. DONALDSON[2], D. S. SEM[3], J. M. YORK[4], M. LADU[4], K. M. FRICK[1];
[1]Dept. of Psychology, Univ. of Wisconsin-Milwaukee, Milwaukee, WI; [2]Chem., Marquette Univ., Milwaukee, WI; [3]Dept. of Pharmaceut. Sciences, Ctr. for Structure-Based Drug Design

and Develop., Concordia Univ. Wisconsin, Mequon, WI; [4]Anat. and Cell Biol., Univ. of Illinois, Chicago, Chicago, IL

Abstract: Among the hallmark pathologies of Alzheimer's disease (AD) is significant synapse loss. The ε4 allele of apolipoprotein E (*APOE4*) increases synapse loss in AD patients. While females are at a greater risk for AD compared to males, female *APOE4* carriers have the greatest risk for AD. Estrogen therapy increases synaptic proteins and dendritic spine density, potentially preventing or reversing AD-induced synaptic changes. However, estrogen treatment is associated with increased risks of cancer due to activation of estrogen receptor α (ERα). Thus, selective ERβ agonists may be safer alternative therapies. To study interactions between *APOE* genotype and estrogen treatment in AD, we used mice EFAD mice ($5xFAD^{+/-}/APOE^{+/+}$), specifically E3/3FAD, E3/4FAD and E4/4FAD. E4/4FAD mice have decreased expression of the synaptic proteins synaptophysin and PSD-95, increased GFAP, and reduced basal dendritic spine density in the mPFC and CA1 region of the dorsal hippocampus relative to E3/3FAD mice (Taxier et al., 2022). Dorsal hippocampal infusion of 17β-estradiol (E2) increases apical CA1 dendritic spine density in E3/3FAD and E3/4FAD females, but not E4/4FAD females (Taxier et al., under review). Thus, the goal here is to determine the extent to which a novel highly selective ERβ agonist, EGX-358, may increases expression of synaptic markers associated with memory in E3/3FAD and E3/4FAD mice. E3/3FAD and E3/4FAD mice were ovariectomized at 5 months of age and then treated orally with vehicle (1% DMSO) or EGX358 (10 mg/kg/day) via hydrogel for 9 weeks. Mice were tested for spatial and object memory, anxiety-like behavior, and vasomotor symptoms following injection of the tachykinin receptor 3 agonist senktide, with testing concluding 2 weeks prior to tissue collection. On the day of collection, brains were removed, flash frozen, and hemisected 1-3 hours after a final EGX358 treatment. Half the brain was Golgi stained for measurement of apical and basal dendritic spine densities on DH and PL/IL mPFC pyramidal neurons. The remaining half was flash frozen for Western blot analysis of the DH and mPFC. Preliminary analyses suggest no effects of EGX358 or *APOE* genotype on DH expression of PSD95 or synaptophysin; ongoing analyses are assessing other proteins associated with synaptic plasticity including phosphorylated CREB, GFAP, and Iba-1. Dendritic spine analyses are also in process and should provide insights into whether ERβ agonism interacts with *APOE* genotype to impact synaptic morphology in a background of AD-like pathology.

Disclosures: M.R. Schwabe: None. **A.W. Fleischer:** None. **R.K. Kuehn:** None. **H.A. Beaty:** None. **E.M. Milkie:** None. **A.L. Schnitzler:** None. **S. Chaudhury:** None. **W.A. Donaldson:** A. Employment/Salary (full or part-time):; Estrigenix. 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.; Estrigenix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix. **D.S. Sem:** A. Employment/Salary (full or part-time):; Estrigenix. 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.; Estrigenix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix. **J.M. York:** None. **M. LaDu:** None. **K.M. Frick:** A. Employment/Salary (full or part-time):; Estrigenix. 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.; Estrigenix. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Estrigenix.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.14

Topic: H.08. Learning and Memory

Support: DGAPA-PAPIIT IA202922

Title: Reconsolidation and extinction of conditioned place avoidance induced by gastric malaise requires cortical β-adrenergic activity.

Authors: *R. SOLÍS GUILLÉN, D. CALDERÓN-REYES, A. HERNÁNDEZ-MATIAS, F. BERMÚDEZ-RATTONI, D. OSORIO-GÓMEZ;
Inst. de Fisiología Celular, Univ. Nacional Autónoma de México, UNAM, Ciudad DE Mexico, Mexico

Abstract: During memory retrieval, memory might undergo two dissociable processes: reconsolidation and extinction. Whereas reconsolidation stabilizes and strengthens existing memories, extinction involves the establishment of a new memory trace in which the conditioned response gradually decreases after subsequent re-exposures to the conditioned stimulus in absence of the unconditioned stimulus. We have previously reported that inhibition of protein synthesis within the insular cortex (IC) impairs both reconsolidation and extinction of conditioned taste aversion induced by gastric malaise. Furthermore, we developed another behavioral task in which gastric malaise is also associated with contextual information, inducing conditioned place avoidance (CPA). Although IC is involved in interoceptive signaling, there is scarce information regarding the relevance of this structure in the establishment of interoception-context relationships. Therefore, we aimed to determine the role of IC in reconsolidation and extinction processes during CPA. For this purpose, male Wistar rats received an injection of lithium chloride (0.4 M; 7.5 ml/kg i.p.) and then were confined in a previously preferred dark compartment. Memory was reactivated 24 hours later for 1 or 10 minutes to induce reconsolidation or extinction, respectively, and assessed the effect of propranolol (10 mg/kg, i.p.), a nonselective β-adrenergic receptor antagonist, in both cases. The test session occurred 24 hours after reactivation. Our results indicate that systemic propranolol impairs both reconsolidation and extinction memory processes when administered before reactivation. Next, we evaluated the specific participation of IC in these same processes through intracortical administration of propranolol (5 µg/µL, 1 µL/hemisphere), observing that such manipulation also hinders reconsolidation and extinction. Thus, reconsolidation and extinction of CPA induced by gastric malaise require noradrenergic activity within the IC.

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Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.15

Topic: H.08. Learning and Memory

Support: Lee Honors College Western Michigan University
College of Arts and Sciences Western Michigan University

Title: Tramadol produces minimal spatial memory impairment as assessed by radial arm maze performance in rats

Authors: *J. M. GRAEBER, R. L. BURROUGHS, K. L. POWELL, L. E. BAKER; Psychology, Western Michigan Univ., Kalamazoo, MI

Abstract: Tramadol hydrochloride is a synthetic analogue of codeine frequently used for pain management with an analgesic potency comparable to pethidine. Tramadol is a mu opioid agonist reported to pose a decreased risk of abuse and a lower incidence of adverse side effects in comparison to other opioid analgesics. However, some reports indicate tramadol may produce cognitive impairment. Despite evidence of widespread use and abuse of tramadol, a review of recent literature has revealed few published studies on the drug's adverse cognitive side effects. The present study assessed the radial eight-arm maze (RAM) performance of male Sprague-Dawley rats following a single acute dose and repeated daily injections of tramadol. Following three days of radial arm maze habituation, rats completed daily training in the RAM for 30 days to establish a performance baseline. On day 31, rats were given a single injection of tramadol (20 mg/kg) or an equivalent volume of 0.9% saline 30 min prior to assessment of maze performance. Once daily injections of tramadol or saline continued for an additional 20 days, while RAM training was suspended. On day 21, RAM performance was re-assessed to determine the effects of chronic treatment. Training continued for an additional 10 days to assess relearning. Both acute and chronic tramadol treatment increased the latency to complete the maze trial. However, working memory and reference memory, and percent correct trials did not differ significantly between treatment and control groups. Moreover, re-acquisition did not differ between the two groups over the 10 days following the end of treatment. These findings indicate tramadol poses low risk for cognitive impairment. Future investigations with a wider dose range, longer treatment duration, and other behavioral assessments are warranted to fully characterize tramadol's neurocognitive effects.

Disclosures: **J.M. Graeber:** A. Employment/Salary (full or part-time):; Western Michigan University. **R.L. Burroughs:** None. **K.L. Powell:** None. **L.E. Baker:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.16

Topic: H.08. Learning and Memory

Support: NASI Senior Scientist fellowship NASI-303/12/2021
UGC-BSR Grant F.18-1/2011
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DST Grant EMR/2015/002178
CSIR-Junior Research Fellowship
CSIR-Senior Research Fellowship
ICMR-Senior Research Fellowship

Title: Mdivi-1 attenuates hippocampal neurodegeneration and memory decline by improving mitochondrial dynamics in scopolamine-induced amnesic mouse model

Authors: *E. MISHRA, M. K. THAKUR;
Dept. of Zoology, Inst. of Sci., Banaras Hindu Univ., Varanasi, India

Abstract: Background Mitochondrial impairment is a remarkable hallmark in amnesic condition and plays a crucial role in pathogenesis of neurological diseases. However, the role of increased mitochondrial fragmentation in scopolamine (SC)-induced amnesia is poorly understood. **Objective** The present study aims to determine the effects of p-Drp1 S616 inhibition by Mdivi-1 on mitochondrial dynamics, neurodegeneration and memory decline. **Methods** We have used 90 male mice (*Mus musculus*) of 10 weeks and divided them into vehicle, SC and SC+Mdivi-1 groups. Initially, mice were subjected to NOR and Y-maze test, and hippocampus was harvested to perform molecular studies. Each experiment was repeated thrice. The effect of Mdivi-1 was tested on mitochondrial ultrastructure and dynamics. Then, the expression of memory, mitochondrial dynamics and apoptosis associated proteins was analysed. Next, we examined dendritic arborization, spine density, myelination, caspase 3 activity and neurodegeneration. Lastly, the effect of Mdivi-1 on mitochondrial function was determined. **Results** The expression of Arc and proBDNF proteins showed a significant increase in SC+Mdivi-1 (66.2%, 74.5%) as compared to SC (39.1%, 37.7%), corroborating the increase in recognition and spatial memory. An improved mitochondrial ultrastructure was attributed to decline in number of fragmented round-shaped mitochondria in SC+Mdivi-1 (130.0%) as compared to SC (194.1%). Reduction in number of fragmented mitochondria was justified by downregulation in the level of p-Drp1 S616 in SC+Mdivi-1 (106.4%) as compared to SC (141.6%). In contrast, increased expression of proteins (Mfn2, LC3BI and LC3BII) in SC+Mdivi-1 (74.9%, 111.1%, 98.9%) as compared to SC (36.9%, 66.8%, 43.5%) indicates healthy mitochondrial dynamics. When compared to SC (21.8%, 42.6%), expression of synaptophysin and PSD95 increased in SC+Mdivi-1 (52.7%, 60.4%), confirming the enhanced dendritic arborization and spine density. Also, decline in pro-apoptotic protein Cyt-c in SC+Mdivi-1 (90.6%) as compared to SC (136.4%) and increase in anti-apoptotic proteins Bcl-2 and pro-caspase-9 in SC+Mdivi-1 (75.3%, 89.6%) as compared to SC (46.5%, 57.2%) suggests

the improved neuronal health. **Conclusion** Mdivi-1 ameliorates mitochondrial ultrastructure and function by regulating mitochondrial dynamics in a healthy way. These alterations result in reduced neurodegeneration, increased neuronal cell density, and myelination, which together improve recognition and spatial memory. Thus, the inhibition of excessive p-Drp1 (S616)-mediated mitochondrial fragmentation might be an efficient therapeutic avenue for amnesic conditions.

Disclosures: E. Mishra: None. **M.K. Thakur:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.17

Topic: H.08. Learning and Memory

Support: CIHR
MSFHR

Title: Type 1 diabetes mediated increased capillary stalling and impaired behavioral performance in mice

Authors: *S. SHARMA, M. CHEEMA, K. A. TENNANT, C. E. BROWN;
Div. of Med. Sci., Univ. of Victoria, Victoria, BC, Canada

Abstract: Introduction Recent work from our lab has shown that the brain capillaries routinely get clogged by cells and debris even under healthy conditions. The present study was undertaken to determine how experimentally induced type 1 diabetes affects this phenomenon, and whether obstructions contribute to cognitive decline. **Methods** C57BL/6 male and female mice were injected with streptozotocin to induce type 1 diabetes. These mice were implanted with cranial windows and cortical volumes were repeatedly imaged from 3-9 weeks after induction of diabetes. To model susceptibilities to short or long lived obstructions, we injected (i.v.) 5µm diameter fluorescent microspheres in diabetic and control mice at 30 minutes and 3 days before euthanasia. The density of microsphere obstructed capillaries was quantified across 15 different brain regions. To determine the impact of diabetes on cognitive and sensorimotor activity, mice were subjected to a battery of behavioural tests. **Results** 2-photon imaging indicated that diabetic mice have higher rates of capillary stalling in somatosensory cortex that became more pronounced with duration of diabetes. The majority of stalls (~60%) in diabetic mice were associated with Rhod6G or CD45 labelled cells, suggesting that leukocytes play a role. Increased stalling also led to greater pruning of cortical capillaries. Consistent with these observations, our fluorescent microsphere obstruction assay yielded significantly higher levels of short and long lived capillary obstructions in diabetic mice, including those treated with insulin. Behaviourally, diabetic mice were not different from controls in tests of ambulatory activity, nor did they show any differences in visual function. However, diabetic mice were significantly impaired in

learning/memory tests such as the novel object recognition, water maze learning or reversal learning. In order to provide a mechanistic explanation, we probed for multiple cytokines/chemokines and found unusually high and sustained levels of IL-10 in diabetic blood serum. We are currently testing endothelial and neutrophil specific knockdown of IL-10 receptors, as well as IL-10R blocking antibodies to determine if IL-10 signalling plays a key role in capillary stalling. **Conclusions** These studies suggests that diabetes is associated with greater risk for capillary obstructions in the brain as well as learning/memory deficits. Our future aims will provide a mechanistic understanding of how diabetes elevates one's susceptibility to capillary obstructions and cognitive decline, and whether manipulating certain immune signalling pathways can alleviate these impairments.

Disclosures: S. Sharma: None. **M. Cheema:** None. **K.A. Tennant:** None. **C.E. Brown:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.18

Topic: H.08. Learning and Memory

Title: Neuroprotective consequences of embelin in ethidium bromide induced model of multiple sclerosis

Authors: *R. RIMPI, Sr.[1,2,3];
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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. Embelin, a para-benzoquinone obtained from dried berries of Embelia ribes has been shown the neuroprotective and anti-inflammatory potential in several experimental model of neurodegeneration. Herein, embelin (1.25, 2.5 and 5 mg/i. p) is assessed for its neuroprotective potential in ethidium bromide induced demyelination in wistar rats. EB (0.1%/10µl/ICP) from day 1st to 7th to induce MS-like symptoms. Behavioral activities were assessed through locomotor activity, Morris-water maze test, rotarod test, and narrow beam walking test on day 1, 7, 14th and 21st. and biochemical alteration were observed in terms of Oxidative and nitrosative markers. Embelin treatment from day 8th to 21st improves behavioral impairments (spatial cognition, memory, grip, and motor coordination) and biochemical alterations compared with EB treated rats. Therefore, embelin appears to improve MS-related motor neuron dysfunctions.

Disclosures: R. Rimpi: None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

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Program #/Poster #: 078.19

Topic: H.08. Learning and Memory

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NCI R01 CA224675
H. Lundbeck A/S generously provided the drug-containing chow

Title: The antidepressant vortioxetine reverses cognitive deficits associated with androgen deprivation therapy in middle-aged rats

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Abstract: Androgen deprivation therapy (ADT) is a treatment for late-stage prostate cancer but is accompanied by cognitive decline. Currently, there are few options for ADT patients that experience cognitive changes. This uncovers a critical gap in quality of life care, as prostate cancer has a five-year survival rate of 98%. In young adult rats, our lab found that surgical castration induces deficits in cognitive domains mediated by the medial prefrontal cortex (mPFC) and hippocampus. Chronic treatment with the antidepressant vortioxetine (VTX; 28 mg/kg/day, 17 days) reversed these impairments. Thus, VTX may be a candidate to improve cognition in ADT patients. One critical factor for the onset of prostate cancer is age, which is accompanied by changes in testosterone levels, decreased cognitive function, and increased inflammatory processes. As the average age of prostate cancer patients is about 65 years old, we evolved our ADT-model to include a clinically-relevant chemical castration method in middle-aged rats, to determine if age can exacerbate the effects of ADT on cognition. ~13 month old rats were injected with the gonadotropin releasing hormone antagonist, degarelix (3 mg/kg, 5% mannitol) and were adminstered VTX through the diet. Animals were tested on the novel object location (NOL) test and the attentional set-shifting test (AST) to investigate changes in hippocampal and mPFC learning, respectively. ADT impaired set-shifting (vehicle-control:degarelix-control, $p<0.05$), and VTX reversed these impairments (degarelix-control:degarelix-VTX, $p<0.05$). High variability contributed to a lack of effect of ADT on the NOL test, but trends indicate that degarelix impaired spatial learning which was reversed by VTX. Evoked local field potentials were used to investigate circuit level changes within the these regions. ADT reduced responsivity in the Schaeffer Collaterals to CA1 pathway of the dorsal hippocampus (vehicle-control:degarelix-control, $p<0.05$) and the ventral hippocampus to mPFC pathway ($p<0.05$). Both were reversed with VTX (degarelix-control:degarelix-VTX, $p<0.05$). Our results suggest that VTX reverses ADT-induced cognitive decline in middle-aged animals

after ADT, indicating that it may be useful in mitigating cognitive changes in older men treated for prostate cancer. Ongoing experiments include investigating pro- and anti-inflammatory factors, which may contribute to cognitive decline in age, by measuring IL-6, IL-1β, TNFα and TNFβ in the mPFC and hippocampus of middle-aged, ADT rats. We will also investigate downstream signaling players such as pJAK2/JAK2, SMAD2, SMAD3, CX3CL1, and CD44.

Disclosures: A. Vaiana: None. **J. Gelfond:** None. **T. Johnson-Pais:** None. **R. Leach:** None. **C. Ramamurthy:** None. **I. Thompson:** None. **D. Morilak:** None.

Poster

078. Memory, Genes, and Molecules

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 078.20

Topic: H.08. Learning and Memory

Support: TL TR002647
R01CA224672

Title: Docetaxel treatment in tumor-bearing rats results in cognitive impairments and changes in inflammatory signaling.

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Abstract: Prostate cancer (PC) is the most common cancer to affect men, with more than 3.1 million men in the United States having been diagnosed with PC at some point in their lifetime. In fact, metastatic PC is the second leading cause of cancer-related deaths. Mainstay treatment of metastatic PC consists of androgen deprivation therapy and chemotherapy, both of which are associated with cognitive impairments. Microtubule stabilizers, like docetaxel, are often used as chemotherapy for PC. Patients who receive docetaxel as therapy experience difficulties in working memory, cognitive flexibility, and attention. These cognitive changes are accompanied by dysfunction in the hippocampus and increases in inflammation, but mechanisms underlying these impairments remain unknown. To study possible mechanisms for chemotherapy-induced cognitive impairments, docetaxel is used to treat a syngeneic rat model of PC. Male Copenhagen rats are implanted subcutaneously with syngeneic Dunning R-3327 G cell tumor fragments or subjected to sham implants, then treated with either docetaxel (3 i.p. injections over 5 days, 4.5mg/kg each) or vehicle. Ater a two week recovery period, hippocampal-mediated visuospatial memory is assessed using the novel object location task. Here, we have prelimary data that docetaxel reduced discrimination in the novel object task primarily in tumor-bearing rats, with less effect in sham controls. Increases in the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor alpha are observed in the plasma of tumor-bearing rats. Moreover, interleukin-6 mRNA is increased in the hippocampus of tumor-bearing rats compared to sham controls.

Results from this study will test the hypothesis that peripheral and central inflammatory signaling associated with prostate cancer may increase brain vulnerability to the potentially detrimental effects of docetaxel, contributing to cancer therapy-induced cognitive impairment.

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Poster

078. Memory, Genes, and Molecules

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Topic: H.08. Learning and Memory

Support: SIP-MDC-20220300

Title: Pharmacological evaluation of new hybrids from sigma-1 antagonists and acetilcholinesterase inhibitors in zebrafish cognition

Authors: *J. SILES-GUEVARA[1], S. A. GIL-LÓPEZ[1], E. ROSALES-ORTEGA[2], G. NAVARRETE-VÁZQUEZ[2], M. DÉCIGA-CAMPOS[1];
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Abstract: Dementia affects memory, thinking, language, judgment, and behavior. Worlwide around 50 million people live with dementia, and this number is projected to increase to 152 million by 2050. It is estimated that between 5% and 8% of the general population aged 60 years and over have dementia at any given time. In this study, new compound hybrids of N-benzylpiperidine were designed, synthesized, and evaluated against cognition in zebrafish. Three compounds, ROE-2, ROE 7 and ROE-13 were evaluated on cognitive impairment in the zebrafish model using a novel method of fear conditioning. First, the normal behavior of fishes was studied in a light-dark tank for 10 min daily for ten days. Posteriorly, fishes were individually subjected to fear conditioning passive avoidance task and evaluated for learned task memory 10 min daily for ten days. Fishes were treated with ROE-2, ROE-7 and ROE-13 for 24 hours previous exposition to scopolamine (200 mM). Donepezil and haloperidol were used as a positive control. In this study, we observe that the latency to enter to dark zone increases, while the average number of entries into the dark and time spent in the dark were significantly decresed. Fishes treated with ROE-2, ROE-7 and ROE-13 groups exposed to scopolamine retained the memory of the learned task. These findings suggest that the new N-benzylpiperidine compounds ameliorated scopolamine-induced cognitive dysfunctions. ROE-2, ROE-7 and ROE-13 might be promising therapeutic new chemical entities for cognitive enhancement in demetia disease.

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Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant R01MH129641

Title: Noradrenergic alpha-2A modulation enhances reward prediction error signaling in theprefrontal cortex and improves reversal learning

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Abstract: Noradrenergic signaling can enhance learning, attention and working memory performance. How these pro-cognitive effects are realized in neuronal network activity has remained unclear. Here, we hypothesized that a noradrenergic drug enhances cognition by enhancing neuronal encoding of learning variables in those prefrontal and striatal areas that support cognitive flexibility.
To test this prediction, we quantified how the alpha-2A sub-receptor specific noradrenergic agonist guanfacine affects reversal learning and neuronal encoding of latent learning variables during reversal learning. Guanfacine is FDA approved for treating attention deficit hyperactivity disorder (ADHD). Following administration of guanfacine or vehicle we extracellularly recorded neuronal activity in the lateral prefrontal cortex (LPFC), anterior cingulate cortex (ACC) and the head of the caudate nucleus (CD) from two healthy adult male rhesus macaques during reversal learning. The task required learning through trial-and-error which of two peripherally presented colored stimuli was rewarded. The reward-associated stimulus reversed un-cued every 30-60 trials.
We found that compared to the vehicle, guanfacine resulted in faster reversal learning and improved performance after learning. Enhanced learning with Guanfacine was linked to better post-error adjustment during the reversal. We used multi-linear regression to evaluate the impact of guanfacine on neuronal encoding of multiple task variables including the chosen stimulus features, reward and error outcomes and latent learning variables such as reward prediction errors (RPE) estimated with a Rescorla Wagner reinforcement learning model. We found that with guanfacine neurons in ACC showed stronger encoding of negative RPE during learning. Additionally, ACC cells with narrow spike waveforms had better error tracking during learning. In PFC as well as in the striatum, guanfacine enhanced the neuronal encoding of outcomes during learning.
These results showcase guanfacine's enhancement of flexible reinforcement learning is linked to enhanced neuronal signaling of prediction errors in those fronto-striatal network areas implicated to enable cognitive flexibility.

Disclosures: **S. Hassani:** None. **T. Womelsdorf:** None.

Poster

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Topic: H.08. Learning and Memory

Support: NIH Grant DA14241
NIH Grant DA050986

Title: Role of cholinergic signaling in acquisition and consolidation of cue-reward learning in mice

Authors: *H. YOUSUF, E. M. GIRARDI, R. B. CROUSE, M. R. PICCIOTTO;
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Abstract: Developing associations between neutral environmental cues and reward availability is dependent on cholinergic signaling. Acetylcholine binds to two families of receptors: nicotinic acetylcholine receptors (nAChRs), and muscarinic acetylcholine receptors (mAChRs), and each family of receptors has diverse subtypes that play distinct roles in learning and memory processes. Specifically, mAChRs have 5 subtypes (M1-M5), and we used an operant learning task to elucidate which receptor subtypes mediate this type of associative learning. Further, we investigated whether cholinergic signaling was involved in acquiring cue-reward contingencies or consolidating these associations in long-term memory. Mice were trained to nose poke during an auditory cue to receive a palatable food reward. Compared to controls, administration of a M1/M3 antagonist (benztropine mesylate; i.p.), or broad mAChR blocker (scopolamine; i.p.) prior to each training session impaired acquisition of the cue-reward contingency. Mice that received scopolamine injections immediately after each behavioral training session obtained significantly fewer rewards compared to saline controls, indicating disruption of memory consolidation. Finally, following successful cue-reward learning across several trials, a single pre-session injection of scopolamine significantly impaired performance of the task, in which mice were unable to accurately nose poke during the tone to receive a reward. Our results reveal the amnestic effects of scopolamine, and that mAChR signaling is required for different stages of cue-reward memory formation. Future work is necessary to investigate the role of nAChR signaling in this form of instrumental learning. Our results may aid in understanding the precise mechanisms that maintain other cue-reward learning disorders, such as addiction.

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Poster

079. Human Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 079.01

Topic: H.07. Long-Term Memory

Title: Preliminary evidence for a role of cognitive maps in action representation

Authors: *I. BARNAVELI[1], D. REZNIK[1], P. HAGGARD[2], C. DOELLER[1,3];
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Abstract: In our everyday life we execute many goal-directed actions and keep track of the relationship between different action plans and desired goals. While there is little doubt that representing the knowledge about potential environmental or bodily outcomes of these action plans is essential for their successful execution, the mechanisms underlying efficient action selection are still not well understood. Recent evidence suggests that cognitive maps supported by the hippocampal-entorhinal system organize knowledge in abstract low-dimensional spaces. The goal of this project is to investigate whether the brain can conceptually organize different action plans using a two-dimensional mapping of expected action outcomes. In particular, our aim is to examine if and how the anticipated outcomes of action plans could be used to abstractly represent and flexibly choose between different action plans along relevant outcome dimensions. To test this,we designed a set of behavioral tasks using immersive virtual reality (VR) with head-mounted display (HMD) and hand controllers. Participants (n=15), aged 18 to 35, were trained to execute different actions by moving two joysticks, that trigger launching of a ball towards them. Participants learned to associate different joystick movements with different probabilities to catch the ball (outcome dimension 1), and for the ball to remain fully visible throughout the flying trajectory (outcome dimension 2). In the subsequent testing phase, participants compared the action sequences based on the learned action-outcome contingencies. We performed multidimensional scaling on the behavioral data from the comparison task and fitted the subjective judgments to the actual outcome space. Our preliminary results indicate that participants' mental map of differences between sampled action sequences correspond to the actual distribution of those sequences on the two-dimensional outcome space. These preliminary findings suggest a map-like representation of action sequences. To explore the neural mechanisms of this map-like representation, we will acquire fMRI data using this paradigm and perform representation similarity (RSA) and adaptation analyses of the neuroimaging data. We hypothesize that encoding mechanisms in hippocampal-entorhinal and parietal-premotor regions are involved in the construction of such a map, thus, supporting behavioral planning by encoding multiple relationships between different action plans, and allowing efficient action selection using a common representational format.

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Poster

079. Human Medial Temporal Lobe

Location: SDCC Halls B-H

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Program #/Poster #: 079.02

Topic: H.07. Long-Term Memory

Title: Creative insight changes representations of knowledge in hippocampus and medial prefrontal cortex

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Abstract: Key in a creative act is the insight, the eye-opening 'aha' moment when we recognize a link between things that we had not seen previously. But what are the brain processes that lead to an insight, and how does insight change them? It has recently been proposed that the hippocampal formation alongside with medial prefrontal cortex (mPFC) may not only guide us in physical space - it could also provide a key metric for human cognition and thought. Accordingly, items of knowledge are neurally encoded along dimensions and indexed by spatial coordinates in the same way as physical items in navigational space. In this study, we recorded fMRI activity patterns evoked by words in the hippocampal formation and mPFC before and after the words were linked to each other in a verbal creative insight task. We first investigated whether the likelihood of a creative insight could be predicted by the neural distances between cue and solution words in the fMRI session preceding the insight task. We found that items were more likely solved if mPFC activity patterns of the cue and solution words had been more similar to each other. Furthermore, we examined how insight changed these neural distances in the fMRI session following the insight task. We found that insight did not generally decrease neural distances between cue and solution words in the second fMRI session. Instead, they only decreased for items in which cue and solution words had been more distant to each other initially. This negative correlation was significantly stronger in real items compared to mock items in both hippocampus and mPFC. Our findings suggest that neural distances between items of knowledge predict and reflect behaviour in a complex cognitive task, supporting the notion of a domain-general cognitive map.

Disclosures: J. Bergmann: None. **C.F. Doeller:** None.

Poster

079. Human Medial Temporal Lobe

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Topic: H.07. Long-Term Memory

Support: ERC Starting Grant NOAM

Title: Retrieval of conceptual goals elicits egocentric-like representations in parietal cortex and modulates the entorhinal grid-like map

Authors: *S. VIGANÒ[1,2], R. BAYRAMOVA[3,1], C. F. DOELLER[1,4], R. BOTTINI[2];
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Abstract: To represent the relations between concepts in memory, humans can recruit the same neural codes used to represent spatial relations in the physical environment. These codes are mostly located within the hippocampal-entorhinal circuit and provide an allocentric, or viewpoint-independent, relational model known as "cognitive map". However, we rarely navigate a space, be it physical or conceptual, without a goal in mind (e.g., a place we want to reach or a concept we want to access). During physical or virtual navigation, goal locations in the environment are also tracked using a complementary reference frame tuned to represent and update their position with respect to the moving subject, using egocentric, or viewpoint-dependent, coordinates. At the same time, experiments in rodents have shown that the allocentric cognitive map is altered in the proximity of goals, with their location being overrepresented by spatially-tuned neurons. Here, using fMRI and a combination of adaptation and multivariate analyses, we found that both these processes occurred also in the human brain when participants (N = 40) mentally navigated two conceptual spaces to compare the presented stimuli to specific goals held in mind. First, the medial parietal cortex represented the relation between currently shown stimuli and the memorized conceptual goals using an egocentric-like reference frame, despite the absence of a physically located, explicitly moving "self". This effect was prominent in the precuneus, where we also found evidence of mental rotation of the two environments, suggesting the involvement of this area in mentally rotating the navigable space to putatively center the focus of attention on the goal concepts that participants wanted to access, independently of the global map. Second, the entorhinal cortex represented a cognitive map of the conceptual spaces using an allocentric grid-like code that generalized across the two environments, but that was partially modulated as a function of goal proximity. This alteration followed the changing goal location across the two geometrically identical spaces, and extended also to the medial prefrontal cortex and the superior parietal lobule, two regions that have previously been associated with representing cognitive maps of physical and conceptual spaces using grid-like codes. These results support and extend the proposal of a phylogenetic continuity between the neural correlates of mammalian spatial navigation and human conceptual memory, suggesting the existence of common mechanisms recruited to represent spatial and non-spatial knowledge across different reference frames.

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Poster

079. Human Medial Temporal Lobe

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Program #/Poster #: 079.04

Topic: H.07. Long-Term Memory

Support: Jebsen Center Alzheimer's disease XXXXX

Title: Entorhinal grid-like representation in early Alzheimer's disease: association with biomarkers concentration and genetic risk

Authors: *M. BECU[1,2], T. BONNEVIE[1,2,3], I. POLTI[1,2], J. JARHOLM[4], G. R. GRØNTVEDT[1,5], G. BRÅTHEN[3,5], T. NAVARRO-SCHRÖDER[1], T. FLADBY[4], C. DOELLER[1,2,6];
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Abstract: Alzheimer’s disease (AD) is characterized by spatial navigation deficits, observed at preclinical stage. These early alterations are supposedly associated to brain damage occurring in the medial temporal lobes, including the entorhinal cortex (EC). In humans, a BOLD response modulation by running direction has been observed in the EC. This so-called grid-like signal is thought to provide a proxy for grid cells, one of the major spatially tuned cells in the brain.The present study investigates the impact of biomarker concentration (amyloid beta$_{42}$, total tau, phosphorylated tau, measured by spinal tap), APOE e4 genetic marker and cognitive decline on grid-like representation in humans, in a sample of 80 subjects (mild cognitive impairment, subjective cognitive decline and controls, age: 47-76 years old) who performed an active navigation task while being scanned (3 Tesla Siemens Skyra/Prisma). Our results show that early AD cases could maintain a minimal level of task efficiency by using the intramaze landmark as a reference to encode object locations in space, likely relying on different neuronal representations. Whether this represents a compensatory strategy to account for structural (eg. gray matter integrity) or functional (e.g. grid-like representation) changes in early AD is considered.

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Poster

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Topic: H.07. Long-Term Memory

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Title: Entorhinal grid-like signals reflect temporal context for human timing behavior

Authors: *I. POLTI[1,2], M. NAU[4], R. KAPLAN[5], V. VAN WASSENHOVE[6], C. F. DOELLER[3,1,7];
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Abstract: The entorhinal cortex (EC) supports the encoding of task regularities. A critical function may be the encoding of temporal context (i.e., forming integrated relational representations of co-occurring events and stimuli). A key neural component in the EC are grid cells, whose activity likely exhibits a six-fold rotational symmetry as a function of gaze direction as measured by functional magnetic resonance imaging (fMRI). Here, we combined fMRI and a time-to-contact estimation task to test whether temporal context modulates this grid-like fMRI activity in the human EC. In addition, we characterized in detail the relationship between trial-wise entorhinal activity and participants' task performance. We found that activity in the EC reflected biases in timing behavior, and that the cross-validated amplitude of grid-like signals indeed depended on the timing errors consistent with temporal-context encoding. These findings suggest that the human EC contributes to adapting internal timing mechanisms to the temporal statistics of the environment in accordance with the predictions from Bayesian models of time perception.

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Poster

079. Human Medial Temporal Lobe

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ERC-CoG GEOCOG 724836

Title: Probing neural representations of scene perception in a hippocampally dependent task using artificial neural networks

Authors: *M. FREY[1], C. F. DOELLER[2], C. BARRY[3];
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Abstract: Deep artificial neural networks (DNNs) trained through backpropagation provide effective models of the mammalian visual system, accurately capturing the hierarchy of neural responses through primary visual cortex to inferior temporal cortex (IT) (Yamins et al. 2016, Zhuang et al. 2021). However, the ability of these networks to explain representations in higher cortical areas is considerably less well researched. For example, DNNs have been less successful as a model of the egocentric to allocentric transformation embodied by circuits in retrosplenial and posterior parietal cortex. These brain regions play a central role in spatial memory and perception, transforming perceptual information into spatial representations found in the entorhinal cortex and hippocampus (Byrne et al. 2007, Bicanski et al. 2019). In humans, the Four Mountains task (Hartley et al. 2007) provides a test of egocentric to allocentric topographical processing - being particularly sensitive to hippocampal damage resulting from disease or trauma. We describe a novel virtual environment, inspired by the Four Mountains task, designed to probe the ability of DNNs to transform scenes viewed from different egocentric perspectives. The 'participant' is required to match scenes that correspond to the same configuration of distinct objects viewed from a different perspective, lures consist of the same or similar objects arranged in other configurations. Using a network architecture inspired by the connectivity between temporal lobe structures and the hippocampus, we demonstrate that DNNs trained using a triplet loss can learn this task. Analysis of the representations learnt in these artificial networks reveals that allocentric responses appear in model layers CA3 and CA1, resembling place and head direction cell responses. Furthermore, we show that feedback signals from medial temporal lobe to visual cortex can be conditioned on novel viewpoints, enabling the reconstruction of the scene from arbitrary angles. These results indicate that the model has learned a generalized representation of the scene in which disentangled object representations are leveraged for the subsequent imagination of novel views.

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Poster

079. Human Medial Temporal Lobe

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Title: Single-neuron representations of ambiguous words in the human medial temporal lobe

Authors: *B. SAMIMIZAD[1], T. P. REBER[1,2], V. BORGER[3], R. SURGES[1], F. MORMANN[1]; [1]Dept. of Epileptology, Univ. Hosp. Bonn, Bonn, Germany; [2]Fac. of Psychology, UniDistance Suisse, Brig, Switzerland; [3]Dept. of Neurosurgery, Univ. Hosp. Bonn, Bonn, Germany

Abstract: Extracting the correct meaning of an ambiguous word from an existing context is crucial for language comprehension and communication. Whether the human medial temporal lobe (MTL) plays a part in language processing or not has been a long-debated question. The human MTL features concept neurons with semantically invariant responses to specific concepts, regardless of context or sensory modality. Due to their ambiguity, homonyms present a prime opportunity to investigate the semantic representation in concept cells. These phonologically and orthographically identical words represent more than one concept, enabling us to observe response modulation by varying the semantic content using biasing contexts, while keeping the perceptual features of a stimulus constant.

For this purpose, we recorded intracranial single-unit activity while subjects performed a context-verification task. We provided context in two forms: once as a sentence and once without syntactic information (as a list of semantically related words). For each homonym we provided two semantically different contexts to bias the meaning selection towards one or the other specific meaning. The target word at the end of the presented context could either be the homonym or one of two synonyms corresponding to each different meaning.

Here we report the result of 22 recording sessions from 15 neurosurgical patients implanted with hybrid Behnke-Fried depth electrodes in the MTL. In our study, we used response-eliciting homonyms which were visually presented in the different contexts described above. This was followed by an audio-screening of target words. We recorded the neural activity of over 1500 units, among which we found a significant number of units with modality-invariant responses ($p<.001$) as well as semantically invariant responses ($p<.001$) (response to homonym and one corresponding meaning).

Our results demonstrate a stronger tuning towards the word-list semantically related to the preferred concept of the responsive unit. With respect to unambiguous words (synonyms), we observed a higher activation in the presence of an incongruent context, which could represent a single-unit correlate of the N400. The group analysis of the semantically invariant units in our data provide evidence in favor of multiple-access theory, indicating that prior context has its effect only after all information is accessed for an ambiguity (Swinney, 1979).

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Poster

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Title: Distinct populations in human MTL combine items and contexts across temporal gaps

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Abstract: The medial temporal lobe (MTL), and particularly the hippocampus, have been proposed to represent items in context to guide the encoding and retrieval of memories. It is currently unclear, however, whether separate or conjunctive (overlapping) representations of content and context contribute to the context-dependent processing of multiple memories involving the same content in humans. We devised a picture comparison task in which contextual questions were to be associated with two subsequent pictures for adequate contextual comparisons of picture contents. Analyzing 3127 neurons recorded from 17 neurosurgical patients, we found that mostly separate populations of visual stimulus-modulated neurons (N = 601) and context-modulated neurons (N = 200) combine representations of pictures and contextual questions across temporal gaps. While the large majority of stimulus neurons were invariant to context (88%) and most context neurons were invariant to stimulus (64%), a small but significant fraction of stimulus neurons represented both (12%) or even specific conjunctions of context and stimulus (5%), particularly in hippocampus (10%). During late picture presentations when questions and picture contents became task-relevant, context neurons encoded associated context, stimulus neurons encoded associated picture contents and contextual question activity was re-instantiated. Overall, stimulus and context neurons contribute to the context-dependent processing of stimuli via re-instatement of associated stimulus-contexts. Their co-activation could both support memory of stimuli in their respective context and further specify processing of contents according to context. Stimulus and context neurons generalized across the respective other dimension and therefore appear well-suited to contribute to flexible decision making by either dynamically broadening or constraining memories through re-instatement or co-activation. Conjunctive representations of stimulus-context, on the other hand, demonstrate pattern separation in humans and potentially specify memories of particular items-in-context or their attributes.

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Poster

079. Human Medial Temporal Lobe

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

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Title: Human single-neuron dynamics during saccadic and smooth-pursuit eye movements

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Abstract: During eye movements such as saccades or smooth pursuit, corollary discharge signals enable the brain to distinguish between sensory effects of self-movements and of movements in the environment, and thereby facilitate stable visual perception and spatial remapping. However, it is unclear whether neurons in the medial temporal lobe (MTL), piriform cortex (PIC) and orbitofrontal cortex (OFC) rely on such signals. Here we asked if there exist dynamics in the single unit activity of these regions that are indicative of a corollary discharge. In up to now 13 experimental sessions, we recorded 1279 units in the medial temporal lobe (amygdala, entorhinal cortex, hippocampus, parahippocampal cortex), the OFC, and the PIC from 8 neurosurgical patients implanted with hybrid Behnke-Fried depth electrodes, and performed simultaneous eye-tracking during a smooth-pursuit paradigm. Subjects were instructed to follow a ring-shaped target moving sinusoidally along the horizontal axis with their eyes, during which on average 770 saccades (SD = 156) were detected per experimental session. In our preliminary dataset, we found a significant proportion of units exhibiting differences in firing rates during perisaccadic periods versus periods of smooth pursuit in the OFC, the PIC, and when considering units from all regions. Of these units, putative interneurons were significantly more likely to exhibit increased perisaccadic firing rates, while putative pyramidal neurons were more likely to show decreased firing rates around saccades. A significant proportion of units in the parahippocampal cortex and when including units from all regions showed firing rates correlated with target direction. The change in neuronal activity around the time of saccades, specifically the increase in putative inhibitory and decrease in putative excitatory unit activity, indicate that these signals could come from corollary discharge, which would be expected to be stronger in the perisaccadic period versus periods of smooth pursuit. Units with firing rates correlated with the target direction could receive directional information through corollary discharge. It remains an open question how these signals are utilized in the investigated regions.

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Poster

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Title: Regional differences in single neuron timescales between static and dynamic stimuli in the human medial temporal lobe

Authors: *A. DARCHER[1], R. GAO[3], V. BORGER[1,2], R. SURGES[1,2], J. H. MACKE[3,4], S. LIEBE[3,5], F. MORMANN[1];
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Abstract: Understanding our environment requires us to integrate information across a multitude of timescales, ranging from milliseconds to hours. Recent work in human and non-human primates has shown that the timescales of neuronal dynamics reflect temporal information about the environment. While hierarchies of neural timescales and their dynamics have been characterized in several brain areas of non-human primates and humans via fMRI and electrocorticography, the extent to which neural timescales vary with those of the environment has not been addressed in human medial temporal lobe (MTL) neurons. Here, we examined the decay of the spike count autocorrelation of over 1500 units in the amygdala, hippocampus, entorhinal cortex, and parahippocampal cortex recorded using hybrid Behnke-Fried depth electrodes from over 35 neurosurgical patients during a paradigm consisting of both static images and a full-length movie, as well as interstimulus baseline periods. To investigate the presence of a temporal processing hierarchy in the MTL, we quantified the timescales of neurons during each of the three experimental conditions and performed regional comparisons.
Across all conditions, we observed consistent differences between MTL subregions, with the shortest neural timescales found in the entorhinal cortex and amygdala, followed by the parahippocampal cortex, and the longest in the hippocampus. Furthermore, we found a distinct hierarchy of timescales within the hippocampus, increasing from anterior to posterior recording sites. Comparing the timescales within a given subregion during the different conditions, we found that they did not significantly change within a neuron based on the stimulus type. However, when examining the stability of neural timescales within neurons across the course of the naturalistic, continuous stimulus, we found that they varied considerably throughout the film presentation. Taken together, these preliminary findings indicate that, while there is a range of timescales present in each region, there may be consistent trends in the differences between these timescales across the subregions of the MTL. Additionally, these findings suggest that the timescales of neurons in the human MTL are consistent across stimulus types.

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Poster

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Title: Concept neurons in the medial temporal lobe represent the semantic building blocks of human memory

Authors: *S. MACKAY[1], T. P. REBER[4], M. BAUSCH[1], M. SOEHLE[2], C. E. ELGER[1], J. BOSTRÖM[3], F. MORMANN[1];
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Abstract: Concept neurons in the human medial temporal lobe (MTL), that respond invariantly to the semantic contents of a presented stimulus regardless of context or sensory modality, have been hypothesized to represent the semantic building blocks for episodic memory, but direct evidence for this hypothesis is lacking to date. We investigated the firing behavior of concept cells and spatially tuned neurons in the human medial temporal lobe (MTL) during associative memory formation.
In a first associative memory paradigm, we presented response-eliciting images (referred to in short as items) within a 3x3 grid on a laptop screen. Several item locations had to be remembered during a distraction task and then recalled. We were able to detect selective neurons responding to items and spatial locations. Spatially tuned neurons responded to one or several grid positions, and were most prevalent in the parahippocampal cortex (PHC). Both types of responses (item and location responses) had memory-predictive properties in that firing rates were higher during successful encoding than during unsuccessful encoding. Furthermore, this effect occurred in distinct brain regions for item and spatial responses, namely in the hippocampus, amygdala and entorhinal cortex for item responses and in the PHC for spatial responses. The second associative memory paradigm employed a similar task but included pharmacologically induced anterograde amnesia, using low doses of propofol. This intervention is particularly interesting because it dissociates conscious perception from memory encoding. Data from this task allowed us to observe that the general effects of propofol on selective responses during the encoding phase were suppression and delay. We investigated the degree to which the responses were suppressed and delayed, and were able to compare these shifts for items that were subsequently remembered

versus forgotten. Responses to subsequently forgotten items were more strongly affected by propofol, i.e. during trials where response properties were altered, the information was less likely to be successfully encoded.
Our results are the first direct evidence of the crucial role of concept neurons for memory formation. Further research is needed to investigate how response strength and timing of concept neurons contribute mechanistically to successful memory encoding.

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Poster

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Title: Semantic tuning curves of single neurons in the human medial temporal lobe

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Abstract: The Medial Temporal Lobe (MTL) plays a crucial role in processing declarative knowledge such as forming and retrieving episodic memories and semantic information. It contains ‘concept cells’ that act as semantic building blocks for episodic memory. They respond invariantly to a specific concept regardless of the context or sensory modality of a stimulus. The span of semantic space such a neuron responds to is, however, currently unknown. Furthermore, there is ongoing debate whether these cells respond in a binary fashion or their response strength varies for different stimuli. The latter may be described by tuning curves, which characterize a graded response of neurons to stimuli dependent on their ‘semantic distance’ to the preferred stimulus. Neurons tuned to specific concepts, such as numbers, have been found in the MTL. However, the existence of semantic tuning curves of neurons in the MTL has not yet been established.
In this study, we investigated semantic tuning by analyzing the scope of the semantic region that elicits a response in a concept cell within the MTL. We used the THINGS database to attribute a

metric for semantic relatedness between concepts. The dataset contains images of objects along with a semantic embedding. We recorded single unit activity from human subjects implanted with intracranial microelectrodes in the MTL. To explore the semantic field a single neuron responds to, we developed an experimental setup which allows for dynamic stimulus selection during the experiment by performing real-time data analysis. More specifically, the experiment starts by presenting a set of stimuli covering the underlying semantic space. Our setup determines the best response eliciting candidates in real-time, directly selects new stimuli in their semantic vicinity, and presents them to the patient. This local search allows us to evaluate the response behavior of neurons in the semantic space around their preferred stimulus. By doing so, we can also search for a local maximum, i.e. the best stimulus in a neighborhood to elicit a response. The system we developed facilitates the investigation of the semantic coding in concept cells and the contribution of semantic features to their responsiveness.
Our data suggests that semantic tuning curves for concept cells do exist. We identified single neurons reacting with varying response strength to several semantically related stimuli. In general, we found that the average response strength of the recorded concept cells increases with semantic similarity to the preferred stimulus. The probability that a neuron responds to another stimulus is also dependent on the semantic similarity to the preferred stimulus.

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Poster

079. Human Medial Temporal Lobe

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Topic: H.09. Spatial Navigation

Support: NIH/NINDS Grant 2R01NS076856

Title: Navigation and mental replay involve different frequencies of theta oscillations

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Abstract: Theta oscillations, semi-periodic fluctuations in the local field potential of the hippocampus, play an important role in supporting spatial learning in humans. Both theta power and frequency increase with increasing movement speed although the functional significance of this effect remains unclear. A separate line of research has shown that hippocampal theta oscillations also contribute to episodic memory and relate to successful encoding of memories. Yet, these two areas of research remain poorly connected and how imagination as part of episodic memory relates to navigation remains unclear. To address this issue, patients with implanted intracranial electrodes navigated specific routes and then imagined the route that they

had just navigated in vivid detail. Consistent with past work, we found a significant correlation between navigation and replay duration, suggesting that the route replay related to the route just navigated, although replay occurred at faster rate than navigation. Electrophysiologically, we found significant increases in theta power in the range of 8 - 14 Hz during replay compared to both movement and standing still during navigation. We used the extended Better OSCillation detection toolbox (eBOSC) to compare the frequency specific amplitude and duration profiles during navigation and replay. The prevalence of oscillatory events in the low theta frequencies (2-4Hz) was significantly greater during navigation than during replay while the prevalence of oscillatory events in the high theta frequencies (8-11Hz) was significantly higher during replay than during navigation. Given previously demonstrated correlations between speed and theta power/frequency, our findings suggest that replay likely involves recapitulation of learned routes but at a faster rate neurally than during navigation. Our findings also imply that part of episodic memory may involve replay of memories but at a compressed rate to which they were originally experienced.

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Poster

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Topic: H.09. Spatial Navigation

Support: NIDS Grant R01NS114913

Title: Evidence for preserved recent and remote spatial memory in amnesic individuals

Authors: *J. D. GARREN[1], A. S. MCAVAN[1], S. THAYER[1], A. P. YONELINAS[2], M. D. GRILLI[1], A. D. EKSTROM[1];
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Abstract: Previous reports suggest that individuals with amnesia show profoundly impaired memory for recent spatial locations but relatively intact memory for remote environments that they learned decades before the brain lesion (Teng & Squire 1999; Rosenbaum et al. 2000). Such findings have often been taken to support retrograde amnesia gradients such that remote memories are relatively preserved compared to recent memories. Yet, studies that have shown severely degraded recent compared to remote spatial memories have relied heavily on verbal reports or map drawing, which may be particularly difficult for individuals with amnesia due to impaired verbal recall and explicit memory processes. Here, we tested the recent and remote memories of individuals with amnesia and medial temporal lobe lesions by having them navigate replicas of the towns and cities they grew up in and currently live in. We included an unfamiliar environment they had never visited as an additional control to assay for new spatial learning and compared the performance of the amnesic individuals against age-matched controls. To build the

three virtual environments (recent, remote, novel), we used accurate 3D blueprints of towns or cityscapes that we obtained from CadMapper and opened them in SketchUp+ to render them as 3D environments before importing them into Unity Landmarks. Each participant was then tasked with navigating between four highly familiar target locations in randomized pairs. We computed the optimal distance path between targets and the traveled path, providing distance error per trial. Preliminary analyses showed that while an amnesic individual performed numerically worse than the age-matched control on the novel city, they were relatively unimpaired at both the recent and remote city. These findings suggest that individuals with medial temporal lobe lesions may show at least partially, if not mostly, intact spatial memory for places they have visited frequently, regardless of the age of the memories, provided rich visual and geometric cues are available to them in virtual reality.

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Poster

079. Human Medial Temporal Lobe

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Program #/Poster #: 079.15

Topic: H.09. Spatial Navigation

Support: 2R01NS076856

Title: The Intersection of Space and Time in Navigation and Episodic Memory

Authors: *E. M. ROBINSON[1], A. MCAVAN[1], M. J. STARRETT[3], K. CHAMBERS[1], E. A. ISHAM[2], A. D. EKSTROM[2];
[2]Psychology, [1]Univ. of Arizona, Tucson, AZ; [3]Univ. of California, Irvine, Irvine, CA

Abstract: The ability to successfully navigate through an environment is an instrumental skill needed for our everyday experiences. A crucial component of navigation includes the ability to integrate both spatial and temporal information to form memories of our spatial environments. However, the way in which environments are encoded in episodic memory with respect to space and time remains unclear. Previous work has shown that spatial representations differ depending on access to body-based, vestibular input during navigation. To that end, we implemented a task that compared immersive and non-immersive navigation conditions (i.e., with and without enriched body-based cues) to further assess how walking affects spatial vs. temporal knowledge. Subjects were randomly assigned to one of two conditions. In the immersive condition, subjects navigated through a virtual city by walking on an omnidirectional treadmill to specific targets by memory. In the non-immersive condition, subjects remained still while navigating through the virtual environment using an Xbox controller without access to body-based information. Following navigation, subjects completed a temporal reproduction task, a judgments of relative direction task, and a verbal episodic memory recall. In the temporal reproduction task, subjects

indicated how long they perceived it took them to travel to the previous target. In the judgments or relative direction task, subjects were asked to imagine they were standing in front of one location, facing another and to point to a third location using a virtual compass. In the verbal episodic recall task, responses were recorded and scored using an encoding method developed from the Levine Method. Our results show that the level of perceptual internal details during episodic memory recall is significantly correlated with reproduced time. This effect was more salient during non-immersive episodic conditions, suggesting that details of time and space may contribute to episodic memory independently. Subjects made more verbal references during recall to body-based information in the immersive condition while subjects made more allocentric references in the non-immersive condition. Critically, a significant effect of condition (immersive vs non-immersive) was found for path error in which walking in virtual reality led to lower path error compared to navigating with a joystick. In contrast, while not statistically significant, subjects made numerically lower temporal reproduction error in the non-immersive condition, suggesting that time and space may be contributing to spatial navigation and memory for navigation independently.

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Poster

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Topic: H.09. Spatial Navigation

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Title: Evidence for flexible navigation strategies during spatial learning involving path choices

Authors: *S. G. DONER[1], J. ZHENG[2], A. S. MCAVAN[3], M. J. STARRETT AMBROSE[5], H. CAMPBELL[3], D. SANDERS[3], A. D. EKSTROM[4];
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Abstract: There is little debate that humans can learn to optimize their paths during spatial learning, but the conditions under which they spontaneously employ shortcuts remain unclear. In a classic study, Tolman et al. (1946) trained rodents to follow a path to a goal location. They found that the rodents chose the optimal path to the goal location when the originally learned route to the goal was blocked and the remainder of the environment was replaced with a sunburst maze. Subsequent studies have found more mixed results , suggesting that non-optimal response strategies are often used. We addressed this issue across four different experiments by creating a

Virtual Reality (VR) replica of Tolman's maze. We tested human participants in this environment under situations in which a light cue was either absent, consistently located from training to test, or moved a large or small distance between training and test. In Experiment 1, we used desktop VR to replicate the finding that in the absence of a light cue, the majority of participants chose the hallway that was closest to their originally learned route. In conditions in which the light moved, participants switched the hallway they searched based on the movement of the light. In Experiment 2, we added distal visual cues to test whether it would result in a greater use of optimal shortcuts. In contrast to predictions, we replicated the same overall results from Experiment 1. In Experiment 3, we tested whether stronger idiothetic cues would result in greater use of optimal shortcuts by immersing participants in the VR environment using a Head Mounted Display (HMD) and having them walk through the environment using an omnidirectional treadmill. In contrast to predictions, we again replicated the same results overall from Experiments 1 & 2. Experiment 3 was a within-subjects design where all participants experienced all conditions. We wondered if learning/repeated exposure effects affected the results of Experiment 3, so we created Experiment 4 as a return to our original between-subjects design. Our overall findings suggest that participants' search strategies are flexible in that they vary depending on external cues and the initially learned path, even when these do not lead to the optimal route to the goal. Moreover, this pattern of strategy selection was robust even in the presence of distal or enhanced idiothetic cues.

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Poster

079. Human Medial Temporal Lobe

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Program #/Poster #: 079.17

Topic: H.09. Spatial Navigation

Support: NSF BCS 16302296

Title: Navigation and spatial memory benefit more from vision than body-based cues in small-scale spaces: a mobile eye tracking study in a real-world environment

Authors: *A. MULLER, J. GARREN, A. D. EKSTROM;
Psychology, Univ. of Arizona, Tucson, AZ

Abstract: Vision is arguably the most important sense humans use to encode information about spatial environments yet body-based walking cues also contribute essential information. The specific contributions of vision and body-based cues to active human navigation and memory, however, remain unclear. Previous research has found evidence for vision being sufficient to learn an environment while other studies show that body-based cues are critical for spatial learning, particularly in rodents. Memory for object layouts learned from a single viewpoint have

shown encoding alignment effects (objects are remembered more accurately from the encoding viewpoint than other viewpoints). It is unknown, however, whether introducing free ambulation will affect the encoding alignment effect. Therefore, the current research aimed to investigate the degree to which body-based cues can enhance memory in small real-world spaces by pairing mobile eye tracking with a navigation and memory task in an ecologically valid design. Thirty participants donned a mobile eye tracker and stood stationary for 30s to encode the layout of 8 items affixed to the walls of a 6m x 6m room. Then they were asked to either remain still while the researcher removed the items (stationary condition) or were guided around the room blindfolded and removed the items (walking condition). Participants then replaced the items in their original positions from either the same location as encoding (same viewpoint) or on the opposite side of the room (different viewpoint). The results showed that all groups performed comparably. Body-based cues did not affect placement error when replacement began from the different viewpoint compared to beginning replacement in the encoding location, suggesting that the initial viewpoint did not have a beneficial effect on encoding. Items next to immovable "wall landmarks" (e.g., door handle, fire alarm) were remembered more accurately than items that were not next to wall landmarks. Objects were replaced more accurately in the vertical than the horizonal axis. We also found a small but significant negative correlation between placement error and total duration of fixations during encoding, showing better placement accuracy for longer total fixation duration, but no correlation between placement error and number of fixations during encoding. Additional eye tracking analyses considering the effects of fixating landmarks and objects and the identities of gazed-upon objects are discussed. These results suggest that for small spaces, vision may be sufficient for learning the locations of items, with eye fixations playing an important role in encoding these object locations.

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Poster

079. Human Medial Temporal Lobe

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Program #/Poster #: 079.18

Topic: H.09. Spatial Navigation

Support: NSF BCS-1630296

Title: Frontal-midline oscillations index the evolution of spatial memory distortions during active navigation

Authors: *Y. K. DU[1], M. LIANG[2,1], A. MCAVAN[1], R. C. WILSON[1], A. D. EKSTROM[1]; [1]Psychology, Univ. of Arizona, Tucson, AZ; [2]Yale Med. Sch., New Haven, CT

Abstract: It remains a largely unresolved question whether we use a fundamentally Euclidean or non-Euclidean structure to organize our spatial memories. Our previous work has shown that rendering a "false" intersection in immersive virtual reality can create the illusion of a crossed

path from an uncrossed path in violation of Euclidean geometry. Here, we employ wireless scalp EEG to investigate the neural correlates of this memory distortion to determine when and how they might come about. Past research suggests that changes in frontal-midline oscillations correlate with movement and other variables related to navigation like viewing landmarks. Other work in cognitive control suggests a potential role in conflict monitoring and updating. We hypothesized that frontal-midline oscillations might differentiate the conditions based on differences in conflict monitoring and spatial updating. Participants walked four-segment paths with 90° turns in immersive VR and pointed to their start location when they arrived at the end of the path. The paths either contained a crossing (True Intersection Cross, TI-C condition; no conflicts) or contained no crossing but two false intersections (False Intersection No Cross, FI-NC condition; conflicts between visual and idiothetic cues). Replicating our previous behavioral work, pointing errors indicated greater memory distortions for the walked paths in the FI-NC condition than in TI-C condition. Our modeling results suggest that the distortion in the FINC condition likely came about from an underestimation of the final walked leg. Power spectral density plots showed low-frequency increases in both conditions, likely related to active movement. Comparing the power spectra, we found that the representational similarity between the first and second times passing the areas where the intersection was rendered was significantly higher in the TI-C than in FI-NC condition. This finding suggests that when participants walked through the second false intersection in FI-NC condition, they possibly detected the conflicting visual and idiothetic cues, resulting in a less similar representation due to spatial updating. When participants were asked to imagine the start of the path from the end, we observed significantly higher frontal-midline beta power in FI-NC than TI-C condition during the earliest retrieval timepoints. These findings may indicate an active reconciliation of the mismatching information in the FINC condition during retrieval. Together, these results indicate that frontal-midline oscillations play important roles in forming and updating of spatial memories and reflect multi-modal cue competition in navigation.

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Poster

079. Human Medial Temporal Lobe

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Title: WITHDRAWN

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.01

Topic: H.07. Long-Term Memory

Support: National Natural Science Foundation of China 31922089

Title: Examining emotional memory processing among insomnia patients

Authors: *S. ZENG[1], H. SIT[1], S. LI[1,2,3], X. HU[1,2,3];
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Abstract: Insomnia is frequently co-morbid with emotional disorders in which disrupted emotional memory is often implicated. Yet, much remains unknown regarding emotional memory processing in the insomnia brain. In this study, we examined emotional memory processing at post-encoding, post-sleep, and 7-day delayed sessions among 12 individuals with insomnia (8 females; M_{age} = 22.83) and 15 healthy sleepers (11 females; M_{age} = 22.13). All participants completed a clinical interview to ascertain their eligibility, followed by encoding 48 pseudo-word + negative/neutral scene picture pairs (24 negative + 24 neutral pairs) in the evening before nocturnal sleep. After one night of electroencephalogram (EEG)-recorded sleep, they were tested on the pictorial memory and associated emotional responses given the cue words at post-sleep and 7-day delayed sessions. EEGs were recorded during all sessions.
We found significant group differences when participants initially viewed the pictures: compared to healthy sleepers, participants with insomnia rated both negative and neutral pictures more negatively (p = 0.019). Multivariate classification on the ERP cortical patterns suggested that while healthy sleepers exhibited robust negative vs. neutral brain representation during 960 – 1820ms post-stimuli (an average of 59% decoding accuracy vs. 50% chance level, $p < 0.001$), participants with insomnia did not show an above-chance level decoding, potentially suggesting a different neural pathway for affective picture processing among insomnia group.
Controlling for the initial valence rating, a linear mixed model with valence rating during memory recall as the outcome suggested that participants with insomnia rated the associated negative pictorial memory more negatively than their healthy counterparts across post-sleep and delayed sessions (p = 0.016), while the ratings for neutral memory was comparable between groups (p = 0.98). In addition, we observed that at the 7-day delayed session, insomnia group reported better negative pictorial memory than neutral ones (p = 0.03), while healthy controls exhibited comparable memory performance (p = 0.11). Corroborating these results, ERP analysis suggested that for frontal 175-250ms P2 activity, insomnia group showed a significantly stronger P2 to negative memory cues than neutral cues on all three recall sessions (p = 0.01), while healthy counterparts did not show such P2 differences (p = 0.97). Overall, our study provided preliminary results suggesting that negative memories may bear more motivational significance in the context of insomnia, which is often linked to emotion dysregulation and mood disorders.

Disclosures: S. Zeng: None. **H. Sit:** None. **S. Li:** None. **X. Hu:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.02

Topic: H.07. Long-Term Memory

Support: National Natural Science Foundation of China 31922089

Title: N400 during social norms learning predicts delayed social conformity

Authors: *D. CHEN, Z. YAO, X. HU;
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Abstract: Compelling evidence suggests that people often feel obliged to change their behavior, preferences, and beliefs to align with perceived social norms. However, whether lab-based social influence could be long-lasting (i.e., more than seven days) remains uncertain. Here, in a pre-registered EEG study, we explored the relationship between ERPs during normative feedback processing and the delayed effect of social influence.We recruited forty-eight participants (37 females; Age, Mean = 23.98, S.D. = 3.13) for a social learning task. Seven participants were excluded due to poor EEG quality. Participants learned about their peer's attractiveness ratings while their brainwaves were recorded in this task. We manipulated the perceived peer's ratings as HIGHER, LOWER, or CONSISTENT compared to participants' initial ratings. Seven days later, participants returned to the lab and rated the attractiveness of the same faces again, followed by recalling the peers' ratings they learned during the first lab visit. We categorized the items into CORRECTLY and INCORRECTLY recalled items based on the discrepancies between recalled and presented peers' ratings. We also computed the absolute difference between recalled and actual peers' ratings as memory error and between participants' and peers' ratings as the conformity level, with higher scores indicating less conformity. We found that participants still showed conformity in the seven-day delayed session, which is memory-dependent: the update of attractiveness rating differed across three conditions, but only for CORRECTLY remembered items, F (2, 54) = 64.08, $p < .001$, $\eta 2 = .505$. Post-hoc comparisons revealed that the update of attractiveness rating of LOWER condition was significantly lower than of HIGHER condition, t (27) = 11.15, $p < .001$ and than of CONSISTENT condition, t (27) = 4.71, $p < .001$, and the rating of CONSISTENT condition was significantly lower than of HIGHER condition, t (27) = 6.67, $p < .001$. In addition, the memory error predicted the conformity level on a single trial level, β = -.057, $p < .001$. Importantly, we found that the central N400, an ERP component implicated in semantic memory and context congruity, predicted both the absolute memory error, β = -.011, p = .053, and the conformity level, β = -.001, p = .004.Our results indicate that 1) N400s during normative feedback processing were associated with both memory errors and updating changes, and 2) the memory of social norms plays a crucial role in the long-lasting effect of social influence. These findings pinpointed a possible neural mechanism of social norm violation detection (i.e., N400) that contributed to long-term social learning and conformity.

Disclosures: **D. Chen:** None. **Z. Yao:** None. **X. Hu:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.03

Topic: G.01. Fear and Aversive Learning and Memory

Support: 31922089 National Natural Science Foundation of China

Title: Pre-sleep testing weakens the target memory reactivation beneficial effect

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Abstract: Memory reactivation during both wake and sleep promotes the formation of long-lasting memory traces. Emerging evidence shows that testing during wakefulness leads to better long-term memory via fast consolidation; cueing previously learned materials during sleep facilitates memory via system consolidation processes. However, whether and how the fast consolidation processes afforded by wakeful testing would influence the subsequent sleep-mediated consolidation remains largely unknown. Using the targeted memory reactivation (TMR) approach, we aimed to investigate the relationship between pre-sleep testing and the TMR effect on long-term memory formation. Participants completed the following sessions in order, 1) learning and tests before sleep, 2) TMR cueing during the NREM sleep, and 3) post-sleep memory tests. Participants learned auditory cue words and picture pairs during the learning period, with half of the pairs tested before they went to sleep. In TMR, we systematically assigned half of the tested pairs and half of the untested pairs to the TMR cueing condition, during which the auditory cues were played during the NREM sleep. Results revealed that pre-sleep tested items showed significantly better long-term memory accuracy than untested items. Moreover, TMR cueing enhanced the subjective remembering for cued than uncued items, especially for pre-sleep tested items. However, there is neither a significant difference in accuracy between cued and uncued items nor a significant interaction effect between testing and TMR cuing effects. In terms of neural activity, we found that TMR cue-locked theta power predicted the TMR benefits for pre-sleep untested items, but not for pre-sleep tested items. These results indicate that pre-sleep testing may weaken the TMR benefits while enhancing subjective memory confidence.

Disclosures: **J. Liu:** None. **X. Hu:** None.

Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.04

Topic: H.07. Long-Term Memory

Support: IBS-R015-D1
NRF-2019M3E5D2A01060299
NRF-2019R1A2C1085566
NRF-2021R1A6A3A13044260

Title: Solving the narrative puzzle: Memory retrieval in the hippocampus during ongoing narrative perception

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Abstract: Humans understand the story by constructing a coherent narrative structure from the perceived events according to their temporal and causal relationships. Previous computational work demonstrated that constructing the narrative structures when perceiving uncertain events entails retrieving past events from memory to piece together with the ongoing event (Qihong et al., 2022). In this study, we examined the neural signatures of narrative memory retrieval during the ongoing narrative perception. We conducted an fMRI experiment in which participants (N=65) watched a 10-min movie consisting of temporally scrambled events and recounted the story in its original sequence. From the scrambled structure of the events, we identified the moments at which the memory retrieval would occur to reconstruct the unscrambled narrative structure of the story. Around the event boundaries that correspond to those moments, we found selective responses for narrative memory retrieval in the hippocampus: Hippocampal activity patterns of the currently perceived event match the pattern representation of the event that immediately follows or precedes the current event in the original sequence of the movie. We further hypothesized that the memory retrieval in the hippocampus would be related to the participant's ability to reconstruct the story. To examine this question, we computed participants' story understanding ability from the amount of recalled information (content score) and the accuracy of reordering the temporal sequence of recalled events (ordering score) by comparing the similarity in the semantic content between the original movie annotations and each subject's recall using a topic modeling method (Heusser et al., 2021). We found that the ordering score was positively correlated with the narrative retrieval response in the hippocampus, whereas the content score was not. Furthermore, to investigate the characteristics of the narrative memory being retrieved, we estimated the coherence of each event in the narrative using the pre-trained deep language model. We found significant narrative coherence between the current and associated past events that evoke retrieval while controlling for the semantic similarities between events. These results suggest that the hippocampus plays a critical role in retrieving past memories during ongoing narrative perception. It also implies that the hippocampal memory retrieval can lead to individual differences in their cognitive abilities to construct the narrative structure in story comprehension.

Disclosures: **J. Park:** None. **H. Song:** None. **J. Choi:** None. **W. Shim:** None.

Poster

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Program #/Poster #: 080.05

Topic: H.07. Long-Term Memory

Support: NIH Grant R01MH119099

Title: Spontaneous memory recall in the dynamic flow of thoughts

Authors: *H. LEE, S. BORN, C. J. HONEY, J. CHEN;
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Abstract: When our minds wander, memories of past events arise amidst other thoughts. What are the cognitive and neural states that trigger episodic memory recall within the flow of spontaneous thoughts? To explore this question, we performed an fMRI experiment in which subjects were asked to verbally describe any thoughts that entered their consciousness for 10 minutes. Subjects' verbal responses were manually segmented into individual "thoughts" based on changes in either the topic or the category of their speech. Five major thought categories were identified: episodic memory, autobiographical semantic memory, non-autobiographical semantic memory, future thinking, and current sensations and feelings. Episodic recall on average accounted for 16% of thoughts, sporadically distributed over the 10-minute session. To test whether episodic recall was triggered more by some categories of thoughts than others, we computed transition probabilities between different categories of thoughts. We found that episodic recall was not triggered by any specific thought category more than expected by chance, including episodic recall itself. We next computed semantic similarity between thoughts using a natural language processing model. Semantic similarity was higher between an episodic memory and its immediately preceding and following thoughts compared to more distant ones, suggesting that episodic memory was triggered by related thought content. This semantic similarity autocorrelation effect was also observed in other categories of thoughts. Finally, we examined brain activation patterns at transitions between different thought categories or topics, focusing on the posterior medial cortex (PMC). PMC previously exhibited an activation pattern specific to major mental context transitions, and the pattern was consistent across different tasks and stimuli (Lee & Chen, 2022, eLife). We found that PMC patterns at topic transitions were positively correlated with the major context transition pattern observed in the prior study more so than category-transition patterns were, implying that topic transitions were experienced as more prominent changes in mental contexts. Non-boundary patterns in the middle of thoughts were not correlated with the major context transition pattern. Together, these results demonstrate that episodic memories are naturally retrieved by shared meanings without any task demands, and suggest that semantic connections may be a major organizing principle of the flow of spontaneous thoughts.

Disclosures: H. Lee: None. **S. Born:** None. **C.J. Honey:** None. **J. Chen:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.06

Topic: H.07. Long-Term Memory

Title: Oscillating Memory: Memory Facilitation through Fixed Frequency Theta Stimulation with Persistence of Effect

Authors: *B. M. ROEDER, M. R. RILEY, R. E. HAMPSON;
Wake Forest Sch. of Med., Winston-Salem, NC

Abstract: Theta oscillation in the hippocampus plays an important role in memory. Analysis of stimulation patterns generated in real time by the Multi Input Multi Output (MIMO) model used in our 2018 report (Hampson et al. J. Neural Eng, 2018, 15:036014) showed a Theta component. This led to testing whether fixed frequency stimulation in the Theta range would enhance memory performance. Other studies exploring fixed frequency stimulation of the hippocampus have primarily used frequencies in the middle of the Gamma range (or higher), with mixed, though primarily detrimental results. A Delayed-Match-to-Sample+Delayed Recognition (DMS+DR) task (with stimulation during the Sample/encoding phase of the DMS portion) was used to determine whether Theta stimulation influences memory function out to a time of 90 minutes.

DMS trials were composed in alternating blocks of stimulated and non stimulated trials. Most patients showed an increase in memory performance on the first block of stimulated trials, with an additional increase in performance on the second stimulation block, as compared to the initial block of non stimulated trials. Additionally, most patients showed an increase in performance on non stimulated trial blocks that followed a stimulated trial block, with maximal facilitation achieved after two stimulation blocks. This persistence of increased effect was not seen in stimulation with the MIMO model (with randomized stim/no-stim trials) where beneficial effects of stimulation only occurred for stimulated trials.

These initial results suggest that reinforcing Theta oscillation in the hippocampus is a viable means of facilitating memory function. As all patients received stimulation at the same Theta frequency the amount of facilitation, and whether facilitation persists, may be dependent on choosing the optimal frequency within the Theta range for individual patients. Neural prosthetics to counter memory dysfunction due to diseases, such as Alzheimer's, or damage from Traumatic Brain Injuries, may require more than one approach to restore functionality to patients. These results offers a new approach in how a neural prosthetic could not only potentially offer facilitation of memory, but to provide persistent facilitation using intermittent stimulation.

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Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.07

Topic: H.07. Long-Term Memory

Support: Intramural Research Program of NINDS

Title: Memory Retrieval Reinstatement of Neural Connectivity Patterns

Authors: *A. PHAN, W. XIE, J. CHAPETON, K. ZAGHLOUL;
NIH, NIH, Natl. Inst. of Neurolog. Disorders & Stroke (NINDS), Bethesda, MD

Abstract: Successful memory retrieval has been shown to occur with reinstatement of neural activity first present during encoding. Previous evidence has shown memory reinstatement of oscillatory power at the level of individual electrodes; however, it is unknown whether groups of electrodes reinstate their network connectivity patterns during memory retrieval. We examined this question using intracranial electroencephalography data captured from 20 participants with medically refractory epilepsy as they performed a paired-associates verbal memory task. We first identified connected electrode pairs by selecting pairs of electrodes across the cortex that exhibited strong correlations with a consistent time lag across random segments of recording data. We next calculated the connectivity of these pairs at the maximal time lag while participants were performing the paired-associates verbal memory task. We calculated the similarity of connectivity patterns across electrode pairs in the temporal lobe between encoding and retrieval periods. Our data revealed a significantly higher encoding-retrieval similarity in the observed connectivity patterns for correct retrieval trials as compared to incorrect retrieval trials. Collectively, these results suggest that successful memory retrieval entails the reinstatement of regional network connectivity patterns initially present during memory encoding.

Disclosures: A. Phan: None. **W. Xie:** None. **J. Chapeton:** None. **K. Zaghloul:** None.

Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.08

Topic: H.07. Long-Term Memory

Support: Charles E. Kaufman Foundation KA2020-114800

Title: Effects of the order of reactivation during sleep on memory and generalization

Authors: *E. M. SIEFERT[1], J. MU[3], S. UPPULURI[2], J. W. ANTONY[4], A. C. SCHAPIRO[2]; [1]Neurosci., [2]Psychology, Univ. of Pennsylvania, Philadelphia, PA; [3]Psychology, Haverford Col., Haverford, PA; [4]Ctr. for Neurosci., Univ. of California, Davis, Davis, CA

Abstract: Neural network models now approach or surpass human level performance in complex learning, memory, and generalization tasks. The success of these models has relied on a critical property of their input: information is presented in interleaved order. Interleaving allows a model to construct representations that reflect the full structure of the inputs, supporting successful generalization. In contrast, when inputs are presented in blocked order, with one set of information seen entirely before a second set, the second set often interferes with the first. Based on these behaviors of neural network models, the Complementary Learning Systems (CLS) theory proposed that in the brain, the hippocampus reactivates recent information in interleaved order during sleep, allowing the neocortex to extract the regularities across this information, promoting understanding and generalization while minimizing interference. Here, we provide the first test of the idea that interleaved memory reactivation during sleep is critical for memory and generalization using the Targeted Memory Reactivation (TMR) method. Participants came into the lab and learned three categories of novel objects. Each object had unique features as well as features shared with members of its category. As participants learned the visual features of an object, they also heard its spoken name. The spoken names of the objects were then used as cues for TMR during a subsequent nap. To optimize the likelihood of the cues leading to reactivation events, we used a real-time closed-loop system to administer cues at the peaks of slow-wave oscillations while avoiding spindle refractory periods. For each participant, TMR cues were administered for one category of objects in interleaved order, one category in blocked order, and one category was left uncued. We evaluated changes across the nap for unique feature memory, shared feature memory, and generalization (the ability to infer missing features of novel objects from the studied categories) as a function of whether objects were in the blocked, interleaved, or uncued category. We report preliminary results suggesting that interleaved reactivation during sleep promotes generalization, but we find no difference in the effect of interleaved or blocked reactivation during sleep on unique or shared feature memory. If these results persist in the full sample, they would support the CLS hypothesis that interleaved reactivation is critical for generalization.

Disclosures: E.M. Siefert: None. **J. Mu:** None. **S. Uppuluri:** None. **J.W. Antony:** None. **A.C. Schapiro:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

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Program #/Poster #: 080.09

Topic: H.07. Long-Term Memory

Support: NRSA F32 (F32EY032352)

Title: Testing theories of neural dynamics during free recall for naturalistic experiences

Authors: *H. R. DIMSDALE-ZUCKER[1], J. A. WILLIAMS[3], K. NORMAN[4], C. BALDASSANO[2];
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Abstract: Free recall provides a window into how the mind stores and organizes our experiences, and has fascinated memory researchers for centuries. Canonical approaches to understanding the organization of free recall have focused on memory for lists of discrete items (Ebbinghaus, 1885; Kahana, 1996). Current models that predict which items will be remembered and their output order make use of multiple features including the temporal order of items at encoding, semantic associations, or associated source information (Howard and Kahana, 2002; Polyn et al., 2009). Yet, our day-to-day world is composed of continuous experiences that are far more complex than the simple lists of memoranda typically used to study free recall and that underpin these models. In order to bridge between the way in which free recall has been studied in classic laboratory tasks and our naturalistic experience of our ongoing world, we propose future modifications to extend these existing models of free recall to continuous narrative events as well as to test novel models based on the types of transitions participants make when recalling (e.g., thematic transitions). Participants (N = 13) watched short video clips (10 clips in each of 12 runs) while undergoing functional magnetic resonance imaging (fMRI). Following free-viewing of each set of 10 clips, participants were then asked to freely recall anything they could remember. Here, we utilize a recently-developed novel application of Hidden Markov Models (HMM; Baldassano et al., 2017), that can segment continuous fMRI data and instantiate different hypotheses about the structure of recall. We find that a simple version of the HMM, which assumes recall progresses exactly in-order from the first item seen at encoding to the last, provides a better fit to the fMRI data from posterior medial cortex than a null HMM in which events are randomly ordered. However, a proof-of-concept version of the HMM given information about the order in which participants recalled clips outperformed the simple encoding-order model in segmenting neural data from recall in a way that matches behavioral recall output (improving the fraction of recall timepoints correctly decoded by 59%). This suggests that recovering event transitions in recall can be improved when imbued with salient information about what may lead to transitions in free recall. Our future extensions testing multiple versions of the HMM that make different assumptions about the dynamic structure of recall will provide a way to test theories of recall dynamics in naturalistic free recall.

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Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.10

Topic: H.07. Long-Term Memory

Support: NIH Grant R15MH114190

Title: Directed Information Flow During Episodic Memory Retrieval At Theta Frequency

Authors: *P. F. BLONIASZ[1,2], K. E. WALSH[2,4], S. C. WYNN[3,5], E. NYHUS[3];
[1]Grad. Program For Neurosci., Boston Univ., Boston, MA; [2]Program in Neurosci. and Program in Digital and Computat. Studies, [3]Dept. of Psychology and Program in Neurosci., Bowdoin Col., Brunswick, ME; [4]Salk Inst. for Biol. Studies, UCSD, San Diego, CA; [5]Ctr. for Human Brain Hlth., Univ. of Birmingham, Birmingham, United Kingdom

Abstract: Episodic memory retrieval enables the recollection of personal experiences and is facilitated by an extensive network of brain regions including the prefrontal cortex, parietal cortex, and hippocampus (frontal-parietal-hippocampal network). Little is known about the functional connectivity of these regions during retrieval. Theta oscillations (3-8 Hz) act as a possible mechanism for the interaction between these brain regions during episodic memory. Theta oscillations modulate interactions between the frontal-parietal-hippocampal network during memory-related cognitive processes (Nyhus & Curran, 2010). However, existing research is correlational. The present research investigates the directional flow of information between the frontal-parietal-hippocampal network during retrieval. Based on previous work (Nyhus & Curran, 2010; Anderson et al., 2010), it was expected that at theta frequency there is directed information flow from the left inferior parietal cortex (IPC) to the right dorsolateral prefrontal cortex (dlPFC). The present study applied a type of Granger causality analysis, specifically renormalized Partial Directed Coherence (rPDC), to measure the directional flow of information in previously recorded electroencephalography (EEG) data during a source (n = 32, 15 female) and item (n = 32, 14 female) episodic memory retrieval task. Using the EEGLAB toolbox groupSIFT (Source Information Flow Toolbox), across-subject rPDC was performed on the EEG data. One connection of interest was from the right precuneus region in the parietal lobe to the right superior frontal lobe (which contains the dlPFC), summed $t = 137$, $p < 0.05$. Directed theta coherence was greater for the hits condition, which requires source monitoring, compared to the correct rejections condition, which does not require as much source monitoring. This suggests the importance of theta oscillations in transient network dynamics during post-retrieval monitoring in episodic memory. Continued research will further analyze this preliminary, exploratory work which identified the potential relevance of the precuneus in the functional network underlying source memory retrieval.

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Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.11

Topic: H.07. Long-Term Memory

Support: R01MH116914

Title: Individual-specific memory reinstatement patterns within human face-selective cortex

Authors: *Y. Y. CHEN[1], A. ARETI[2], B. L. FOSTER[1];
[1]Neurosurg., Univ. of Pennsylvania, Philadelphia, PA; [2]Rice Univ., Houston, TX

Abstract: Humans have the remarkable ability to recall past events vividly. According to the theory of sensory reinstatement, brain regions involved in the initial sensory processing of an item are later reactivated to support successful memory retrieval. Prior work in human functional neuroimaging has shown evidence for the reactivation of sensory cortices during retrieval across different modalities. However, such evidence was often assessed at a coarse anatomical scale. Therefore, careful examination is required to elucidate how reinstated neural patterns during retrieval conform or systematically differ from established functional neuroanatomy for sensory encoding. To precisely determine the configuration of sensory reinstatement responses, we leveraged the well-known organization of face-selective areas in the human visual system and examined the retrieval-based reactivation of face stimuli within and across these areas. Using fMRI, we first identified face-selective areas while participants (n = 17) viewed images from 10 visual categories. Multiple face-selective areas were identified within each participant's native brain space: two fusiform (mFus and pFus) and one inferior occipital gyrus (IOG) face areas. While the specific location of the identified areas varied across individuals, their relative topography was consistent. Next, we probed memory processes using a paired-associates task. During encoding, participants studied word-famous face pairs. During retrieval, participants were presented with only the cue word (from the encoding set or lures) and were required to retrieve the associated famous face. We examined responses during encoding and retrieval within the identified areas. During retrieval, all face-selective areas showed increased responses beyond baseline levels and significantly greater responses than a neighboring control region selective for 'place' stimuli. While the reinstatement response within each face area differed between individuals, responses in mFus and pFus conformed to their encoding response pattern. Moreover, reinstatement responses in mFus were positively correlated with individual memory performance. These findings suggest encoding-retrieval sensory reinstatement recapitulates individual-specific functional neuroanatomy, but with a distinct bias in the relative activation pattern across the encoding substrate during memory retrieval. Therefore, consideration of individual-specific functional neuroanatomy will be critical in identifying common principles of the transformation between perceptually and mnemonically driven activation within the sensory cortex.

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Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.12

Topic: H.07. Long-Term Memory

Title: Attention modulates single-unit sequences in human anterior temporal lobe during memory encoding

Authors: *K. K. SUNDBY[1], J. WITTIG[2], A. VAZ[3], K. ZAGHLOUL[2];
[1]Natl. Inst. of Neurolog. Disorders and Stroke, [2]Natl. Inst. of Neurolog. Disorders and Stroke, Bethesda, MD; [3]Duke Univ. Sch. of Med., Duke Univ. Sch. of Med., Durham, NC

Abstract: Attention has been shown to augment memory, yet the neural mechanism remains poorly understood. In the current study, we examine how attention modulates single-unit spike sequences that occur during encoding. Prior work found that populations of neurons recorded from human anterior temporal lobe (ATL) fire in bursts repeatedly throughout encoding and that the sequence of neurons firing is unique to the item being encoded. Further, the similarity between burst sequences within a given trial, i.e. for a given memory item, was predictive of later recall. Thus, prior work suggests that the repetition of memory-specific spike sequences may critically contribute to memory formation. We posit that attention augments encoding, at least in part, by enhancing the consistency of spike sequences during encoding. To test this, we recorded spiking neurons from the ATL in 9 medication resistant epileptic patients as they performed a word memorization test. The task included three conditions: pre-cued attention trials with a pre-word cue prompting the participant to pay attention to the subsequent word, post-cue attention trials with the attentional cue occurring after word presentation, and non-cued trials. We quantified within trial sequence similarity for each trial with multiple bursts using the matching index (MI) with a higher MI indicating greater sequence similarity. We expected pre-cued attention trials to yield more reliable spike sequences (i.e., greater similarity or a higher MI) during encoding compared to non-cued trials. As predicted, we found increased MI for sequences during encoding for correct cued trials compared to correct non-cued trials. Preliminary data also reveals a late increase in sequence similarity on post-cue trials which may reflect the retroactive replay of memory-specific sequences in response to a late attentional cue. Together these data may help elucidate one of the neural mechanisms by which attention regulates memory.

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Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.13

Topic: H.07. Long-Term Memory

Title: Predicting semantic representations over time in the medial temporal lobe

Authors: *S. H. SOLOMON[1], A. C. SCHAPIRO[2];
[2]Psychology, [1]Univ. of Pennsylvania, Philadelphia, PA

Abstract: Semantic similarities change across contexts and across time. At the zoo, an owl is more similar to a parrot than it is to a bat. Lost in the woods at midnight, however, owls and bats become more similar. How does the brain capture the transience of context-dependent semantics while maintaining the stability of semantic knowledge learned over a lifetime? Due to its role in memory encoding as well as its sensitivity to recent environmental statistics, we hypothesized that the medial temporal lobe (MTL) may be a site of context-dependent semantic representations and that the stability of semantic representations may vary across MTL regions. To test these predictions, we applied a semantic encoding model to an experiment in which eight participants each completed at least 30 fMRI sessions in which they viewed thousands of natural images (Natural Scenes Dataset; NSD). The NSD stimuli were drawn from the Common Objects in Context (COCO) dataset and are tagged with the potential 80 COCO object categories present in each image (e.g., person, giraffe, umbrella). Within each fMRI session, our semantic encoding model estimated each voxel's response to each of the 80 object categories based on one half of the data, and estimates were tested on the unseen half. The encoding model can also generate predictions regarding multivoxel patterns evoked by each image within a region of interest (ROI). A wholebrain searchlight analysis revealed successful prediction across large swaths of visual and temporal cortex, and ROI analyses revealed successful prediction within several MTL regions (i.e., hippocampal subfields, entorhinal, parahippocampal, and perirhinal cortex). Further, because the fMRI sessions spanned ~250 days for each participant, we are able to assess the similarity of object representations within and across sessions to explore the stability of semantic representations within the MTL and across the brain.

Disclosures: S.H. Solomon: None. **A.C. Schapiro:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.14

Topic: H.07. Long-Term Memory

Support: NIH Grant R01MH100121 (ARP)
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NIH Grant F32HD095586 (NLV)

Title: Differentiation of related events in hippocampus supports memory reinstatement in development

Authors: *N. L. VARGA[1], H. ROOME[2], R. J. MOLITOR[3], L. MARTINEZ[1], E. HIPSKIND[1], M. L. MACK[4], A. R. PRESTON[1], M. L. SCHLICHTING[4];
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Abstract: We often experience related events featuring the same people or places. Although children can form and retrieve memories for such events, less is known about the neural representations that support organization of related experience early in life. Past behavioral and neural studies hint that children may be limited in the organizational schemes to which they have access. That is, whereas adults can flexibly integrate or differentiate related events in hippocampus, sometimes simultaneously, children may be less likely to link related events. Here, we test developmental differences in related representation in hippocampus, offering insight into the organizational schemes that underlie successful memory at different ages. Children (7-10 years) and adults learned that several objects were paired with a shared face or scene. At test, participants were cued with an object, followed by a delay in which they were instructed to hold the associated face or scene in mind in preparation for an upcoming memory decision. Memory performance was high in all participants, suggesting that both groups formed and retrieved the memories. To ask how those related memories were organized in hippocampus, we compared the pattern of fMRI activation for object cues that were related through a common item with those that were unrelated. Children relied uniquely on differentiation, such that related memories were represented as *less* similar—an organizational scheme that may be especially important early in a life as a way of keeping overlapping events distinct. In contrast, adults made use of both differentiated and integrated schemes. In addition to assessing related representation in hippocampus, we examined the fidelity with which the associated faces and scenes were reinstated in neocortex. In line with prior encoding data showing that children and adults form similar representations of specific items in neocortex, both age groups showed high-fidelity reinstatement of the associated items at retrieval. Further, greater hippocampal differentiation of related objects tracked higher-fidelity neocortical reinstatement of the associated faces and scenes among children on a trial-by-trial basis, consistent with prior observations of a correspondence between hippocampal—neocortical traces in adults. Together, the findings suggest that differentiation supports children's ability to organize and retrieve related memories. Although differentiated neural organization that serves to keep events separate may aid memory when retrieving specific events, this scheme could also limit children in other ways, such as hindering extraction of commonalities during novel inference.

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Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

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Support: Natural Sciences and Engineering Research Council of Canada (NSERC)
Discovery Grant RGPIN-04933-2018
Canada Foundation for Innovation JELF
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University of Toronto
NSERC Postgraduate Doctoral Scholarship
Ontario Graduate Scholarship

Title: Cognitive orientation during learning impacts subsequent neural representation of conceptual information

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Abstract: Past work has shown that while both conceptual and perceptual features of experience are represented in the brain, the specific nature of such representations may also depend on cognitive orientation. For example, in some regions conceptual codes only emerge when participants are concurrently performing a conceptual orienting task. However, whether such experience orienting to conceptual or perceptual features has a lasting impact on stimulus representation remains unknown. Here, we used fMRI to address this question. We manipulated participants' (N=42 young adults) attention to the conceptual (story) or perceptual (artist style) features of storybook illustrations using a feature repeat detection task. Participants then completed a recognition task that presented the studied illustrations along with lure illustrations that matched studied illustrations on either conceptual (story) or perceptual (artist) dimensions. We used representational similarity analysis within a searchlight to identify where in the brain studied illustrations were represented similarly to lures on the conceptual (story) or perceptual (artist) dimensions. We found that illustrations depicting the same story were represented more similarly than illustrations showing different stories in many regions across the brain: these conceptual codes were exhibited in medial prefrontal, superior and medial parietal, as well as lateral occipital cortex. However, no regions reliably coded perceptual style. Interrogating how conceptual representations varied as a function of orientation showed that superior and medial parietal along with visual cortex represented conceptual features only following conceptual orientation. By contrast, there were no regions in which the representation of perceptual features was sensitive to prior orientation. Our results show the presence of conceptual representational codes that show both invariance across and sensitivity to prior experience. Consistent with previous work on parietal mechanisms that support attention to certain features through a top-down influence on visual processing, here we demonstrate that this attentional enhancement is also reflected in subsequent representations of the experience. More broadly, these findings suggest that how people engage with a stimulus initially can have a lasting impact on how it is stored in the brain. These observations may moreover suggest a mechanism by which memories can become broad enough to encompass new but similar experiences, which may in turn support behavioural generalization.

Disclosures: S. Vijayarajah: None. **M.L. Schlichting:** None.

Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.16

Topic: H.07. Long-Term Memory

Support: NSERC Discovery Grant RGPIN-2016-05

Title: Adults and children rely on different brain regions to support specific and general statistical learning

Authors: *T. FOREST, M. L. SCHLICHTING, A. S. FINN;
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Abstract: Statistical learning allows humans—from neonates to adults—to extract meaning from the regularities surrounding us. But our ability to do this across ages belies important differences in how statistical learning changes with development. Recent work shows children and adults do not form the same memories for their statistical experiences: adults remember general and specific aspects of repeated experiences but children remember just the specific. Here we test the hypothesis that these differences arise from ongoing neural development of regions which support general and specific memories (like the mPFC and hippocampus, respectively), and have been linked statistical learning in adults. To do this, 9-10-year olds and adults watched a stream of shapes made up of triplets that were always presented in the same order (e.g. ABC, DEF) followed by a memory test for general and specific aspects of the triplet structure. After this, participants watched these shapes again during fMRI in the original structured order and in a random order. To directly examine specific and general memories, we also compared neural representations of same triplet shapes before and after learning in 6 preregistered ROIs. We found that both adults and children engaged regions previously associated with statistical learning (IFG, hippocampus, lateral occipital cortex, basal ganglia) more when viewing shapes in their structured order than in a random order. A direct comparison between age groups showed that adults engaged anterior frontal regions more than children, and represented group information in the late-developing vmPFC. Children, instead, engaged more posterior cortical regions, and represented group information in the IFG and posterior hippocampus (which they also activated more than adults when watching structure). In the posterior hippocampus only, children showed a unique signature of group knowledge in which shapes in the same statistical groups came to be represented as *less* similar following learning, rather than more, suggesting they formed particularly distinct representations of shapes which appeared in the same group. Together these data provide the first demonstration that similar performance on statistical learning tasks in children and adults may be underpinned by different neural representations—while both children and adults demonstrated general statistical knowledge, this was supported by earlier developing brain regions in the children, with distinct consequences for the nature of the general memories formed. Broadly, these results highlight that statistical learning changes across childhood as a function of ongoing neural development.

Disclosures: T. Forest: None. **M.L. Schlichting:** None. **A.S. Finn:** None.

Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.17

Topic: H.07. Long-Term Memory

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NSERC CGS-D
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Canada Foundation for Innovation and Ontario Research Fund (36601)
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Wilfred and Joyce Posluns Chair in Women's Brain Health and Aging from the Posluns Family Foundation

Title: Hippocampus-related exception categorization varies across the menstrual cycle

Authors: M. PEROVIC[1], *E. M. HEFFERNAN[1], G. EINSTEIN[1,2], M. L. MACK[1];
[1]Psychology, [2]Dalla Lana Sch. of Publ. Hlth., Univ. of Toronto, Toronto, ON, Canada

Abstract: Rule-plus-exception categorization has conflicting demands: the learner must not only generalize across rule-following stimuli to understand the category rule but also form distinct representations of rule-violating exceptions. Emerging evidence suggests that the distinct encoding of conjunctive features required for exception learning is a hippocampal process. Notably, the structural integrity of hippocampal white matter, specifically the connections between subfields of the trisynaptic pathway (TSP), has been linked to individual differences in one's ability to categorize exceptions. Hippocampus is not a fixed entity; for example, estradiol - an ovarian hormone with levels that vary distinctly throughout phases of the menstrual cycle - induces numerous changes in TSP-related subfields and their connections. If and how these neural dynamics are reflected in behaviour remains poorly understood. Here, we test the prediction that estradiol-induced fluctuations in hippocampal function, as inferred by typical hormone levels throughout the menstrual cycle, specifically impact exception learning. Participants performed a rule-plus-exception task in which exceptions items were introduced after category rules were learned. Consistent with our predictions, participants in a high estradiol phase outperformed participants in a low estradiol phase and demonstrated faster learning of exceptions than a male group. To characterize potential mechanistic accounts of these learning changes, we leveraged a hippocampus-related clustering model of human learning to simulate exception learning in the context of low and high estradiol. The estradiol-dependent changes in exception learning were best explained by differences in the model's ability to encode a distinct memory trace in response to a surprising event. Notably, in participant and model, the categorization of rule-following stimuli, which relies on generalization rather than distinct encoding, exhibited no such menstrual cycle- nor parameter-dependent shifts. These findings further our understanding of computational mechanisms of hippocampus that support exception learning and offer valuable insight into how cognition varies throughout the fundamental biological cycles of the human experience.

Disclosures: M. Perovic: None. **E.M. Heffernan:** None. **G. Einstein:** None. **M.L. Mack:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 080.18

Topic: H.07. Long-Term Memory

Title: Multiple methods for memory integration in the human brain

Authors: *J. NICHOLAS[1], N. D. DAW[2], D. SHOHAMY[1];
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Abstract: Flexible decisions require drawing on associations between past memories to infer possible outcomes. There are at least two distinct ways in which relationships between previously experienced stimuli can be used to support choice under novel circumstances: prospectively and retrospectively. Prospective memory integration allows inferences to be drawn at choice time, whereas retrospective memory integration relates stimuli before a decision is actually faced. While prior studies indicate that humans can make use of both prospective and retrospective integration, the circumstances under which the brain preferences one over the other remain unclear. Based on computational theory (Mattar & Daw, 2018), we hypothesized that competition for associative strength between individual memories should modulate the direction of memory integration used for choice. Forty healthy young adults (aged 18-35) completed a novel learning and decision making task in the fMRI scanner. Participants first learned a series of associations between antecedents and consequents represented by initially neutral images (e.g. A1-C1 and A2-C2). They next learned to associate a subset of the consequents with a rewarding outcome (e.g. C1-reward; C2-no reward). Finally, participants made decisions between two choice options, which were novel combinations of the previously experienced images (e.g. between C1 and C2 or A1 and A2). Stimulus-stimulus associations were also trained under two different conditions. In the forward condition, two antecedents preceded a single consequent, whereas in the backward condition one antecedent preceded two possible consequents. We predicted that each condition should be easier to solve using the corresponding direction of memory integration, whereas the other direction should be disfavored due to competition from the additional stimulus. Participants' choices on probe trials indicated that they successfully learned to both associate consequent images with reward (β=6.7, 95% CI=[4.3,9.1], $p<0.001$) and to preferentially choose antecedent images associated with rewarding consequents (β=2.0, 95% CI=[1.3,2.7], $p<0.001$). Further, choice accuracy did not differ between both forward and backward conditions (β=0.3, 95% CI=[-0.4,1.0], $p=0.38$), demonstrating that participants successfully used inference to support choice in both cases. Finally, using fMRI, we explored the possibility that the hippocampus was involved in these decisions. These results indicate that separate mechanisms for memory integration may support flexible decision making under differing circumstances.

Disclosures: **J. Nicholas:** None. **N.D. Daw:** None. **D. Shohamy:** None.

Poster

080. Human Memory Dynamics

Location: SDCC Halls B-H

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Program #/Poster #: 080.19

Topic: H.07. Long-Term Memory

Support: NIH Grant 1U19NS107609-01
NINDS R01 NS021135

Title: Awake ripples enhance emotional memory encoding in the human brain

Authors: *H. ZHANG[1], I. SKELIN[2], S. MA[5], M. PAFF[3], M. A. YASSA[4], R. T. KNIGHT[6], J. LIN[7];
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Abstract: Multiple mechanisms have been proposed to support the prioritized encoding of emotional experiences, including neuromodulatory effects on plasticity and interplay between the hippocampus and the amygdala. Sharp-wave/ripples (SWRs) are transient hippocampal oscillations (80-150 Hz), associated with synchronous neural activation in the hippocampus and the amygdala and implicated in binding anatomically distributed memory traces. Moreover, behaviorally relevant reactivation of emotional memory occurs during awake SWRs (aSWR), and aSWR disruption immediately post-experience interferes with memory utilization. In this study, we tested the hypothesis that post-encoding aSWRs could facilitate better emotional memory discrimination, through the coordinated hippocampal-amygdala memory reinstatement. We recorded intracranial electroencephalographic (iEEG) recordings in the human hippocampus and amygdala (n = 17, 20 electrodes respectively) in 7 presurgical epilepsy patients (4 females, age 33 +/- 16) performing an emotional memory encoding and discrimination task. We first show that stimuli with higher arousal ratings were associated with better discrimination performance (p = 0.047, logistic linear mixed-effect model). Next, we demonstrated that during the immediate post-encoding period (i.e., shortly after stimulus presentation and before the next stimulus), the number of aSWRs predicts both stimulus arousal rating (p = 0.03) and probability of later discrimination (p = 0.03, Wilcoxon signed-rank test). Next, we implemented representational similarity analysis to quantify memory reinstatement for each trial. We observed that the post-encoding aSWR is associated with reinstatement in the hippocampus and the amygdala (p < 0.05, permutation test). This reinstatement exhibited region-specific activity. Specifically, amygdala reinstatement was associated with higher arousal (p = 0.035), whereas hippocampal reinstatement was linked to later correct discrimination (p = 0.008, cluster-based permutation test). We then tested whether memory reinstatement occurs simultaneously in the amygdala and hippocampus, by computing joint memory reinstatement, which was predictive of memory discrimination during the post-encoding period (p < 0.05, permutation test). Finally,

mutual information analyses revealed that amygdala reinstatement unidirectionally influenced hippocampal reinstatement (p = 0.038, cluster-based permutation test). Together, these findings provide evidence that aSWRs mediate memory reinstatement in the amygdala and hippocampus accounting for better remembering of emotional experiences.

Disclosures: H. Zhang: None. **I. Skelin:** None. **S. Ma:** None. **M. Paff:** None. **M.A. Yassa:** None. **R.T. Knight:** None. **J. Lin:** None.

Poster

080. Human Memory Dynamics

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Program #/Poster #: 080.20

Topic: H.07. Long-Term Memory

Support: NSERC Grant RGPIN-2021-02721
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Title: Memory in action: Theta differences localized to anterior temporal lobe vary as a function of memory-guided auditory benefit

Authors: *M. FISCHER, M. MOSCOVITCH, C. ALAIN;
Psychology, Univ. of Toronto and Rotman Res. Inst. - Baycrest Hosp., Toronto, ON, Canada

Abstract: How do our memories prepare us to act? Contextual memory acquired via exposure facilitates target detection. However, it remains unclear what mechanisms enable this benefit. We created long-term associations between audio-clips and the location of an embedded tone to examine auditory incidental memory-guided attention. At learning, participants (N = 91; 60 F) were exposed to 80 different audio-clips (half included a lateralized tone) and told to make judgements about the embedded tone. A surprise memory test followed, in which participants pressed a button to respond to a lateralized *faint* tone (target) embedded in each audio-clip. They also indicated if the clip was (i) old/new; and (ii) if the tone was on left/right/not present when they heard the clips at learning. We measured neuroelectric activity during exposure (i.e., incidental learning) and surprise memory test (i.e., retrieval) phases, using high-density electroencephalography recordings.
The results revealed good explicit memory for the clip and for the presence/absence of the tone, but *not* for the *specific location of the tone*. Target detection at test was faster for clips associated with the location of the target (memory-cue) than for those that were not (neutral-cue). Time-frequency analyses in the theta-band during the cue period revealed reduced theta power for memory-cue trials compared to neutral-cue ones. This difference was source localized (multiple source bilateral beamformer in the time-frequency domain) to the left temporal pole. Importantly, detection benefits varied as a function of the difference in left temporal pole activity between conditions.

Auditory memory may facilitate detection via enhanced memory retrieval by theta in the temporal pole. Reduced theta in the memory-cue condition may be indicative of retrieval during the trough of the theta band. Further, these findings suggest that explicit memory for both the clip and the presence or absence of the tone, but an implicit memory of the specific spatial location of the tone was sufficient to facilitate target detection.

Disclosures: **M. Fischer:** None. **M. Moscovitch:** None. **C. Alain:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.01

Topic: H.10. Human Learning and Cognition

Support: ANR-18-CE22-0016

Title: Psychophysics in the wilderness: naturalistic optical flow generated by high-speed train motion modulates estimation of time-to contact-judgment.

Authors: *W. VALLET[1], G. LEMAITRE[2], V. VAN WASSENHOVE[1];
[1]NeuroSpin, CEA DRF/Joliot, Cognitive Neuroimaging Unit, INSERM, Univ. Paris Saclay, 91191 Gif-sur-Yvette, France; [2]SNCF, DTIPG, Direction de la Recherche, Saint-Denis, France

Abstract: Introduction: The wish to carry experiments outside the laboratory has enabled major advances in cognitive neuroscience but it also raises a central question: in a real-world experimentation, how can researchers combine ecological validity with controlled experimental parameters? In the present study, a first aim was to make operational an experimental design in which we could integrate controlled parameters from the ecological environment during a classic psychophysics task. A second aim was to explore the impact of naturalistic optical flow generated by high-speed train motion on time-to-contact (TTC) estimate. **Methods**: 64 participants completed a TTC task using acoustic stimuli simulating a straight path collision trajectory. Participants had to press a keyboard when they considered that the sound reached its target position. To explore the impact of the optical flow generated by motion in train, the design integrated one factor of *Motion* with 3 levels (*no motio*n in the lab; *congruent* or *incongruent* motion in the trains with participants facing forward or backward, respectively) and one factor of *Duration* with 3 levels (*0.5s, 1.2s* and *2.1s*). **Results:** The analysis showed no main effect of motion ($F(2, 180) = 1.57$, $p = .21$). The main effects of TTC were significant indicating that participants performed the task correctly since they differentiated the durations ($F(2, 2) = 393.24$, $p < .001$, $\eta^2_p = .81$). A significant interaction between duration and motion was found ($F(2, 4) = 4.72$, $p < .001$, $\eta^2_p = .09$). The post hoc-decomposition indicates that participants significantly underestimated the longest TTC during congruent motion compared to *no motion* condition (*Congruent motion*: $M = 1.53$s, $SD = 0.33$s; *No motion*: $M = 1.83$s, $SD = 0.31$s; [$t = -4.33$, $p_{Bonf} < .001$]. The difference between *Incongruent motion* and *No motion* was not significant

(*Incongruent motion*: M = 1.79s, SD = 0.18s; [t = -0.64, $p_{Bonf} > .05$]. **Conclusion:** Taken together, these results indicate the reliability of the TTC estimate across ecological and laboratory contexts and the possibility to implement ecological parameter in the design. Regarding the task, the results suggest the ecological benefits of optical flow for possible threat detections when navigating in real-world environment.

Disclosures: W. Vallet: None. **G. Lemaitre:** None. **V. van Wassenhove:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.02

Topic: H.10. Human Learning and Cognition

Title: Thalamic Spectral Entropy Characterizes Temporal Dynamics of Cognitive Control

Authors: B. REID[1], M. HEDLUND[1], N. KABOODVAND[1], P. BHANOT[2], D. A. PURGER[1], J. PARVIZI[1], *V. BUCH[1];
[1]Stanford Univ., Palo Alto, CA; [2]Brown Univ., Providence, RI

Abstract: Cognitive control states are critical for the planning and execution of goal-directed behavior and are thought to be directly influenced by the spatiotemporal dynamics of large-scale brain networks. Though critical, the role of the human thalamus in information transfer for cognitive control is not well understood. Spectral entropy (SE) is an information theoretic metric that quantifies a signal's frequency information distribution. In human sEEG, such a measure could provide critical insights into how specific hubs may govern the spectral information dynamics across the brain. A unique cohort of nine focal epilepsy patients implanted with sEEG electrodes, providing coverage of key functional networks, including thalamic nuclei, allows for characterizing cognitive control states through entropic methods. During electrocorticography recording, these patients participated in a true/false math-based cognitive task. SE was calculated for each contact in four canonical frequency bands (θ/α: 3-12 Hz, β: 12-30 Hz, γ: 30-55 Hz, γ_h: 70-150 Hz) during the pre-trial and early intra-trial period. Temporal dynamics of SE, which we hypothesize to be indicative of cognitive control states, were visualized in the fastest and slowest 1/3 of correct response times within each task session. Thalamic activity expresses frequency-dependent characteristic SE patterns throughout the cognitive task that distinguish cognitive performance: increased pre-trial theta/alpha SE, increased pre-trial beta SE, decreased pre-trial gamma SE, and an intra-trial delay in gamma SE returning to baseline. These characteristic relationships to cognitive control demonstrated in the thalamus extend to the global average of nodes. Beta-band SE in Pulvinar nodes exceptionally distinguished cognitive performance, driven by a reduction of SE during the pre-trial period. SE features extracted from the human thalamus characterize cognitive control states that may drive performance on a math-based task and demonstrate strong influence over the macro-scale cognitive networks involved in task completion.

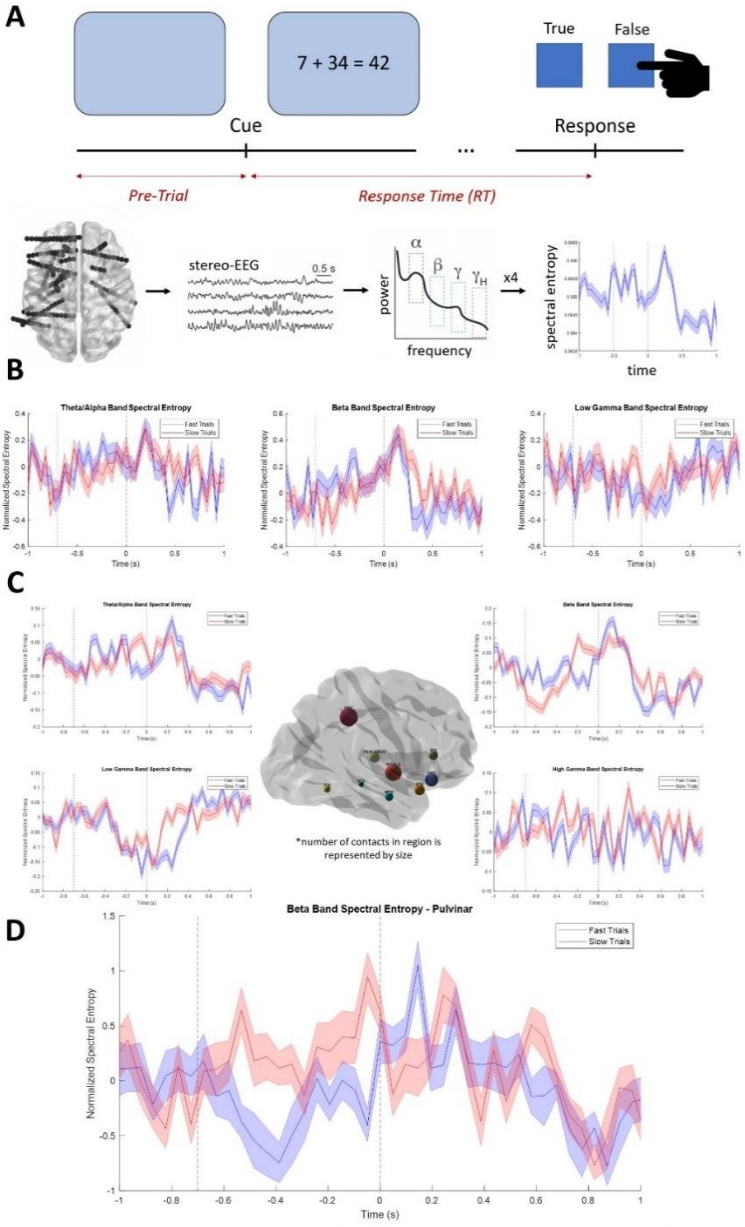

Fig 1. Epileptic patients participated in a math-based cognitive task during sEEG recording, and SE was extracted from each contact (A). Thalamic SE characterizes cognitive performance during this task primarily in the 700ms pre-trial window (B). Recordings were taken from many brain structures across all subjects, and trends from Thalamic SE are seen on a global scale (C). Thalamic performance distinction is driven by a reduction of SE in the Pulvinar (D).

Disclosures: **B. Reid:** None. **M. Hedlund:** None. **N. Kaboodvand:** None. **P. Bhanot:** None. **D.A. Purger:** None. **J. Parvizi:** None. **V. Buch:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.03

Topic: H.10. Human Learning and Cognition

Title: Evidence of entrainment and beta-band oscillations in a tempo matching task

Authors: *C. MONDOK, M. WIENER;
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Abstract: Numerous theories have been developed to explain how the perception of time and rhythm is organized in the brain, central of which is the notion that our sense of time and rhythm relies on neural circuits that involve the supplementary motor area (SMA). Though the SMA appears to have a prominent role in rhythm and timing, its exact function is unknown. One theory is that the SMA matches rhythms by synchronizing firing rates to the perceived beat, which replicates the beat internally and allows for entrainment, the human ability to sync to the beat effortlessly. Further, rhythms can be predicted using a forward predictive model by allowing one to anticipate incoming auditory input and thus focus on predictive errors that may arise leading recent theories to suggest that this region is critically involved when adjusting to a change or violation of an ongoing rhythm, which are suggested to manifest at beta (20Hz) and gamma (40Hz) frequencies. Notably, the SMA is involved in rhythmic entrainment regardless of whether a movement is required or not. Yet, whether the SMA is required for actively comparing rhythms, or merely representing them, is unknown. To address this, we conducted a study with human participants (n=10) performing a rhythm matching task while recording Electroencephalography (EEG). In this task, participants were given two different tempos simultaneously and were asked to modulate the rate of a variable tempo (1.67-2.34Hz, between trials) to match a constant target tempo (2Hz). In the behavioral data, some participants tended to anchor their resolved BPMs to that of the comparison tempo such that trials faster/slower tempos remained above/below the target tempo, which presented itself in a linear fashion across tempos. Evidence of entrainment was observed at the target oscillation (2Hz) as well as increased phase coherence at the target and comparison tempos for onset-related activity with the maximal values at the frontocentral electrodes. Additionally, there was a sustained 20Hz oscillation throughout the listening period in the frontal region as well as a 45Hz (gamma) desynchronization approximately 8s into the trial. Lastly, at the moment of synchrony, when subjects had judged that both tempi were equal, we observed an Event-Related Potential (ERP) with a positivity at 200ms associated with gamma desynchronization at 40Hz in the frontocentral region as well as a negativity at 600-800ms associated with a 20Hz (beta) positivity occurring over the left motor regions. Altogether, these results support the role of the SMA and associated motor regions during the comparison of rhythms and shed light on the neural underpinnings of beat perception.

Disclosures: C. Mondok: None. **M. Wiener:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.04

Topic: H.10. Human Learning and Cognition

Title: Influences of timing on presentation order effects

Authors: *R. GALLAGHER, W. H. ALEXANDER;
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Abstract: Goal directed behavior at times relies on the ability to quickly integrate multiple sources of information. Recent research has demonstrated that altering the presentation order of information may elicit differences in behavior. This implies that information mapping is governed by temporal abstraction, defined as the time elapsed between events. The more temporally abstract events are with respect to one another, the less direct influence they generally have. Alternatively, abstraction can also be defined structurally as it relates to the ability of an information source to minimize prediction uncertainty. Concrete information directly minimizes uncertainty, while abstract information does so indirectly, by modulating uncertainty associated with the concrete source. In agreement with predictions of a computational model of PFC, when both structural and temporal abstractions are in agreement, faster learning and overall higher accuracy is observed during behavioral experimentation. The purpose of this study was to further examine the temporal dynamics that govern integrating multiple sources of information. To do this, we manipulated the presentation duration of the first stimuli, the inter-stimulus interval, and second stimuli/response deadline in conjunction with presentation order. As seen in previous studies, *slower* and *more accurate* responses were observed when abstract information was presented first. Additionally, *higher accuracy* was observed throughout both conditions when the duration of either stimuli presentation or inter-stimulus interval duration was extended. Manipulating the relative timing and duration of the presentation of different sources of information modulated the strength of the order manipulation, shedding new light on how abstract and concrete sources of information are integrated.

Disclosures: R. Gallagher: None. **W.H. Alexander:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.05

Topic: H.10. Human Learning and Cognition

Support: ERC Grant No 682117 BiT-ERC-2015-CoG
FARE Grant R16X32NALR

Title: The topographic representation of time and its link with temporal context and perception

Authors: *G. FORTUNATO[1], S. KULASHEKHAR[1], S. MAASS[2], H. VAN RIJN[2], D. BUETI[1];
[1]Cognitive Neurosci., Intl. Sch. for Advanced Studies (SISSA), Trieste, Italy; [2]Exptl. Psychology, Univ. of Groningen, Groningen, Netherlands

Abstract: Neuronal tuning and topography are mechanisms widely used in the brain to represent not only sensory information but also abstract features like numerosity and time. In humans, temporal topography has been shown recently in a wide circuit of brain regions, from lateral occipital to inferior parietal and premotor regions. However, it remains unclear whether chronotopic maps are specific to vision, whether they map time in an absolute or relative fashion, and to what extent they reflect objective or subjective, perceived time and whether they are influenced by temporal context. Here we asked human participants to reproduce the durations of sounds in two, partially overlapping, temporal contexts while we recorded high-spatial resolution fMRI. Both model-based and data driven analysis approaches show the presence of auditory chronomaps in the auditory parabelt, intraparietal sulcus, and in the supplementary motor area (SMA). Most importantly, when the same physical duration is presented in different temporal contexts, and thus perceived differently, different neuronal units respond to it. Those units were also spatially shifted on the cortical surface according to the relative position of the perceived duration within each context. Finally, voxels did not change their preferences across contexts and their pattern of activity was more similar within rather than across them, suggesting a pivotal role of the context in shaping the maps. These results highlight two important properties of human chronomaps: their flexibility of representation due to perception and their dependency on temporal context.

Disclosures: G. Fortunato: None. **S. Kulashekhar:** None. **S. Maass:** None. **H. Van Rijn:** None. **D. Bueti:** None.

Poster

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Topic: H.10. Human Learning and Cognition

Support: ERC Grant No 682117 BiT-ERC-2015-CoG
FARE Grant R16X32NALR

Title: The more numerous the longer: how the interaction between numerosity and time affects their cortical representation

Authors: *V. CENTANINO, G. FORTUNATO, I. TOGOLI, D. BUETI;
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Abstract: If you are stuck in a traffic jam, the more numerous the queuing cars, the longer you expect to wait. Time and numerosity are stimulus dimensions often associated and whose

interaction can lead to misjudgments, like for example when at the shopping till you erroneously believe the shorter queue to be the faster. The nature of numerosity and time interaction at brain level is far from clear. Neuroimaging studies have shown that the cortical representation of these magnitudes is organized in partially overlapping topographic maps located in a wide network of brain areas from occipital to frontal cortex. However, in these studies, time and numerosity are always manipulated separately. Here we used high spatial resolution fMRI to investigate how numerosity and time maps change when these dimensions are varied together on the same visual stimulus in a congruent (the more the items, the longer the display time) or incongruent (more items, shorter time) manner. Compared to baseline conditions, where only one dimension was changed at a time, numerosity and duration maps changed in tuning preferences. These changes were particularly pronounced in the congruent condition and whereas affecting all maps, they were more pronounced in the comparison between parietal and frontal ones. Finally, while tuning became more sensitive to both magnitudes, the topography degraded. Overall, our results show that the neural representation of time and numerosity is not independent if these dimensions vary together and reveal the flexibility of this representation as a function of magnitude integration.

Disclosures: **V. Centanino:** None. **G. Fortunato:** None. **I. Togoli:** None. **D. Bueti:** None.

Poster

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Topic: H.10. Human Learning and Cognition

Support: JSPS KAKENHI (22H00502)
JSPS KAKEINHI (19H01087)
JSPS KAKEINHI (17KK0004)

Title: Effects of spatial distance between motor effectors on effector-specific acquisition of multiple prior distributions in a human coincidence timing task

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Abstract: The brain optimizes the performance of sensorimotor tasks by acquiring a prior distribution of tasks, which it integrates with sensory inputs (Bayesian estimation). Notably, multiple events possibly occur in our daily life (e.g., speedball/slowball in hitting). Therefore, the brain would likely acquire multiple prior distributions in the real world. We recently found that in a coincidence timing task, two different prior distributions can be acquired separately when they are assigned to two different motor effectors such as the right and left hands (motor-effector specificity). Moreover, increasing the anatomical (intrinsic) difference between effectors (e.g.,

foot vs. hand) increased the rate of effector-specific acquisition of the priors. In the present study, we investigated the effects of spatial (extrinsic) distance between motor effectors on effector-specific acquisition of multiple priors. In tactile temporal order judgment between hands, temporal precision was better with greater hand distance (Shore et al. 2005), Based on this finding, we formulated a working hypothesis that greater hand distance enhances the effector-specific acquisition of multiple priors. In the experiments, each participant completed 640 trials of a coincidence timing task. Target time intervals were sampled from short-time (424-988 ms, mean: 706 ms) or long-time (1129-1694 ms, mean: 1412 ms) prior distributions. Each of the two priors (short/long) was assigned to one of two stimulus locations (left/right). Participants performed the task using the left (right) index finger when the stimuli were presented on the left (right) side. In the group with greater hand distance (19 cm), the long-time prior was acquired; however, the short-time prior could not be acquired within 640 trials. In the group with smaller hand distance (3.5 cm), participants acquired the short-time prior as well as the long-time prior. Thus, contrary to our working hypothesis, our results suggest that greater hand distance inhibited effector-specific acquisition of multiple priors. We discuss the mechanism behind these results.

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Poster

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Program #/Poster #: 081.08

Topic: H.10. Human Learning and Cognition

Support: JSPS KAKENHI Grant 22H00502
JSPS KAKENHI Grant 19H01087

Title: Intra- and Inter-trial aftereffects of prior synchrony/asynchrony on tactile synchrony judgment

Authors: *K. WIDJAJA[1], K. SAITO[2], K. KANNAGA[3], D. YOSHIOKA[1], Y. ITAGUCHI[4], M. MIYAZAKI[1,2,3];
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Abstract: Prior experience can influence human perception of time. In this study, we conducted two experiments with different designs to investigate the effect of prior synchrony/asynchrony on tactile synchrony judgment (SJ). In Experiment 1, participants (n = 12) engaged in an SJ task where they received two pairs of tactile stimuli in each trial: an adaptor stimulus pair and a test stimulus pair. Participants were instructed to judge the synchronicity of the test stimulus pair. The stimulus onset asynchrony (SOA) for the adaptor stimulus pair (A-SOA) was either ±100 (asynchronous) or 0 (synchronous) ms, whereas the SOA for the test stimulus pair (T-SOA) was ±80, ±30, ±10, or 0 ms. The interstimulus interval (ISI) between the two pairs was set to be

either 500, 1000, or 2000 ms. Participants completed 12 sessions (63 trials each) of the task. We observed a positive aftereffect of prior asynchrony on tactile SJ where the proportion of "synchronous" response was less when the adaptor was asynchronous compared with when it was synchronous. The aftereffect appeared under the ISI of 500 and 1000 ms but disappeared when the ISI was 2000 ms. Moreover, no differences were found in the proportion of synchronous response when the adaptor was synchronous across all ISIs, indicating that prior asynchrony caused the aftereffect. This aftereffect is consistent with the prediction based on the Bayesian estimation model. Experiment 2 was then conducted using a trial-by-trial design where no adaptor stimulus pairs were provided. Newly recruited participants (n = 14) engaged in the same SJ task, except they had to judge every pair of the tactile stimuli. The SOA for the stimuli was the same as the T-SOA in Experiment 1, and trials were separated by an interval of three seconds. Participants completed four sessions (21 trials each) of the SJ task. Data were analyzed based on synchrony and asynchrony of the SOA in the previous trial. In contrast to Experiment 1, we found a negative aftereffect where the proportion of synchronous responses was higher when the previous trial had an asynchrony SOA than when the SOA was synchrony. The aftereffect was similar to the negative aftereffect found in the previous studies using multisensory stimuli. Thus, we found that intra- and inter-trial aftereffects occurred in an opposing manner in Experiments 1 and 2, respectively. Our results suggest that different adaptation mechanisms operate on short- and long-time scales in tactile synchrony judgment.

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Poster

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Location: SDCC Halls B-H

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Program #/Poster #: 081.09

Topic: H.10. Human Learning and Cognition

Title: The impact of mechanically induced movement tremor on auditory and visual time perception

Authors: *R. DE KOCK[1], K. GLADHILL[4], W. ZHOU[2], W. M. JOINER[3], M. WIENER[5]; [2]Neurobiology, Physiol. and Behavior, [3]Dept. of Neurobiology, Physiol. and Behavior, [1]Univ. of California, Davis, Davis, CA; [5]Dept. of Psychology, George Mason Univ., [4]George Mason Univ., Fairfax, VA

Abstract: Previous studies have shown a strong relationship between the motor system and time perception. We have found that manipulating arm movements during timing can both improve and bias time perception. We sought to further characterize this relationship by testing whether adding noise to the movement (applying an artificial "tremor") would in turn decrease timing precision. Our predictions are guided by a Bayesian optimal cue combination framework, whereby a timed perceptual event and added movement represent two channels of information

that are weighted by their reliability and integrated for a combined duration estimate. Accordingly, increasing movement "noise" should decrease its reliability and decrease overall timing precision. In two separate experiments, we tested participants on their ability to time auditory (Experiment 1) or visual (Experiment 2) intervals between 1000-4000 ms. Participants (n=24 in Experiment 1, n=10 in Experiment 2) manipulated a robotic arm along a flat workspace with an upward-facing display indicating their current position by a cursor. They began in a central location, then were cued to begin moving continuously throughout the trial. In the auditory task, they heard a 440 Hz tone for a variable duration (1000-4000 ms), and in the visual task, the screen turned from black to gray for a variable duration (1000-4000 ms). Participants then categorized the duration as 'short' or 'long' by reaching to one of two circular targets equidistant from the starting position (choice assignment randomized between subject). An artificial tremor was applied to the handle that varied in frequency (4, 8 and 12 Hz) and amplitude (low, medium, and high - 1, 3 and 5N). We analyzed measures of bias (bisection point) and precision (coefficient of variation) as a function of frequency and amplitude. In the auditory task, timing precision decreased with an increase in tremor frequency, in line with the Bayesian model predictions. In the visual task we observed a similar pattern for only the "high" amplitude tremor. A drift-diffusion model fit to these data revealed that the decrease in precision effect could be explained by an increase in within-trial noise. These results may shed light on known relationships between movement and timing deficits in patients with Parkinson's Disease and other conditions exhibiting motor tremors.

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Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

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Program #/Poster #: 081.10

Topic: H.10. Human Learning and Cognition

Support: NIH Grant NS116589

Title: Multiplexing working memory and time: encoding retrospective and prospective information in neural trajectories.

Authors: S. ZHOU[1], M. J. SEAY[2], J. TAXIDIS[2], P. GOLSHANI[3], ***D. V. BUONOMANO**[2]; [1]Univ. of California, Los Angeles, Univ. of California, Los Angeles, Los Angeles, CA; [2]UCLA, UCLA, Los Angeles, CA; [3]UCLA Dept. of Neurol., UCLA Dept. of Neurol., Los Angeles, CA

Abstract: Working memory (WM) refers to the ability to transiently store information, and subsequently use this information in a flexible manner for goal-oriented behaviors and decision making. Timing, here, refers to the ability to track elapsed time after a stimulus, in order to anticipate subsequent external events or generate appropriately timed motor responses. While it

is widely recognized that the ability to transiently store information about the past and prospectively anticipate external events are among the most fundamental computations the brain performs, the fields of WM and timing have evolved mostly independently from each other because they have been seen as distinct cognitive functions with different underlying neural mechanisms. Yet, both WM and timing share critical computational features: both require transiently storing information, retrospective information in the case of WM and prospective information in the case of timing (e.g., when a delayed reward will occur). In some cases, these properties are mirror images of each other. For example, a timer, such as an hourglass, can be seen as encoding a transient memory that it was recently flipped over and of generating a prediction as to when an external event may occur.
To explore the hypothesis that WM and timing may rely on shared neural mechanisms, we used two modified delayed-match-to-sample (DMS) psychophysical tasks: the first task contained task-relevant WM but task-irrelevant timing component—where the subject was asked to do a standard DMS task but with one cue always followed by a short delay and the other cue long delay; the second task contained task-relevant timing but task-irrelevant WM components—where the subject has to associate the length of the delay with the identity of the second cue to respond. In both cases the task-irrelevant component influenced performance. RNN simulations revealed that cue-specific neural sequences, which multiplexed WM and time, emerged as the dominant regime that captured the behavioral findings. However, task structure and model hyperparameters accounted for a diversity of experimentally-observed neural signatures, including ramping activity. Analysis of RNN structure revealed distinct microcircuit connectivity motifs responsible for generating fixed-point or dynamic attractors. Neural sequences relied primarily on inhibitory connections, and could survive the deletion of all excitatory-to-excitatory connections. Our results suggest that in some instances WM is encoded in time-varying neural activity because of the importance of predicting when WM will be used.

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Poster

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Topic: H.10. Human Learning and Cognition

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Cooperative Study Program of National Institute for Physiological Sciences
Takeda Science Foundation
Grant-in-Aid for Scientific Research (15K12055) of the Japan Society for the Promotion of Science

Title: The retrieval process coordinated by respiration via the right temporoparietal junction.

Authors: *N. H. NAKAMURA[1], M. FUKUNAGA[2], T. YAMAMOTO[2], N. SADATO[2], Y. OKU[1];
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Abstract: The phases and rhythms of breathing result in a variety of body and brain states. Although breathing aligns the detection of sensory information and voluntary actions with brain activity, how such respiratory coordination influences cognitive performance and brain functioning remains unclear. Here, by investigating simultaneous functional magnetic resonance imaging and respiratory measures according to the nasal cannula in participants performing a delayed matching-to-sample recognition memory task, we show that respiration organized retrieval and recognition processes for performance, but did not the sampling of visual test cues. Specifically, when an expiration-to-inspiration (EI) transition point (or onset of inspiration) occurred between a test cue presentation and a motor response during retrieval, accuracy was decreased with a longer reaction time. Notably, respiratory coordination with the retrieval process facilitated task performance in response to activation of the anterior cluster of the right temporoparietal junction (TPJa), a node of the ventral attention network. This activation was retrieval-dependent and was absent during the encoding or delay process in the task. These findings suggest a potential respiratory role in cognitive function along with a brain mechanism and shed light on the mind-body interactions that underlies breathing for successful performance and improvement of our quality of life.

Disclosures: N.H. Nakamura: None. **M. Fukunaga:** None. **T. Yamamoto:** None. **N. Sadato:** None. **Y. Oku:** None.

Poster

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Topic: H.10. Human Learning and Cognition

Support: ICMR extramural funds

Title: Impact of gamma knife radiosurgery (GKRS) on the neurocognitive status in adults with intracranial lesions

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Abstract: Gamma Knife is a sophisticated instrument that offers radiation treatment with high precision and accuracy to treat several kinds of intracranial disorders. Though dose fallout from GKRS is sharp, still a smaller biological equivalent dose of radiation is administered to healthy brain tissues. Low doses of radiation can induce a wide array of cognitive impairments and

deficits even without any significant alterations in brain structures. In this study, we attempt to investigate the effect of GKRS on the cognitive functions of patients with benign disorders. 94 patients undergoing primary GKRS were enrolled in a prospective trial between 2017-2019. The patients underwent clinical (subjective), radiological (1.5 T MRI), and a multi-domain neurocognitive battery assessment including global screening (MMSE), verbal memory (HVLTR), visual memory (BVMTR), executive functions (TMT, Stroop CW), phonemic fluency (COWA), semantic processing (ANT), processing speed (Coding WAIS 3), and intelligence (PGI BBD general intelligence). The patients were assessed at three-time points; baseline before GKRS, at 6 months, and 12 months post GKRS. The mean education level of our cohort was 12.35 and the mean age was 35.80. Baseline neurocognitive statistical analysis was done on the basis of (a) brain disorder characteristics 1. hemisphere involved (left n=40 and right n=54), 2. lesion type (vascular malformations n=47 and benign tumors n=47), 3. lesion site (supratentorial n=49 and infratentorial n=45), and 4. lesion volume (<3 cc n=51 and >3 cc n=43) and (b) sociodemographic variables 1. Gender (male=42 and female=52), 2. age group (18-30 n=37, 31-50 n=42, and 51-65 n=15), and 3. education level group (4-8 n=17, 9-12 n=36, 12 above n=41). It was observed that the higher education group performed significantly better in all the cognitive domains than the patients having lower education. Changes in cognitive performance were not seen in all the groups of brain disorder characteristics at baseline. At 6 months follow-up, highly significant improvement was observed in almost all the cognitive domains. No statistically significant difference was observed in the domains of verbal delayed memory, phonemic and semantic fluency at 6 months follow-up. At 12 months post GKRS (n=60), a similar trend of improvement was seen in all the cognitive domains when compared to baseline. Symptomatically, the patients showed improvement over the period of 12 months. Apart from radiological improvement (resolution of lesions), significant improvement of neurocognitive domains ensures a landscape of better neurocognitive outcomes and quality of life after GKRS. Long-term followup is underway.

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Poster

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Topic: H.10. Human Learning and Cognition

Support: NSF Award # 1849067
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Title: Differential effects of TMS on motor system structures on accuracy and precision in a Ready, Set, Go reaching task

Authors: *K. A. GLADHILL, M. WIENER;
Psychology, George Mason Univ., Fairfax, VA

Abstract: Time and movement are a necessity for mobile organisms in everyday actions in order to interact with a dynamic world. In fact, it has been shown that brain areas involved in these processes greatly overlap. For example, motor system structures such as the supplementary motor area (SMA), traditionally thought to be dedicated to motor control, are active during timing tasks even when no overt motor response is necessary. However, despite the SMA being highly implicated in time perception, many transcranial magnetic stimulation (TMS) studies in which the SMA has been stimulated have not revealed consistent results. One possibility is that the SMA is crucial when the encoded time intervals need to be adapted or changed. To that end, we employed a Ready, Set, Go (RSG) task in which participants (n=16) made timed reaching movements to indicate the "Go" beat of a 3-part sequence. Importantly, participants experienced two different conditions, in separate blocks: 'same as' and '1.5x's'; In the 'same as' condition participants reproduced the same duration between the first and second beat (ready and set) with that between the second and third (set and go) by reaching the visual target. In the '1.5x's' participants reproduced 1.5x's the duration between the first and second beat with that between the second and third by reaching the visual target. Therefore, in the '1.5x's' condition participants had to mentally transform the duration to calculate what 1.5x's the first duration would be. In addition to performing this task, participants also received transcranial magnetic stimulation (TMS), using continuous theta burst stimulation (cTBS) on two different brain areas prior to the task in two different sessions: the SMA and the dorsal premotor cortex (PMd). We found that when the SMA was stimulated participants under-reproduced the target duration, but only in the '1.5x's' condition with no effect of PMd stimulation; however, for PMd stimulation, we instead observed a decrease in timing precision, also exclusively in the '1.5x's' condition, with no effect of SMA stimulation on precision. Therefore, stimulating different motor system structures affected the participants' ability to transform the duration in different ways (SMA = decreased accuracy; PMd = decreased precision) depending on the structure that was stimulated. These findings suggest a dissociation between motor system structures when mentally transforming time estimates, and further shed light on the lack of positive TMS results when stimulating the SMA in timing studies.

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Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

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Topic: H.10. Human Learning and Cognition

Support: NIH Grant R01-GM103894

Title: The effect of music listening on brain activity under propofol sedation

Authors: *Z. HUANG[1], A. G. HUDETZ[2];
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Abstract: Background. Real-world events are processed via hierarchically organized temporal receptive windows (TRWs) in the brain (Hasson et al., 2008, PMC2556707; Honey et al., 2012, PMC3517908; Murray et al., 2014, PMC4241138). The relative length of a TRW reflects the timescale on which a specific brain region typically processes information, which can be indexed by a frequency-domain measure of BOLD-fMRI signals, i.e., mean frequency (MF) (Huang et al., 2018, PMC5830518). For example, higher MF (shorter TRW) is associated with rapid sensory information process in early sensory areas, whereas lower MF (longer TRW) is associated with slower integration of perceptual and cognitive events in higher order brain areas. How sedation distorts the perception of complex, temporally structured stimuli is unclear. **Methods.** Twenty-three healthy volunteers (ages between 18-34 years old; 15 women) were studied via fMRI during conscious baseline, loss of responsiveness (LOR) induced by propofol sedation, and recovery of responsiveness (ROR). A 16-min fMRI scan with presentation of music of four different genres was conducted for each period (baseline, LOR and ROR). The music evoked activity was quantified by inter-subject correlation of the BOLD signals. In addition, the MF was calculated as the sum of the product of the spectrogram power intensity and the frequency, divided by the total sum of spectrogram power intensity. **Results.** We found a spatial hierarchy of statistically significant inter-subject correlation to music starting in the primary auditory cortex (A1) and secondary/tertiary auditory cortex (A2/A3), along the anterior insular cortex (AIC) and up to the right dorsal lateral prefrontal cortex (rDLPFC) during conscious conditions (baseline and ROR). In contrast, during LOR, significant inter-subject correlation was seen in A1 only. Furthermore, a significant decrease of MF in A1 and A2/A3, and a significant increase of MF in AIC and rDLPFC were found during LOR compared to conscious baseline and ROR ($p<0.05$, corrected). **Conclusions.** During loss of responsiveness induced by propofol, primary sensory areas preserve shared neural activity across subjects in response to the acoustic features of music, whereas higher order brain areas (e.g., AIC and DLPFC) fail to process long-timescale information integration such as the musical structures. These effects are accompanied by a prolongation of TRW in sensory areas, and a shortening of TRW in higher order brain areas. Together, the results suggest that disparate changes of TRWs along the brain's spatial hierarchy may account for the loss of conscious experience in sedation.

Disclosures: Z. Huang: None. **A.G. Hudetz:** None.

Poster

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Program #/Poster #: 081.15

Topic: H.10. Human Learning and Cognition

Title: Cognitive Perception of Unfamiliar Electro-cutaneous Grip Force Response by an ERP P300 Component Analysis

Authors: *R. LI[1], K. DING[1], Z. OU[1], N. V. THAKOR[1,2];
[1]Biomed. Engin., [2]Electrical and Computer Engin., Johns Hopkins Univ., Baltimore, MD

Abstract: Sensory feedback is an integral part of two-way upper-limb prosthesis, but the underlying cognitive processing remains inadequately explored. Transcutaneous electrical nerve stimulation (TENS) has been employed to elicit graded grip force response, yet researchers showed that subjects often reported difficulty generating more than 3 force levels. Our hypothesis was that event related potential (ERP) would reflect different levels of stimuli when force level responses could not. We recruited six intact limb individuals for this study. Firstly we identified sensitive locations and stimulation threshold for pulse widths. Then a brief training phase was conducted where subjects produced graded grip force response using a dynamometer based on TENS at threshold and two higher pulse widths which represented three stimulation intensities. We recorded electroencephalogram (EEG) during the main experiment consisting of three blocks of grip force trials. For block 1, subjects gripped the dynamometer according to the same three levels of TENS in training phase. Block 2 presented passive stimulations, with no force response required, using the previous three intensity levels secretly added by a subthreshold level. In block 3, the same three stimulation levels were applied with a highest pulse width introduced without informing the subjects. Kruskal Wallis tests with post-hoc analysis were performed to compare force levels, reaction times, and P300 positive areas based on stimulation intensities within each block. We ran Monte Carlo simulations for both force response and reaction time of each pulse width on blocks 1 and 3 for each subject. In block 3 stimulation intensity was shown to impact force response (average $p < 1.45e-17$) and reaction time ($p < 5.37e-04$). We observed both an increasing trend of force response and decreasing trend of reaction time as stimulation intensity increased, but the force level difference between high and highest level was not statistically significant from post-hoc analysis ($p > 0.3$). When comparing block 3 with block 1 we observed reversal of the difference of P300 positive area for CPZ electrode between low and high intensity stimulations ($p < 0.059$). Another interesting observation was the "U shape" distribution of P300 area as intensity level increased for both CPZ and PZ electrodes. This demonstrated that although subjects were not trained on the highest stimulation and couldn't generate statistically largest force, ERP was applicable for identifying the highest stimulus. The result suggested ERP components could be employed to probe cognitive processing of sensory feedback.

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Poster

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Program #/Poster #: 081.16

Topic: H.10. Human Learning and Cognition

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UV-PTC-898

Title: Mathematical anxiety during arithmetic processing in university students.

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Abstract: Mathematical anxiety during arithmetic processing in university students.Samuel, Zamora-Lugo [1], Martín, Cadena-Barajas [2], Tamara, Cibrián-Llanderal[3]
[1] PhD Program, Instituto de Neuroetología, Universidad Veracruzana.[2] Facultad de Estadística e Informática, Universidad Veracruzana.[3] Neurofisiología y Neurobiología de la Conducta, Instituto de Neuroetología, Universidad Veracruzana.
Introduction. Quantifying the elements around us is a basic ability for humans and other species, this ability is called numerical processing and allows us to perceive approximate quantities of a set of objects. In humans, this numerical processing is the basis where a more complex numerical capacity is constituted, independent of that obtained in formal education. **Objective.** To describe and observe if math anxiety affects arithmetic processing in university students. **Method.** 45 university students were evaluated, with a mean age of 23.38 (SD ± 5.5), 33.3% male and 66.7% female. The instruments used were the Mathematical Anxiety Index, the Beck Anxiety Inventory with validation for the Mexican population and finally arithmetic processing task. An arithmetic processing task was designed in MATLAB 2019a software, using the toolbox called Psychtoolbox 3.0.15. The participant solved 80 arithmetic operations with a result and with the first or second operand missing (2 + ? = 5). The 80 operations were distributed as follows: 40 additions and 40 subtractions, operations (? + 2 = 17). **Results.** The mean Beck Anxiety Inventory score was 21.7 (SD ± 11.8). 11.1% obtained a rating of Low, 44.4% Mild, 28.9% Moderate and 15.6% Severe. The average of the Response Times was 4.6 seconds (SD ± 1.8) and the average of Correct Answers was 70.8 (SD ± 7.7). A first comparison of the scores of the Mathematical Anxiety Index (MI), Correct Answers, Response Times (RT) and the Beck Anxiety Inventory (pBAI) was made between the Gender factor (Women and Men). According to the results obtained by the Wilcoxon rank sum test, it was observed that women had higher AMI scores compared to men ($w= 128.5$, $p<0.05$) and no difference was observed between the scores of BAI between men and women ($F_{(1,43)} = 0.07$, $p = 0.8$). It was observed that women required more
TR ($w= 130$, $p < 0.05$) to solve the operations, although this did not affect their performance (see figure 11). The mean RT for women was 5.01 s (SD ± 1.8) and for men it was 3.9 s (SD ± 1.4). **Conclusion.** A positive relationship is observed with higher MAthematic Anxiety scores, higher Response times, more likely in women, but it did not mean that these scores affected the performance of the arithmetic task.
Keywords: : Anxiety, Education, Mathematics, University students.

Disclosures: S. Zamora Lugo: None. **I. Cibrian-Llanderal:** None. **M. Cadena-Barajas:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.17

Topic: H.10. Human Learning and Cognition

Support: KIST Institutional Program (2E31084)
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Title: Neural correlation of speech envelope tracking for MCI: A preliminary study

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Abstract: Decline in hearing ability has been recognized as one of the significant risk factors for dementia. Several studies have attempted to show the relationship between the ability to process speech in noise and the decrease in cognitive performance (Mamo et al., 2019, Pronk et al., 2019). Also, Decury et al. (2019) have investigated the effects of age on speech understanding, cognition, and neural tracking of the speech envelope using EEG (electroencephalography). They found that the enhancement of envelope tracking is correlated with the increase in speech understanding and that such association is more robust for older adults. Here we propose a preliminary study to examine the difference in the speech perception and neural envelope tracking between individuals with MCI and healthy older adults (demographically matched, normal hearing). Since speech perception in noise demands more cognitive processes (attention, working memory, and language processing), we hypothesize that the degree of the changes in envelope tracking would be different for the person with MCI compared with the healthy adults with normal hearing. The stimuli consisted of matrix sentences either masked by SSN (Speech Shaped Noise) or ISTS (International Speech Test Stimuli) presented under several noise conditions. We investigated the correlation of noise levels, cognitive abilities, and central auditory processing tasks on neural envelope tracking. We expect that our preliminary results could explain the possible relation between speech perception and the decline in cognitive abilities.

Disclosures: H. An: None. **J. Lee:** None. **Y. Lim:** None.

Poster

081. Human Cognition and Imaging: Timing and Temporal Processing

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 081.18

Topic: H.10. Human Learning and Cognition

Title: Is $1/f^a$ noise a ubiquitous phenomenon in human cognition?

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Abstract: Spectral analysis of the time series of response times (RTs) in various cognitive tasks has provided evidence for long range time dependence (LRD), known as $1/f^{a}$ noise, and represented by a straight line fit in the log-log scale of the RT power spectral density (with slope α between 0.5 and 1.5). The presence of 1/f noise was theorized to be ubiquitous in human cognition providing a signature of the complex underlying system. Proponents of this theory claim that LRD is present in all RT tasks. However, it can be masked by white noise infusion in certain experimental manipulations such as for example by including random variation of inter-trial intervals (ISIs). Opposing this theory, others argue that 1/f noise in RT time series is an epiphenomenon of short-range processes where each RT is dependent on one or few preceding RTs. These processes can be modelled using Autoregressive Moving Average (ARMA) models. In this study we measured RTs of 12 participants in series of 210 visual stimuli separated by a constant ISI. Each participant performed 8 such series with ISI of 0.5s, 0.6s, 0.8s, 1s, 1.5s, 2s, 3s and 5s. Mean RT, autocorrelation function and periodogram was calculated for each RT series of each ISI and participant. Mean RT increased from close to zero for the first 4 ISIs indicating predictive behavior, to values larger than 0.25s for the 3 largest ISIs, indicating reactive behavior ($F_{7,14} = 33.4$ $p < 0.0001$). ISI significantly modulated both the autocorrelation function value at lag 1 ($F_{7,20} = 101.3$ $p < 0.0001$), and the slope α of the linear fit on the log-log scale of the periodogram ($F_{7,21} = 74.8$ $p < 0.0001$). More specifically there was no significant autocorrelation and slope a was zero for the largest 3 ISIs (2s,3s and 5s) confirming the absence of LRD. The times series for smaller ISI where significant autocorrelation at lag 1 was present along with values of a in the 0.5 to 1.5 interval, were further analyzed using ARMA models. Our results demonstrate that LRD of RT manifested as 1/f noise is not a ubiquitous phenomenon in human cognition and is observed in specific cases possibly related to predictive behavior. Moreover, ARMA models of short-range dependence can adequately explain time dependence of RTs in these specific cases.

Disclosures: A. Smyrnis: None. **N.P. Smyrnis:** None.

Poster

082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 082.01

Topic: H.12. Aging and Development

Title: Impact of COVID-19 pandemic confinement on the physical and social activity of Mexican elders

Authors: *J. G. MARTÍNEZ-GALINDO[1], R. M. SALINAS-CONTRERAS[1], M. KIVIPELTO[2,3], F. MANGIALASCHE[2,3], A. L. SOSA[1];
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Abstract: Background: Never have we experienced social isolation on such a massive scale and for so long as during the COVID-19 pandemic lockdown, which brought changes in the lifestyle of adults, including elders. And although this isolation was necessary, we know that social engagement as well as other activities that were limited during the pandemic, such as physical activity may help decrease risk of further cognitive decline. **Method:** For this study, 75 urban-Mexican elders cognitively health (age mean:71.2 + 5.7, with an average schooling of 9.6 + 4.9 years, 58% women), responded face to face to a survey designed by the WORLD-WIDE-FINGERS-SARS-COV-2 Initiative, which has the objective of measuring the direct and indirect effects of the pandemic in old age, focusing on changes in lifestyle and psychosocial factors, included physical and social activities before and after the pandemic quarantine. **Result:** As expected, changes in physical and social activities were observed; 42% of the sample indicated that they felt their physical activity had reduced, when asked about the frequency of physical exercise, they did before and after the pandemic, a significant decrease ($Z = 3.06$, $p = .003$) was observed. In addition, 73% of respondents claimed to have less contact (even at a distance) with friends and family, and 22% of the sample reported increased feelings of loneliness. **Conclusion:** Mental health in older Mexicans has been negatively affected during the social distancing for COVID-19 pandemic. Therefore, a multifactorial program that includes physical and psychosocial intervention is highly recommended for this population after 2-years confinement, to reduce risk factors for dementia and mild cognitive impairment.

Disclosures: J.G. Martínez-Galindo: None. **R.M. Salinas-Contreras:** None. **M. Kivipelto:** None. **F. Mangialasche:** None. **A.L. Sosa:** None.

Poster

082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 082.02

Topic: H.12. Aging and Development

Title: Anti-aging activity of Resveratrol in the hippocampus of Wistar rats

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Abstract: An organism undergoes a series of morphological, physiological, and biochemical modifications in aging. Brain aging is considered the main cause of the cognitive decline and a

risk factor for developing a neurodegenerative disorder. Various theories related to aging have been proposed. One of the most studied is oxidative stress, in which the accumulation of free radicals promotes the progression of oxidative processes such as lipoperoxidation and macromolecules damage. The endogenous antioxidant system maintains the neural homeostatic state. However, their effectiveness decreases with age, and external support is required through preventive therapies such as exogenous antioxidants. Resveratrol is an important antioxidant that has shown anti-aging functions. In this work, we aimed to evaluate the effect of chronic administration with Resveratrol on cell damage associated with aging in the hippocampus of Wistar rats. 216 Wistar male rats 3 months old were randomly divided into control, vehicle (ethanol 7.5%), and resveratrol group (10mg/kg of weight + ethanol 7.5%). The administration periods were for 2, 4, 6, 8, 10, 12, 16, and 18 months. The open-field and novel object recognition tests were performed. In the hippocampus, nitrites, lipoperoxidation, the enzyme activity of superoxide dismutase, catalase, and the glutathione system were analyzed. Subfields morphology was evaluated by hematoxylin and eosin staining. The results showed that chronic treatment with Resveratrol at a dose of 10 mg/kg of weight has a protective effect on hippocampal cells because it improves cognitive performance in the short-term and long-term memory, decreases oxidizing molecules, and enhances the antioxidant system. Additionally, Resveratrol treatment decreases cell damage from the hippocampus's CA1, CA3, and DG subregions. This work evidences the neuroprotective role of Resveratrol on the hippocampus during aging.

Disclosures: I. Cesar Arteaga: None. **D. Juárez Serrano:** None. **A. Díaz Fonseca:** None. **A. Navarro Cruz:** None. **O. Vera López:** None. **S. Trevino Mora:** None.

Poster

082. Cognitive Disorders in Aging

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Program #/Poster #: 082.03

Topic: H.12. Aging and Development

Support: NIH Grant AG054617
NRSA T32 USC 5T32MH111360-05

Title: Hippocampal volume mediates the relationship between depression and fluid intelligence differentially by sex and APOE4 carrier status

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Abstract: Sex differences are a notable feature of both depression and Alzheimer's disease (AD), with evidence suggesting women have an increased lifetime risk relative to men.

However, little is known about the neural mechanisms linking these diseases. Hippocampal atrophy resulting from neurotoxic insults related to depression pathology may elevate AD risk. The apolipoprotein E (APOE) ε4 allele, a major genetic risk factor for sporadic AD, has been associated with late-life depression and disproportionate AD risk in women compared to men. The goal of this study was to investigate whether hippocampal volume mediates the relationship between depression symptom severity and fluid intelligence, and whether this association differed by sex and APOE ε4 carrier status. UK Biobank subjects were selected based on availability of demographic variables, mental health assessments, structural MRI scans, APOE genotype, and completion of the fluid intelligence test. Fluid intelligence assessed verbal and numeric reasoning. Current depression symptom severity was derived from the Patient Health Questionnaire. The effect of depression symptom severity on fluid intelligence via hippocampal volumes was tested using causal mediation analyses in R. Models were adjusted for age, sex, APOE genotype, total brain volume, education, handedness, and the Townsend Deprivation Index, which is a measure of material deprivation. To evaluate sex-specific differences, sex and APOE genotype stratified analyses were completed. The sample included 17,677 middle-aged and older adults (54.8% women, mean age±SD= 64.2±7.5 years, 21.2% APOE ε4 carriers, 60.8% non-APOE ε4 carriers). Hippocampal volume mediated the relationship between depression symptom severity and fluid intelligence in women. However, APOE ε4 carrier status attenuated this association, such that women APOE ε4 carriers did not show this relationship. Women non-APOE ε4 carriers demonstrated an indirect effect of depression symptom severity on fluid intelligence, (mediation effect = -0.003, 95% CI (-0.007, 0.00), p=0.034) with 14.8% (p = 0.028) of the effect mediated. There were no associations observed in men. These findings provide support for a mechanistic link between depression and cognition via a hippocampal pathway for women that is not observed in women APOE ε4 carriers. Overall, this study provides new insight into sex and genetic differences in the etiology of how depression relates to cognition and may confer risk for AD.

Disclosures: **N.E. Ortega:** None. **V. Aslanyan:** None. **J. Pa:** None.

Poster

082. Cognitive Disorders in Aging

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Program #/Poster #: 082.04

Topic: H.12. Aging and Development

Support: NSF DRL 1631563

Title: Variables associated with inter-individual variation in cerebellar anatomy in chimpanzees

Authors: *A. N. PARKS[1], K. L. BRYANT[2], T. M. PREUSS[3], E. E. HECHT[4];
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Abstract: It is now well-established that the cerebellum is functionally multifaceted, contributing to cognitive, emotional, and autonomic utility just as it does to its more historically appreciated roles in sensorimotor and vestibular function. While emerging research is now investigating the expanded roles of the human cerebellum in healthy brain function and disease, these topics in humans' closest relatives, chimpanzees, remain mostly unaddressed. Chimpanzee brain research offers insight into both the evolution of cognitive complexity in humans as well as conferring a more closely related model system by which to understand cerebellar function and pathology. Here, we assess several metrics of inter-individual variation in chimpanzee cerebellar anatomy and relate said variation to behavioral metrics. We report (a) variability and asymmetry in cerebellar lobules, (b) lobule variation as a result of age and sex differences, and (c) correlations between cerebellar lobule variation and cognitive and behavioral differences. These results have implications for the evolvability of cerebellar lobule size, asymmetry, and plasticity and could deliver insight into the evolution of cerebellar traits that make humans susceptible to neurocognitive decline that results from cerebellar pathology. Future work should assess the heritability of chimpanzee cerebellar variation in comparison to humans.

Disclosures: **A.N. Parks:** None. **K.L. Bryant:** None. **T.M. Preuss:** None. **E.E. Hecht:** None.

Poster

082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

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Program #/Poster #: 082.05

Topic: H.12. Aging and Development

Title: Norepinephrine mediates effects of chronic amphetamine on improved memory performance in aged rats.

Authors: *S. SCOGNAMIGLIO, G. DEZFULI, K. J. KELLAR;
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Abstract: Understanding the neural mechanisms of normal and pathological aging is increasingly important as the average lifespan continues to rise. Memory impairment is attributed to normal aging processes, with the hippocampus and the cerebral cortex playing key roles in the encoding and retrieval of memories. Norepinephrine (NE) is an important modulator of memory, especially emotional memory (Tully *et al.*, 2010). Previously, we demonstrated that acute treatment with amphetamine (0.5mg/kg) enhances memory performance on the novel object recognition (NOR) task in aged Fischer 344 rats, with a reversible and short-term effect (Scognamiglio *et al.*, 2021). We also demonstrated that chronic amphetamine treatment has a more robust and possible long-term effect on memory performance in aged rats. The purpose of this present study is to investigate whether the effect of amphetamine on object recognition memory is NE-dependent.
To determine the extent to which NE mediates the cognitive effects of amphetamine, we first examined the effect of systemic administration of the β-adrenergic antagonist propranolol alone

on object recognition memory in aged rats. Then, we examined the effect of systemic co-administration of propranolol and amphetamine in the NOR task in the same group of animals. The present studies found: **(a)** acute propranolol at doses of 0.5mg/kg does not affect the baseline scores of aged rats on the NOR task **(b)** propranolol co-administrated with amphetamine abolishes the effect of amphetamine on memory performance in the NOR test. An additional goal of this study is to assess the outcome of chronic amphetamine treatment on a cellular level by analyzing dendritic spines in the cerebral cortex. The results of those studies will be reported.

Disclosures: S. Scognamiglio: None. **G. Dezfuli:** None. **K.J. Kellar:** None.

Poster

082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

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Program #/Poster #: 082.06

Topic: H.12. Aging and Development

Support: Bell Family Endowed Chair
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Title: An initial validation of the Cleveland Clinic Virtual Reality Shopping platform

Authors: *J. L. ALBERTS[1,2], M. MCGRATH[1,3], C. WALTZ[1], A. ROSENFELDT[1], K. OWEN[1], K. HASTILOW[1], L. SCELINA[1], K. SCELINA[1], M. KOOP[1];
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Abstract: Background: Impaired performance of instrumental activities of daily living (IADLs) has been proposed as a prodromal marker of neurological disease. Existing assessments of IADL function rely on self-reported questionnaires or lengthy performance-based evaluations. Neither approach offers objective, unbiased measures of IADL performance. Recent advancements in virtual reality provide opportunities to safely and accurately replicate complex IADL environments in a clinical setting. The Cleveland Clinic Virtual Reality Shopping (CC-VRS) platform combines a full-scale grocery shopping environment with an omnidirectional treadmill, tasking users with physically navigating a store while looking for items on a shopping list.
Purpose: The purpose of this study was to examine the convergent, discriminant, and ecological validity of the CC-VRS platform.
Methods: Fifteen healthy young adults (8 male, 7 female; average age 25.1 ± 4.0 years), 14 individuals with Parkinson's disease (7 male, 7 female; average age 66.7 ± 7.9 years), and 14 age- and gender-matched healthy participants (7 male, 7 female; average age 66.1 ± 8.9 years) completed this study. All participants completed the full CC-VRS platform, including one Basic and one Complex shopping scenario. The store route, shopping items, and distractors were consistent across all participants. Participants also completed traditional tests of cognitive function (i.e., Montreal Cognitive Assessment, symbol digit matching, Trail Making Tests, Rey's

Verbal Learning Test), motor control (Mini Balance Evaluation Systems Test), and IADL function (Lawton IADL Questionnaire). Shopping task outcomes were compared to clinical measures of cognitive and motor function, and CC-VRS performance was compared across participant groups.
Results: Performance of cognitive aspects of the CC-VRS were positively correlated with traditional outcomes of cognition across all three participant groups, particularly in the domains of executive function and memory. The CC-VRS showed improved discrimination between PD and healthy control participants as compared to the Lawton IADL Questionnaire. On average, participants reported minimal to mild symptoms of motion sickness, and rated the CC-VRS between "good" and "excellent" on a usability scale.
Conclusions: The CC-VRS platform assesses both cognitive and motor components of IADL function. The CC-VRS is well-tolerated among populations of adults with and without neurological disease, and may offer improved sensitivity to subtle declines in IADL function compared to traditional questionnaires.

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Poster

082. Cognitive Disorders in Aging

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NVIDIA Corporation

Title: Smart-kage: a fully automated system for life-long continuous phenotyping of mouse cognition and behaviour

Authors: H. HO[1], ***N. KEJZAR**[1], H. SASAGURI[2], T. SAITO[3], T. SAIDO[2], B. DE STROOPER[4], M. BAUZA[5], J. KRUPIC[1];
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Abstract: Comprehensive ethologically-relevant behavioural phenotyping in rodent experiments is essential for deciphering the neural basis of animal cognition. Automated home-cage-based testing platforms present a valuable tool to fulfil this need. However, they often involve complex animal training routines, water or food deprivation, and probe a limited range of behaviours. Here, we present a new fully automated AI-driven home-cage system for cognitive and behavioural phenotyping in mice ("smart-Kage"). The system incorporates spontaneous alternation T-maze, novel-object recognition and object- in-place recognition tests combined with monitoring of an animal's position, water consumption, quiescence and locomotion patterns, all carried out continuously and simultaneously in an unsupervised fashion over long periods of time (>8 months). Mice learnt the tasks rapidly without any need for water or food restrictions. We applied an ethomics approach to show that combined statistical properties of multiple behaviours can be used to discriminate between mice with hippocampal, medial entorhinal and sham lesions and accurately predict genotype of Alzheimer's disease mouse model ($App^{NL\text{-}G\text{-}F}$) on an individual animal level, surpassing the performance of several gold standard cognitive tests. This technology could enable large-scale behavioural screening for genes and neural circuits underlying spatial memory and other cognitive processes.

Disclosures: **H. Ho:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. F. Consulting Fees (e.g., advisory boards); Cambridge Phenotyping Ltd. **N. Kejzar:** A. Employment/Salary (full or part-time):; Cambridge Phenotyping Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. **H. Sasaguri:** None. **T. Saito:** None. **T. Saido:** None. **B. De Strooper:** None. **M. Bauza:** A. Employment/Salary (full or part-time):; Cambridge Phenotyping Ltd. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. **J. Krupic:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Cambridge Phenotyping Ltd. F. Consulting Fees (e.g., advisory boards); Cambridge Phenotyping Ltd.

Poster

082. Cognitive Disorders in Aging

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Topic: H.12. Aging and Development

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Dale and Deborah Smith Center for Alzheimer's Research and Treatment, and the Kenneth Stark Endowment

Title: Effects of Visceral Fat Removal on Insulin Sensitivity and Cognition in male APPNL-F/NL-F and C57BL/6 mice.

Authors: *S. MCFADDEN[1], C. A. FINDLEY[3], M. R. PECK[6], A. BARTKE[2], K. N. HASCUP[4], E. R. HASCUP[5];
[1]Southern Illinois Univ., SPRINGFIELD, IL; [2]Southern Illinois Univ., Carbondale, IL; [3]Pharmacol., [4]Neurology, Ctr. for Alzheimer's Res. and trEatment (CARE)Disease and Related Disorders, [5]Neurology, Neurosci. Institute, Ctr. for Alzheimer's Dis., Southern Illinois Univ. Sch. of Med., Springfield, IL; [6]Neurol., Southern Illinois Sch. of Med., Springfield, IL

Abstract: Effects of Visceral Fat Removal on Insulin Sensitivity and Cognition in male APP$^{NL-F/NL-F}$ and C57BL/6 Mice.
S.A. McFadden[1], C.A. Findley[1,2], M.R. Peck[1], Andrzej Bartke[1,4], K.N. Hascup[1,2,3], E.R. Hascup[1,2]
[1]Neuroscience Institute, Dale and Deborah Smith Center for Alzheimer's Research and Treatment, Depts. of Neurology, [2]Pharmacology, and [3]Medical Microbiology, Immunology and Cell Biology, and [4] Internal Medicine, Southern Illinois University School of Medicine, Springfield, IL, USAVisceral fat (VF) contributes to the development of insulin resistance, type 2 diabetes, and dyslipidemia that are risk factors for developing Alzheimer's disease (AD). Previous work from our laboratories demonstrate that VF pad removal (VFR) improves insulin sensitivity and glucose tolerance in a mouse model of successful aging. We hypothesized that VFR would improve metabolism and slow disease progression in an AD mouse model.At 16 months of age, after the onset of plaque deposition and cognitive decline, male APP$^{NL-F/NL-F}$ and C57BL/6 (genetic background controls) underwent VFR or sham surgery. In the VFR group, perigonadal and perinephric adipose tissue was removed. For the sham operation, similar incisions were made and the same adipose depots were mobilized but not removed. Insulin and glucose tolerance tests (ITT, GTT) were conducted at 18 months of age to examine metabolic function followed by cognitive assessment using novel object recognition (NOR). Preliminary metabolic data support improved insulin sensitivity in the C57BL/6 VFR group when compared to sham control mice. This trend was not seen with APP$^{NL-F/NL-F}$ VFR compared to sham. Reduced glucose tolerance was observed in the C57BL/6 VFR mice, while the APP$^{NL-F/NL-F}$ VFR improved glucose tolerance. NOR performance was improved in C57BL/6 VFR mice compared to the sham group. This improvement was not observed in APP$^{NL-F/NL-F}$ VFR mice. VFR at 16 months significantly improved insulin sensitivity in C57BL/6 mice, but this was not seen in APP$^{NL-F/NL-F}$. This supports that VFR during later AD progression does not improve insulin signaling in male APP$^{NL-F/NL-F}$ mice. Lack of improved NOR performance in APP$^{NL-F/NL-F}$ VFR mice, but improvement in C57BL/6 VFR, demonstrates changes in peripheral insulin signaling may contribute to VFR-related cognitive decline. Future studies investigating VFR at 4 months of age could help elucidate the influence of VF on insulin signaling and cognition before AD onset.
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Poster

082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 082.09

Topic: H.12. Aging and Development

Support: Alz Society Grant ASC 20-21
NSERC Grant RGPIN-2017- 06057
NSERC Grant RGPIN-2021-02721

Title: Time-of-day effects on inhibitory control in healthy aging and amnestic mild cognitive impairment

Authors: *R. CHOW[1], R. RABI[1], S. PARACHA[1], S. GARDNER[1,2], L. HASHER[1,3], N. D. ANDERSON[1,4], C. ALAIN[1,5];
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Abstract: Much research has demonstrated the effects of circadian mismatch on cognition in older adults, with worse performance across cognitive domains during one's non-optimal relative to their optimal time-of-day. Executive functioning deficits have been shown in healthy older adults and older adults with amnestic mild cognitive impairment (aMCI), a prodromal stage of Alzheimer's disease (AD) — however, time-of-day effects on such deficits are less clear and are lacking in empirical support from neural measures. Therefore, the aim of this research was to examine how circadian mismatch affects inhibitory processing in a) healthy aging and b) persons with aMCI using neural and behavioural indices. We report data from 51 younger adults, 52 healthy older adults, and 54 older adults with aMCI. Participants were randomly assigned to complete a Flanker task during their optimal or non-optimal time-of-day, while electroencephalography was recorded. To reflect the predominant chronotype of their respective populations, the younger adult sample were all of afternoon-to-evening chronotype, and both older adult samples were of morning chronotype. Time-of-day effects (i.e., optimal versus non-optimal) on neural and behavioural metrics were analysed between a) healthy younger and healthy older adult samples, and between b) healthy older adults and individuals with aMCI. Analyses of electrophysiological correlates of inhibition (i.e., N2 and P3 event-related potential components) showed that age differences in P3 amplitude between younger and older adults were apparent only during the non-optimal time-of-day. Meanwhile, individuals with aMCI showed reduced P3 amplitudes compared to healthy older adults, which were modulated by time-of-day. This finding was accompanied by individuals with aMCI showing greater Flanker effects in reaction times than healthy older adults, with worse inhibition during the non-optimal compared to optimal time-of-day. In summary, healthy older adults and individuals with aMCI demonstrated behavioural deficits of inhibition, which were accompanied by neural evidence for impaired inhibitory processing — particularly in the late afternoon and early evening times. Findings provide early evidence of sundowning effects in the prodromal stage of AD, and reinforce the need to assess and control for time-of-day in aging research.

Disclosures: R. Chow: None. **R. Rabi:** None. **S. Paracha:** None. **S. Gardner:** None. **L. Hasher:** None. **N.D. Anderson:** None. **C. Alain:** None.

Poster

082. Cognitive Disorders in Aging

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Program #/Poster #: 082.10

Topic: H.12. Aging and Development

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PAPIIT-DGAPA IA208120
PAPIIT-DGAPA IA208022
CONACyT Fellowships 778596

Title: Effect of D-serine on cognitive reserve in aged rats

Authors: *B. VÁZQUEZ-PRIETO[1], L. NAVA-GÓMEZ[2,3], I. CALERO-VARGAS[2], S. ALCAUTER-SOLÓRZANO[4], M. LÓPEZ-HIDALGO[2];
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Abstract: Aging-associated cognitive decline is associated with structural and functional changes in the brain. One of the theories explaining the differences between the trajectories of this decline is the cognitive reserve, where differences in physiology and anatomy allow some individuals to cope better than others. NMDA receptors have a pivotal role in many cognitive functions, for its activation, it requires besides the binding of glutamate, the binding of the co-agonists D-serine. During aging, D-serine levels are decreased which has been linked to cognitive deficits. Previous results from our lab show that D-serine supplementation to senescent rats restores cognitive flexibility, functional brain connectivity, and the neuronal spine density that is affected by age. However, it is not clear how D-serine may affect cognitive flexibility and functional brain connectivity in middle-aged rats in a longitudinal study. Here, we analyzed the effect of oral D-serine supplementation on the decline of cognitive flexibility in late middle-aged rats (18 months old) in a longitudinal study. The rats were trained in a reversal-learning task and the number of perseverations was quantified as an inverse measure of cognitive flexibility. Middle-aged rats (12 months) had lower performance than young rats (6 months), indicating a decline in cognitive flexibility. The rats were then randomly assigned into two groups, 1) The D-serine group, which was supplemented with D-serine (300 mg/kg) in the drinking water for two months before the cognitive flexibility test, and 2) the control group, which receives only regular drinking water. Rats from both groups were followed for 6 months and had a second evaluation of cognitive flexibility performance when they were 18 months old. We found that late middle-aged rats improve their performance in the second evaluation, however, 26% of middle-aged rats supplemented with D-serine, significantly decreased their cognitive flexibility. We then wonder how the performance of the rats and the D-serine effect was related to the brain functional connectivity measured during resting-state with fMRI. We observed a decreased functional

connectivity between brain areas associated with cognitive flexibility in those rats affected by D-serine supplementation in their number of perseverations. These results indicate that the D-serine effect may produce a detriment to cognitive functions.

Disclosures: B. Vázquez-Prieto: None. **L. Nava-Gómez:** None. **I. Calero-Vargas:** None. **S. Alcauter-Solórzano:** None. **M. López-Hidalgo:** None.

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082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 082.11

Topic: H.12. Aging and Development

Title: Prior Head Injury Impacts Cognitive Aging in Adults At-Risk of Alzheimer's disease

Authors: *L. K. CHIU, A. NORIEGA DE LA COLINA;
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Abstract: ABSTRACT Background: Prior head injury is a modifiable risk factor for Alzheimer'sDisease (AD). A history of serious traumatic brain injury (TBI) diminishescognitive reserve and accelerates the normal process of cognitive decline.However, most head injuries are categorized as mild, and it remains unclear ifmild TBI contributes to neurocognitive symptoms of AD. This study characterizesmild TBI based on severity, timing, and frequency, and relates such features tocognitive outcomes in older adults at-risk of AD. By identifying keycontributing features of prior head injuries, we can better assess the risk ofdeveloping symptoms of AD and provide early intervention. **Method:** Cognitivelyhealthy participants (n = 304) from PREVENT-AD (PResymptomatic EValuationof Experimental or Novel Treatmentsfor AD), an open science dataset of adults over the age of 55 witha parental or multiple-sibling history of AD. A subset of participants (n =115) reported one (n = 59) or multiple (n =56) prior head injuries. Of these,76 individuals reported period of loss of consciousness (n = 40) and/or periodof memory loss or altered mental state (n = 61). To assess cognitive function,Rey's Auditory Verbal Learning Test, Trail Making Test, and Color-WordInterference Test were administered. Clinical measures of neuropsychology forapathy, depression, and anxiety were also collected. Groupdescriptive statistics and linear regressions with age and education ascovariates were carried out in R. **Results:** A difference in processing speed was found between controlswithout prior mild TBI and a subgroup of participants with at least one headinjury with a reported loss of consciousness. The number of head injuries waspositively correlated with inhibition. Participants with a reported mild TBIafter mid-life (age $\geq$ 35) had lower inhibition and cognitive flexibilitymeasures. Time since last head injury was reported was negatively correlatedwith working memory. Participants with prior head injury also scored higher onthe geriatric depression scale than those without. **Conclusion:** These findings show that different features of a mild TBIdrives different outcomes in cognition. Based on reported loss ofconsciousness, number of prior head injuries, and age of last head injury, eachcharacteristic had a greater impact on

processing speed, inhibition control,cognitive flexibility, and working memory. Ultimately, these cognitive andneuropsychological differences form a base to relate structural and functionalneuroimaging data to further inform how prior head injuries lead to abnormalcognitive aging in older adults at-risk for AD.

Disclosures: L.K. Chiu: None. **A. Noriega de la Colina:** None.

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082. Cognitive Disorders in Aging

Location: SDCC Halls B-H

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Program #/Poster #: 082.12

Topic: H.12. Aging and Development

Title: Associations between objectively measured sleep parameters and cognition in healthy older adults: a meta-analysis

Authors: *S. QIN[1], R. LEONG[1], J. ONG[1], M. W. CHEE[2];
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Abstract: Sleep measured objectively by polysomnography or actigraphy is multidimensional. Although many studies have examined associations between sleep macrostructure, microstructure and cognition in older adults, results are influenced by different sleep parameters and different cognitive domains assessed across studies. The current meta-analysis quantified and summarized associations between objectively measured sleep parameters and cognitive domains in healthy older adults, across 72 independent studies. Both macrostructure (e.g., sleep duration, continuity, and stages) and microstructure (e.g., slow wave activity and spindle activity), and their associations with cognitive performance in older adults were examined. For macrostructure sleep initiation and continuity measures, especially restlessness at night, were strongly and consistently associated with cognition in older adults. Specifically, lower restlessness at night was associated with better memory performance, while lower sleep onset latency was associated with better executive functioning. Both percentages in N2 and REM sleep were significantly associated with cognition, while percentage in N3 was not. The association between microstructure and cognition in older adults was marginally significant, and was significantly moderated by age of participants in a positive direction. Taken together, results show that different sleep parameters are associated with different cognitive domains, and that the influence of microstructure on cognition may be stronger in older-old adults. The current meta-analysis emphasizes the value of objectively assessing multidimensional aspects of sleep in understanding the relationship between sleep and cognition in healthy older adults.

Disclosures: S. Qin: None. **R. Leong:** None. **J. Ong:** None. **M.W. Chee:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

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Program #/Poster #: 083.01

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

Title: Highly-multiplexed Single Cell Spatial Biology for Neuroscience Research

Authors: *H. LIU[1], A. PRATAPA[1], D. KLYMYSHYN[1], A. C. TORRES[2], L. HEINRICH[2], F. ZAFAR[2], A. KHAN[3], B. SCHUELE[2], O. BRAUBACH[1];
[1]Akoya Biosci., Marlborough, MA; [2]Dept. of Pathology, Stanford Univ. Sch. of Med., Stanford, CA; [3]Cell Sci. Imaging Facility (CSIF), Beckman Ctr., Stanford Univ., Stanford, CA

Abstract: Highly-multiplexed immunofluorescence imaging methods enable researchers to visualize dozens of biomarkers in situ and at single cell resolution. These technologies have already transformed cancer and immunology research. Yet, only few of these methods have penetrated neuroscience research, despite a rapidly growing interest in cataloging neuronal and glial diversity in normal and diseased brain tissues. The PhenoCycler-Fusion spatial biology platform is a revolutionary multiplex immunofluorescence technology that allows visualization of 100+ antigens on a single tissue section. The technology is imaging-based and widely deployable across different applications and tissue types, ranging from mouse fresh frozen to human FFPE brain tissues. Conventional neuronal, neural stem cell, and glial cell antibodies can be readily modified to become compatible with the PhenoCycler experimental workflow. By means of diverse application data, ranging from neuronal phenotyping of cultured cells, to mapping cortical phenotypes in murine brains, and characterizing inflammatory microglia in post-mortem human brains, we explore how spatial biology can be deployed in various types of discovery and translational neuroscience research.

Disclosures: **H. Liu:** A. Employment/Salary (full or part-time):; Employment. **A. Pratapa:** A. Employment/Salary (full or part-time):; Employment. **D. Klymyshyn:** A. Employment/Salary (full or part-time):; Employment. **A.C. Torres:** None. **L. Heinrich:** None. **F. Zafar:** None. **A. Khan:** None. **B. Schuele:** None. **O. Braubach:** A. Employment/Salary (full or part-time):; Employment.

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083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.02

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

Title: Dissecting mammalian brain architecture with Veranome Biosystems' spatial biology toolkit

Authors: *M. OTERO-GARCIA[1], E. TEO[2], H. SOH[2], L. ZHEN[2], B. HILBUSH[1];
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Abstract: The spatial architecture of the brain is intrinsically related to its function. The properties of the central nervous system arise from the interactions of hundreds of neuronal and non-neuronal cell types, organized in precisely defined structures and circuits. Understanding brain function, development and disease will require spatially resolved measurements of multiple molecular features in combination with morphology, activity and connectivity properties of individual cells. In the past decade, single-cell transcriptomic methods have accelerated our understanding of the brain cell-type composition, but at the cost of losing sight of tissue architecture. Currently, spatial transcriptomics is promising to bring these discoveries back into their anatomical context. Here, we have generated a large corpus of data spanning cortical, thalamic, and hindbrain regions from the mouse brain using Veranome Biosystems' VSA-1 platform. The multi-omic analysis of nearly 250,000 cells provides a basis for building an integrative taxonomy that captures several biological dimensions *in situ* and in parallel, including transcriptomic profiles, proteomics, morphological features and spatial relationships. Using Veranome's highly multiplexed FISH assay, transcriptional profiles were generated for hundreds of genes with single cell resolution. Spatial transcriptomic analysis and multi-omic visualization were done with data imported into Veranome's desktop software. We surveyed over 50 regions of interest (typically 1 to 4 μm^2), cataloguing an array of cell type-specific clusters and differentially expressed genes. Multi-omic analysis, gene co-expression network and spatial proximity analyses further refined taxonomic classification for some neuronal subtypes. We identified potentially novel interneuron and projection neuron subtypes based on transcriptomics and spatial data. Our data demonstrate that the VSA-1 is a powerful spatial multi-omic platform for interrogation of multiple biological dimensions required for taxonomic classification. The results indicate that improved cell type and cell state classification accuracy can be achieved by application of imputation methods and spatial pattern detection algorithms. Application of Veranome's toolkit will advance the building of an integrative taxonomy of the mammalian brain and enable researchers to track cellular responses to drugs, pathogens and disease progression.

Disclosures: **M. Otero-Garcia:** A. Employment/Salary (full or part-time):; Veranome Biosystems. **E. Teo:** A. Employment/Salary (full or part-time):; Veranome Biosystems. **H. Soh:** A. Employment/Salary (full or part-time):; Veranome Biosystems. **L. Zhen:** A. Employment/Salary (full or part-time):; Veranome Biosystems. **B. Hilbush:** A. Employment/Salary (full or part-time):; Veranome Biosystems.

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083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.03

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

Title: Analyzing the molecular basis underlying anatomic and functional complexity of the mouse brain with MERSCOPE™

Authors: *R. CHEN, B. WANG, Y. CAI, L. COLBERT, J. HE;
Vizgen, Cambridge, MA

Abstract: Single-cell sequencing has provided numerous novel insights into cell heterogeneity and cell-type-specific adaptation of the nervous system. However, most sequencing-based techniques require cell dissociation and consequently lose the spatial information of the analyzed cells, which prevents directly connecting the cell types to specific anatomic and functional features. The rapid development of spatially resolved genomic assays enables molecular analysis in the tissue context, with the potential of revealing how single-cell gene activity orchestrates the structure and function of complex tissues like the nervous system. Here, we demonstrated the use of the MERSCOPE™ Platform to generate a transcriptionally defined and spatially resolved single-cell mouse brain atlas. By performing multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) assays with a 500-gene panel designed for cell typing, we obtained over one million cells with precise gene expression and spatial information along the anterior-posterior axis of the mouse brain. Clustering analysis of the gene expression data resolved all major cell populations as well as detailed neuron and non-neuron subtypes across different brain regions. Importantly, all these molecularly determined cell types were precisely mapped to their original locations. By assessing the relationship between molecular and anatomic features of identified cell types, we found that both projection and interneurons subtypes exhibit significant variation in gene expression and spatial distribution along multiple axes of different brain structures. Most notably, in addition to well-established cortical layers, excitatory neuron subtypes exhibit significant variation along the anterior-posterior axis. Furthermore, by combining cell typing with neural tracing and behavioral assay, the anatomic and functional features of molecularly defined cell types could be directly assessed through MERFISH imaging. Altogether, our work not only created a molecularly defined and spatially resolved mouse brain cell atlas, but also demonstrated the power of MERFISH measurements generated by the MERSCOPE™ Platform in analyzing the molecular basis underlying the anatomic and functional complexity of the nervous system.

Disclosures: **R. Chen:** A. Employment/Salary (full or part-time):; Vizgen. **B. Wang:** A. Employment/Salary (full or part-time):; Vizgen. **Y. Cai:** A. Employment/Salary (full or part-time):; Vizgen. **L. Colbert:** A. Employment/Salary (full or part-time):; Vizgen. **J. He:** A. Employment/Salary (full or part-time):; Vizgen.

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083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

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Program #/Poster #: 083.04

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

Support: R21 NS112886
1R34NS111654

Title: Robotag-seq: robot assisted cellular barcoding for transcriptome-wide sequencing of anatomically identified cell populations in intact tissue

Authors: J. O'BRIEN[1], L. GENGELBACH[2], *M. S. RIEDL[3], R. SCHORN[3], L. CAYE[3], B. AUCH[2], A. G. J. SKORPUT[3], G. WILCOX[3], D. M. GOHL[4], L. VULCHANOVA[3], S. KODANDARAMAIAH[1];
[1]Mechanical Engin., [2]Genomics Ctr., [3]Neurosci., [4]Genomics Ctr; Genetics, Cell Biology, and Develop., Univ. Minnesota, Minneapolis, MN

Abstract: Single cell transcriptomics is a powerful tool for measuring the gene expression patterns within single cells, and it has revealed numerous insights that were previously hidden by the averaging of gene expression patterns across cell populations. Contemporary methods for single cell transcriptomics lack the ability to integrate spatial information with transcriptomic measurements at scale. For instance, in-situ hybridization, sequencing, or capture approaches are generally unable to conduct large-scale transcriptome-wide measurements at cellular resolution and/or they are depth-limited to only analyzing cells near the tissue surface. Here, we propose a workflow that enables barcode tagging of specific cell populations in intact tissues or tissue slices. During subsequent transcriptomic profiling of dissociated cells or isolated nuclei from the injected tissues, the injected barcodes can be used to relate the transcriptomes with the pre-identified cells thereby achieving large-scale, transcriptome-wide sequencing at cellular resolution. We have developed a robotic microinjector that can detect and microinject 100's of pre-identified cells in intact neural tissue with oligonucleotide barcodes. We have demonstrated successful automated microinjection of a fluorescently labeled dextran into neurons in mouse striatal and spinal cord dorsal horn slices with success rates of 96% and 68%, respectively. Moreover, we are capable of performing high-throughput automated microinjections at a rate of approximately 100 injected neurons per hour which will facilitate transcriptome-wide profiling at scale. We have visually confirmed microinjection-mediated labelling of neuronal nuclei in mouse striatum using fluorescently labeled antibodies targeting the nuclear pore complex and oligonucleotide barcode-antibody conjugates (using the same monoclonal antibody). We have also observed labeling of nuclei via microinjection of unconjugated oligonucleotide barcode. These successes demonstrate the proof-of-concept that we can use high-throughput automated microinjection for cell and nuclei labeling as precursor to transcriptomic profiling. We are currently optimizing the processes for nucleus and cell isolation, enrichment, and subsequent transcriptomic profiling. When fully developed, we anticipate this approach to be a generalized strategy for barcode tagging specific cell populations for whole transcriptome analysis in a variety of tissues and will enable high-throughput correlation of single cell gene expression with anatomical and physiological features.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

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

Support: NIH R01 MH 087463
NIH K99 AG 068306
HAWK-IDDRC P50 HD103556

Title: Neuroestimator: using deep learning to quantify neuronal activation from single-cell and spatial transcriptomic data

Authors: *S. CHATTERJEE[1], E. BAHL[2], M. ELSADANY[2], Y. VANROBAEYS[2], L.-C. LIN[3], K. GIESE[5], T. ABEL[6], J. J. MICHAELSON[4];
[2]Interdisciplinary Grad. Program in Genet., [3]Iowa Neurobank Core, Univ. of Iowa, [4]Dept. of Psychiatry, Univ. of Iowa, [1]The Univ. of Iowa, Iowa City, IA; [5]King's Col. London, London, United Kingdom; [6]Dept. of Neurosci. and Pharmacol., Univ. of Iowa, Iowa City, IA

Abstract: Neuronal activity-dependent transcription directs molecular processes that regulate synaptic plasticity, brain circuit development, behavioral adaptation, and long-term memory. Single cell RNA-sequencing technologies (scRNAseq) are rapidly developing and allow for the interrogation of activity-dependent transcription at cellular resolution. Here, we present NEUROeSTIMator, a deep learning model that integrates signals of activation distributed throughout the broader transcriptome to estimate neuronal activation in a way that is robust against differences in species, cell type, and brain region. We demonstrate this method's ability to accurately detect neuronal activity in previously published single cell and time course studies of activity-induced gene expression. Further, using spatial transcriptomic techniques, we demonstrate the model's ability to identify patterns of learning-induced activation. In conclusion, NEUROeSTIMator is a powerful and broadly applicable tool for measuring neuronal activation, whether as a critical covariate or a primary readout of interest.

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083. Single Cell Profiling Techniques in Health and Disease

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

Support: NIH 1UG3NS111688
Genomic Sciences Training Program NHGRI Training Grant 5T32HG002760

Title: Transcriptomic profiling of the mouse hippocampus after intracerebral injection of Cas9 nanocapsule genome editors

Authors: *K. GIMSE[1], Y. WANG[1], J. A. FELTON[1], J. M. METZGER[1,2], M. EMBORG[1,2], J. E. LEVINE[1,2], S. GONG[1], K. SAHA[1];
[1]Univ. of Wisconsin Madison, Madison, WI; [2]Wisconsin Natl. Primate Res. Ctr., Madison, WI

Abstract: Evaluating the editing efficiency and cell-type specificity of genome editors is a critical task for developing somatic cell genome editing strategies, especially for those that target the brain. Standard methods rely on deep sequencing at the on-target site combined with immunohistochemistry within treated animal model systems to enumerate the types of edited cells in select tissues after administering genome editors. Animal reporter systems that express fluorescent proteins after successful on-target genomic editing provide robust platforms to evaluate the number of edited cells but typically incorporate limited opportunities to co-register cell-type markers with the reporter protein. Thus, molecular characterization of an edited cell typically is limited to 2-4 cell-type markers that can be imaged simultaneously with the fluorescent reporter. Here, we have utilized single nuclei RNA sequencing (snRNA-seq) to deeply characterize the transcriptome of edited cells within the hippocampus of Ai14-tdTomato reporter mice. We performed snRNA-seq on nuclei isolated from the hippocampus after intracranial injection of nanoparticle genome editors. Our research team has developed a biodegradable nanocage (NC) capable of delivering preassembled SpyCas9 protein-gRNA ribonucleoprotein complexes (RNPs). RNPs targeting the Ai14 loxP-STOP cassette were encapsulated into these NCs and delivered into the hippocampus via intracranial injection. Two weeks post-injection, nuclei were isolated from the hippocampi of treated mice and snRNA-seq was performed. We observed the capture of ~500-15000 unique transcripts per nuclei and robust *Ai14-tdTomato* reporter expression in nuclei from neuronal, glial, and oligodendrocytic cells. Additionally, differential cell-type specific transcriptomic shifts were identified primarily in immune and cell signaling pathways, when comparing treatments with NC or recombinant adeno-associated viral vectors (rAAV) encoding Cre recombinase. Overall, transcriptional profiling provides a high-resolution and complementary method to examine the cellular outcomes from genome editing within animal reporter systems. We anticipate these results will inform the clinical development of genome editing therapeutics that target the brain.

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083. Single Cell Profiling Techniques in Health and Disease

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Program #/Poster #: 083.07

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

Support: NIH Grant 1R01AG075820-01

Title: Novel parallel affinity purification of cell-specific RNA and protein in vitro and in vivo using TurboID proximity labeling

Authors: *C. C. RAMELOW, S. SUNNA, S. RAYAPROLU, M. MCGUIRK SAMPSON, N. T. SEYFRIED, S. A. SLOAN, S. RANGARAJU;
Emory Univ., Emory Univ., Atlanta, GA

Abstract: The development of new tools for complementary molecular profiling of neurons and glia at the RNA and protein level is critical to unveil biological and therapeutically relevant insights into cellular disease mechanisms. We utilized the biotin ligase, TurboID, to establish a method for concomitant cell type-specific analyses of the transcriptome and proteome with the goal of applying this in an in vivo context. We have found that TurboID biotinylates several RNA-binding proteins (RBPs) and ribosomal proteins in vitro and in vivo. Therefore, we hypothesized that transcripts associated with these proteins may also be enriched simultaneously. To test our hypothesis, we created a mouse microglial BV2-TurboID cell line that stably expresses TurboID fused to a nuclear export sequence to efficiently label the proteome. We homogenized control BV2 and BV2-TurboID cells, enriched for biotinylated proteins with streptavidin beads while maintaining RNA-protein interactions, and then eluted RNA. Quality control studies showed low RNA yield from control BV2 cell streptavidin pulldowns, and high levels of RNA from BV2-TurboID cell pulldowns. After confirming high enrichment of RNA from BV2-TurboID pulldowns, our project aimed to determine whether the enriched transcriptome was representative of the whole cell transcriptome and if LPS-driven transcriptional changes could be captured. RNA-sequencing was performed on whole cell RNA from control BV2 and BV2-TurboID cells as well as streptavidin pulldowns from both cell types with or without LPS treatment. Transcript abundances of 11,922 genes were highly comparable between control BV2 whole cell and BV2-TurboID pulldowns treated without LPS (r=0.98) and with LPS (r=0.99), confirming capture of a representative transcriptome. The effect of LPS on microglial activation on the whole cell transcriptome was also reliably captured at the pulldown level in BV2-TurboID cells (r=0.87). Our results indicate that our novel concomitant RNA and protein profiling approach captures a representative transcriptome and recapitulates the effect of LPS treatment on BV2 cells at the whole cell and TurboID pulldown level. We have extended this approach in vivo and confirmed the ability to enrich RNA from both neuronal and astrocytic TurboID labeled animals. On-going studies using this new parallel RNA and protein profiling method will resolve neuronal and astrocyte transcriptomes and proteomes under homeostatic and neuroinflammatory conditions in vivo.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

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Program #/Poster #: 083.08

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

Title: Understanding microglia-neuron crosstalk by characterizing microglial contamination in human and mouse Patch-seq datasets

Authors: K. ARBABI[1], Y. JIANG[2], D. HOWARD[1], D. FELSKY[3], ***S. TRIPATHY**[1,3]; [1]Univ. of Toronto, [2]Immunol., Univ. of Toronto, Toronto, ON, Canada; [3]Krembil Ctr. for Neuroinformatics, Ctr. for Addiction and Mental Hlth., Toronto, ON, Canada

Abstract: Microglia are dynamic immune cells with diverse functional roles, including the regulation of neuronal excitability. Here, we leveraged an inconvenient truth of neuronal Patch-seq datasets — that they routinely display evidence of contamination by surrounding microglia — to better understand aspects of microglia-neuronal crosstalk. We first quantified the presence of microglial transcripts in Patch-seq datasets of human supragranular glutamatergic neurons and mouse GABAergic interneurons, and observed extensive off-target contamination. Variation in microglial contamination was explained foremost by donor identity in human samples, whereas neuronal identity contributed most among mice. Differential expression testing and enrichment analyses suggest that microglial contamination in Patch-seq is likely reflective of activated microglia and that these signatures are distinct from those captured via single-nucleus RNAseq. Finally, neurons with greater microglial contamination differed markedly in their electrophysiological characteristics, including lowered input resistances and more depolarized action potential thresholds. Our results suggest microglial contamination contributes to cell- and donor-related electrophysiological variability and sheds light on how microglia might impact neurons in vivo.

Disclosures: K. Arbabi: None. **Y. Jiang:** None. **D. Howard:** None. **D. Felsky:** None. **S. Tripathy:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.09

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

Title: Nuclei Isolation from OCT embedded human brain tissue enables multiomic readouts

Authors: *L. ALVARADO, **C. MCCONNELL HOLLYER**, M. GIBBONS, S. TAYLOR; 10x Genomics, Pleasanton, CA

Abstract: Single cell analysis of neuronal samples can provide unique insights on brain biology. However, neuronal tissue sample preparation can present specific challenges due to large cell

sizes, activation of neuronal cells during enzymatic incubation, and sensitive cell types lost during the dissociation process. Nuclei isolation provides an alternative approach but come with its own challeges as protocols are complex, time consuming, and require previous hands-on experience. The recently introduced 10x Genomics Chromium Nuclei Isolation kit provides a simplified, high throughput sample processing solution from frozen tissue that is compatible across all existing 10x Genomics single cell assays. Here, we processed flash-frozen (FF) and OCT-embedded human brain from a single post-mortem donor with the stock nuclei isolation kit protocol. The resulting single nuclei suspensions were run through Chromium Single Cell Multiome ATAC + Gene Expression assay. Similar cell populations were recovered for both FF and OCT samples, and though overall data complexity (i.e. genes/cell) was greater for the FF sample, feature expression correlation was high for both GEX (R2 > 97%) and ATAC (R2 > 99%) data. Together these data suggest that nuclei extracted from OCT embedded human brain using the Chromium Nuclei Isolation kit accurately recapitulate the cell type composition and gene expression signature of FF tissues. Importantly, this creates the opportunity to pair single nuclei data generated on the Chromium platform with spatial gene expression data generated on the 10x Visium platform on serial sections - allowing for more accurate spot deconvolution in Visium analysis.

Disclosures: **L. Alvarado:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **C. McConnell Hollyer:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **M. Gibbons:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics. **S. Taylor:** A. Employment/Salary (full or part-time):; 10x Genomics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); 10x Genomics.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.10

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

Title: Single-cell and single-nuclei RNA sequencing of mouse mid-brain using Pre-templated Instant Partitions (PIPseq)

Authors: *C. KUGLER[1], P. FRAZEL[2], A. MAY-ZHANG[1], R. MELTZER[1], K. FONTANEZ[1], S. A. LIDDELOW[2];
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Abstract: In neuroscience, single-cell RNA sequencing (scRNAseq) along with single-nuclei RNA sequencing (snRNAseq) have greatly improved our ability to identity, distinguish, and discover novel and rare cell types. However, even the largest individual scRNA-seq datasets only represent about 1% of the over 100 million cells in the adult mouse brain. PIPseq is a novel single-cell sequencing technology that makes scRNAseq accessible and affordable to all researchers and facilitates the characterization and interrogation of larger and more numerous neuronal datasets. PIPseq has sample preparation improvements over other commercial applications by allowing samples to be “instantly partitioned” into single cell reaction vesicles without the use of microfluidics. PIPseq also has the advantage of convenient stable stopping points where sample collection can occur at the site of sample collection and downstream processing can occur in a core lab or at Fluent BioSciences service laboratory. Here, we compare the performance of PIPseq single-cell analysis to 10x 3’ V3.1 in mouse neuronal tissue (freshly dissected and dissociated postnatal day (P)30 mouse forebrain). Additionally, we performed PIPseq on nuclei extracted from frozen mouse brain to compare to whole- cell transcriptomics. Analysis from both PIPseq and 10x processed samples was able to resolve all expected neuronal cell types. The proportion and variety of the common cell type markers derived from each scRNAseq method was comparable, with no drop-outs or biases observed. The differentially expressed genes from each technique were highly concordant and replicated what is commonly described in the literature and outlined in the Linnarsson Brain Atlas. Single-nuclei preparations in PIPseq also recapitulated the cellular and transcriptomic diversity observed with whole-cell preparations. In summary, these experiments demonstrate the efficacy, sensitivity, and flexibility afforded by PIPseq for expanding the applications of single cell and single nuclei transcriptomics in neuroscience.

Disclosures: C. Kugler: None. **P. Frazel:** None. **A. May-Zhang:** None. **R. Meltzer:** None. **K. Fontanez:** None. **S.A. Liddelow:** None.

Poster

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Program #/Poster #: 083.11

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

Title: Understanding the chromatin architectural landscape of retinal development at the single-cell level

Authors: *V. TRINH, R. P. CARMEN, C. P. SANTIAGO, L. JIANG, J. P. LING, S. BLACKSHAW;
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Abstract: Higher-order chromatin organization is important for understanding how gene expression is regulated during neuronal developmental. Little is known about how chromatin architecture affects gene expression in mouse rod photoreceptors, where chromatin is highly

compacted and euchromatic/heterochromatic regions are structurally inverted. Previous studies have analyzed chromatin architecture in the retina; however, studies were completed in bulk. We conduct single-cell Hi-C to identify chromatin domains, compartments, and interaction profiles that correlate with transcriptional profiles during different retinal cell developmental stages, and to understand cell-to-cell heterogeneity. Hi-C was used to measure genome-wide chromatin interactions in bulk and at the single-cell level using the 10x Genomics system on retina of CD1 mice at P0 and adult ages. Pseudo-bulk single-cell Hi-C data of mouse retina was compared to bulk and previously established single-cell Hi-C data of mouse photoreceptors. Retinal cells at P0 and adult were distinguishable through differential chromatin compartmentalization and promoter-enhancer interaction profiles. Understanding the chromatin regulatory landscape of retinal development will provide insights into the chromatin changes that occur during retinal disease and aging. Identifying key chromatin interaction regions that regulate developmental genes may guide therapeutic strategies that aim to reverse the chromatin signatures associated with disease and aging.

Disclosures: **V. Trinh:** None. **R.P. Carmen:** None. **C.P. Santiago:** None. **L. Jiang:** None. **J.P. Ling:** None. **S. Blackshaw:** None.

Poster

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Program #/Poster #: 083.12

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

Support: NIH Grant 1R01-NS100919
NIH Grant 1R01-NS101461

Title: Single-nuclei paired multiomic analysis of young, aged, and Parkinson's disease human midbrain reveals age- and disease-associated glial changes and their contribution to Parkinson's disease

Authors: M. SONG[1], L. ADAMS[1], Y. TANAKA[2], ***Y.-S. KIM**[1];
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Abstract: Age is the primary risk factor for Parkinson's disease (PD), but how aging changes the expression and regulatory landscape of the brain remains unclear. Here, we present a single-nuclei multiomic study profiling shared gene expression and chromatin accessibility of young, aged, and PD post-mortem midbrain samples. We profiled 69,289 high-quality nuclei from 31 individuals (9 young donors, 8 aged donors, 14 PD patients). To investigate how aging process affects PD pathogenesis, we combined the snRNA and snATAC sequencing datasets and established a combined pseudopathogenesis (cPP) trajectory. cPP trajectory reveals all glial cell types are affected by age, but microglia and oligodendrocytes are further altered in PD. Using

this analytical strategy, we identified three distinct subsets of oligodendrocytes in the human substantia nigra. Most cells exhibited a low cPP score and had a transcriptome signature characteristic of healthy, canonical oligodendrocyte function. All donors, regardless of age or disease status, had a large population of healthy cells. We present evidence for a novel disease-associated oligodendrocyte subtype characterized by a high cPP score and identify genes lost over the aging and disease process, including CARNS1 and RBFOX1, which may predispose healthy cells to develop a disease-associated phenotype. We also identified genes such as QDPR and SELENOP altered during PD. We confirmed our bioinformatics findings with RNA-FISH on FFPE human substantia nigra sections. Peak-gene association analysis from paired data identifies 89 PD-associated SNP loci, including five in MAPT, that show differential association with gene expression in disease-associated oligodendrocytes. Our study suggests a previously undescribed role for oligodendrocytes in aging and PD pathogenesis.

Disclosures: **M. Song:** None. **L. Adams:** None. **Y. Tanaka:** None. **Y. Kim:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.13

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

Title: Simultaneous detection of neural activity and temperature in a single neuron

Authors: *J. WU[1], Y. SHINDO[1], K. HOTTA[1], C. Q. VU[2], K. LU[2], T. WAZAWA[2], T. NAGAI[2], K. OKA[1];
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Abstract: Temperature is a fundamental parameter in biological processes because it affects so many biochemical reactions inside living cells, including enzymatic activity, gene expression, and diffusion of biomolecules. Within the cell, heat is generated from the process of energy production as uncompensated heat. Therefore, the intracellular temperature is expected to increase when neurons fire. The firing activates ion currents to flow through voltage-gated channels and lots of ATP was consumed and synthesized for maintenance of ion gradient by ion pumps. A recent study using fluorescent thermometer nanosheets showed no temperature rise was observed, even though electrical stimulation generated clear calcium mobilization in neurons. (Oyama et al., 2020), because this thermometer detected the extracellular temperature. In this study, we provide a system that can measure both intracellular calcium mobilization and intracellular temperature in living neurons using a fluorescent protein-based ratiometric thermometer B-gTEMP and fluorescent protein calcium ion sensor B-GECO, simultaneously. B-gTEMP is composed of green and red fluorescent proteins with different temperature sensitivities, and the fluorescence ratio changes depending on the temperature. Because B-GECO emits blue fluorescence, those sensors can be applied for simultaneous imaging. We focus on the

neuronal firing-related temperature changes by the thermogenesis. We recorded the intracellular calcium mobilization and the temperature during the firing of the hippocampal culture neurons induced by veratridine which is an activator of voltage-gated sodium ion channels and KCl which can depolarize the membrane potential. The response of intracellular calcium and the temperature has a positive correlation when stimulated by veratridine and KCl. The veratridine stimulation in a calcium-free medium showed no response in both the intracellular calcium and temperature. These results indicate that the temperature of neurons is strongly related to calcium mobilization. When neurons fire, calcium ions flow into the neurons through voltage-gated calcium channels, activate intracellular downstream signals, and trigger neurotransmitter release. Calcium ions also are accumulated into mitochondria and endoplasmic reticulum, and some are extruded to extracellular space (Bers et al., 2002, Roberto et al., 2017). Our results indicate that any of these processes would be responsible for heat generation during the firing of neurons.

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Poster

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Program #/Poster #: 083.14

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

Support: NIMH-IRP

Title: Identification and spatial mapping of major neuronal cell types in human mediodorsal thalamus

Authors: *A. SCHULMANN[1], S. MARENCO[2], P. K. AULUCK[2], N. FENG[2], Q. XU[2], Y. LENG[3], N. AKULA[1], Y. PATEL[2], B. K. LIPSKA[2], S. K. WILLIAMS AVRAM[4], R. E. EDELMANN[4], S. ROY[4], T. B. USDIN[4], M. A. PENZO[3], F. J. MCMAHON[1];
[1]Human Genet. Br., [2]Human Brain Collection Core, [3]Unit on the Neurobio. of Affective Memory, [4]Systems Neurosci. Imaging Resource, NIMH, Bethesda, MD

Abstract: Background: The mediodorsal thalamus (MDT) and its reciprocal connectivity with the prefrontal cortex have been implicated in the pathogenesis of severe mental illness, particularly schizophrenia, autism spectrum disorder, and bipolar disorder with psychotic features. Despite the importance of human MDT, little is known about its neuronal cell type composition. Recent studies in rodents showed unexpected diversity of cell types in mouse MDT, suggesting similar or greater cell type diversity in human MDT. **Methods**: MDT was dissected in six postmortem human brain specimens from adults without any major psychiatric disorder. Nuclei isolation by sucrose-gradient centrifugation was followed by single-nucleus RNA-seq on the 10X Chromium platform. Data were analyzed using CellRanger and Seurat. Multiplex RNA fluorescent in situ hybridization (RNA-FISH) via the RNAscope HiPlex

platform was performed on coronal thalamic sections at multiple anterior-posterior levels and imaged on a widefield microscope system followed by endogenous signal subtraction. **Results**: Most glutamatergic neurons in these samples were dominated by a transcriptional gradient that included several functionally relevant genes, such as neurotransmitter receptors and ion channels. These genes overlapped with those that distinguish primary, secondary, and tertiary thalamic profiles in mice. One glutamatergic subtype, expressing *TLL1*, clustered separately from the main gradient and did not map onto any neuronal subtypes previously found in mice. GABAergic neurons in human MDT expressed reelin and did not form discrete subclusters. Multiplex RNA-FISH of major cell type marker genes revealed spatial separation of glutamatergic cell populations. The main transcriptional gradient mapped roughly onto the mediolateral axis. **Conclusion**: Human MDT is composed of highly heterogeneous cell types. Glutamatergic neurons in MDT separate along a transcriptional gradient that maps onto anatomical space and include a discrete subtype that may be specific to higher mammals. These results provide a reference dataset for cell type-specific studies of human MDT in neuropsychiatric disorders.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

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Program #/Poster #: 083.15

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

Title: Ribo-stamp - a new method for measuring translation in neurons at the single-cell level

Authors: *F. ZAMPA[1], S. L. SISON[2], E. KOFMAN[2], P. JAGANNATHA[2], J. NARITOMI[2], R. J. MARINA[2], K. BRANNAN[2], G. LIPPI[1], G. YEO[2];
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Abstract: A detailed census of the identity and function of brain cell types is necessary to understanding the complex biology of behaviors and diseases. Gene expression programs sculpt neuronal cell-type specification, development and integration in mature neuronal networks. RNA sequencing (RNA-seq) is currently used to map brain transcriptomes as proxies of developmental and functional states and advances in single-cell technologies have greatly increased the resolution of cell classifications. However, transcript profiles do not capture the numerous post-transcriptional mechanisms that regulate gene expression, a concern for neuronal cells which heavily rely on translational controls to regulate key cellular features. Available methods to infer the translatome rely on purification of ribosome-associated mRNA from cell populations or from

single cells, suffering from transcript biases, limited coverage or scalability. Moreover, unbiased single-cell proteomics lags next-generation sequencing depth. To overcome these limitations, we have developed a novel technology called Ribosome Surveying Targets by Antibody-free Mutation Profiling (Ribo-STAMP), which allows for simultaneous measurement of both the transcriptome and the translatome. In Ribo-STAMP, a fusion of the cytidine deaminase APOBEC1 with a ribosomal protein is expressed in cells, resulting in cytosine into uracil (C-to-U) edits that permanently mark translated mRNAs and that are identified by standard RNA-seq analyses using mutation-aware transcript mapping algorithms. Here, we demonstrate the applicability of Ribo-STAMP for the analysis of the brain translatome at the single-cell level. We first optimize the performance of Ribo-STAMP in basal conditions, by testing the combination with different ribosomal subunits. We further demonstrate that expression of Ribo-STAMP is not toxic to neurons. Finally, we deliver Ribo-STAMP *in vivo* and pair it with single-cell RNA-seq to detect the translatome of individual neurons from the mouse hippocampus. In Ribo-STAMP expressing cells, we detect edit deposition on accepted cell type-specific markers. Moreover, edited genes positively correlate with published cell type-specific translatome datasets. Results from this study indicate that Ribo-STAMP can be used for cell-type identity mapping throughout the brain. Understanding the translational profiles of different neuronal subtypes will facilitate to provide insights into how the brain functions in a healthy state and how it may go awry during disease.

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Poster

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

Support: NIH Grant 1R21MH119650-01A1

Title: Differential transcriptomic profiles in amygdala are nuclei-specific in a nonhuman primate model of maternal immune activation

Authors: *E. L. CARLSON[1,3], B. P. ANDER[2,3], S. KAMBOJ[4,5], A. S. FOX[4,5], M. D. BAUMAN[3,5,1], C. M. SCHUMANN[1,3];
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Abstract: Children prenatally exposed to maternal infection during pregnancy have an increased risk of neurodevelopmental disorders (NDDs), including autism spectrum disorder and schizophrenia. Rodent models demonstrate that maternal immune activation (MIA) by itself is

capable of altering brain development and behavior in offspring, and evidence suggests that the amygdala, a key modulator of emotional response, is particularly affected. However, the precise MIA-driven cytoarchitecture and gene expression changes and how they manifest in more complex primate neurobiology remain unclear. As part of the UC Davis Conte Center, we developed a preclinical nonhuman primate MIA model using the viral mimic PolyIC to induce a transient immune response during the first trimester. Offspring (N=8, 4 male saline control, 4 male PolyIC treated) underwent extensive neuroimaging, immunological, and behavioral testing from birth until brain acquisition at 4 years of age. Precision sampling techniques utilizing adjacent stained sections as an anatomical reference were applied to isolate fresh frozen tissue discretely from central and lateral amygdala nuclei. Samples were processed into single nuclei suspensions upon which single nuclei RNAseq (sn-RNAseq) was performed. We found that central and lateral amygdala nuclei have unique cellular population profiles and patterns of differential gene expression (DGE) between cells of the same type within a cohort. Additionally, patterns of DGE between MIA and CTL were observed in a subregion-specific manner. Foremost, these results add to the growing body of evidence demonstrating that prenatal immune exposure results in lifelong changes in amygdala development in primates and likely contributes to deviations in typical social behavior observed in the MIA-exposed nonhuman primate cohort. Secondly, these findings demonstrate the individuality of amygdala nuclei and highlight the need for precise subregion- and cell-specific sampling. Subtle yet critical treatment related effects could be lost when brain regions are sampled in bulk without respect for their complex anatomical subdivisions and cell types.

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Poster

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Thomas D. and Virginia W. Cabot Professorship
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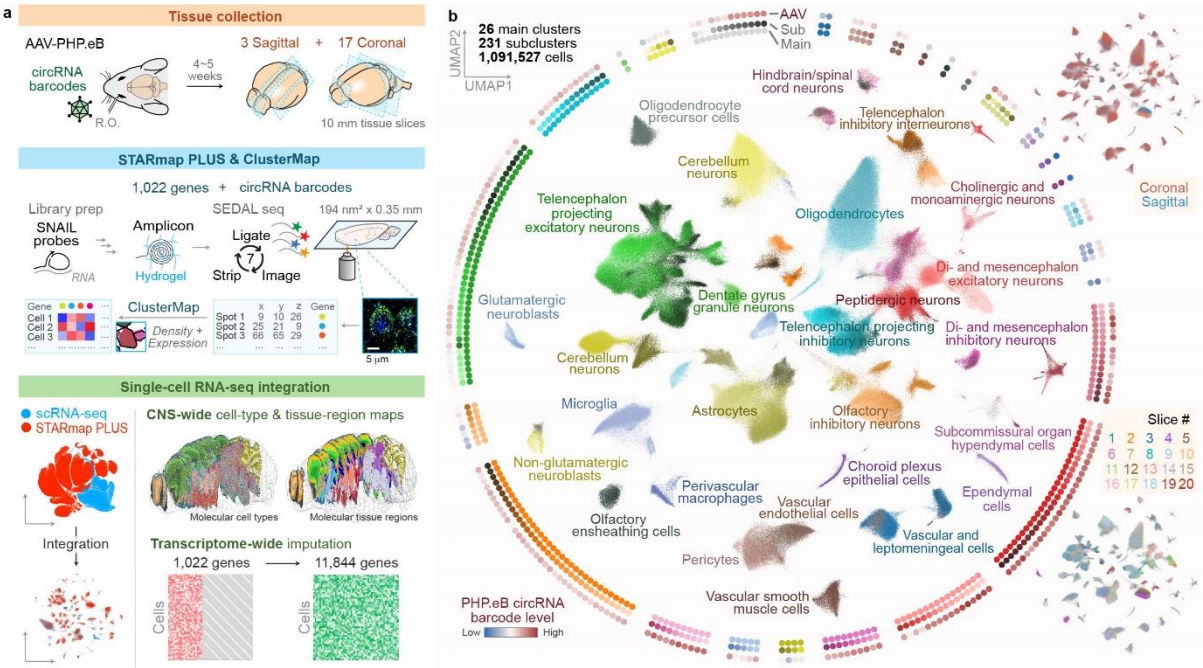
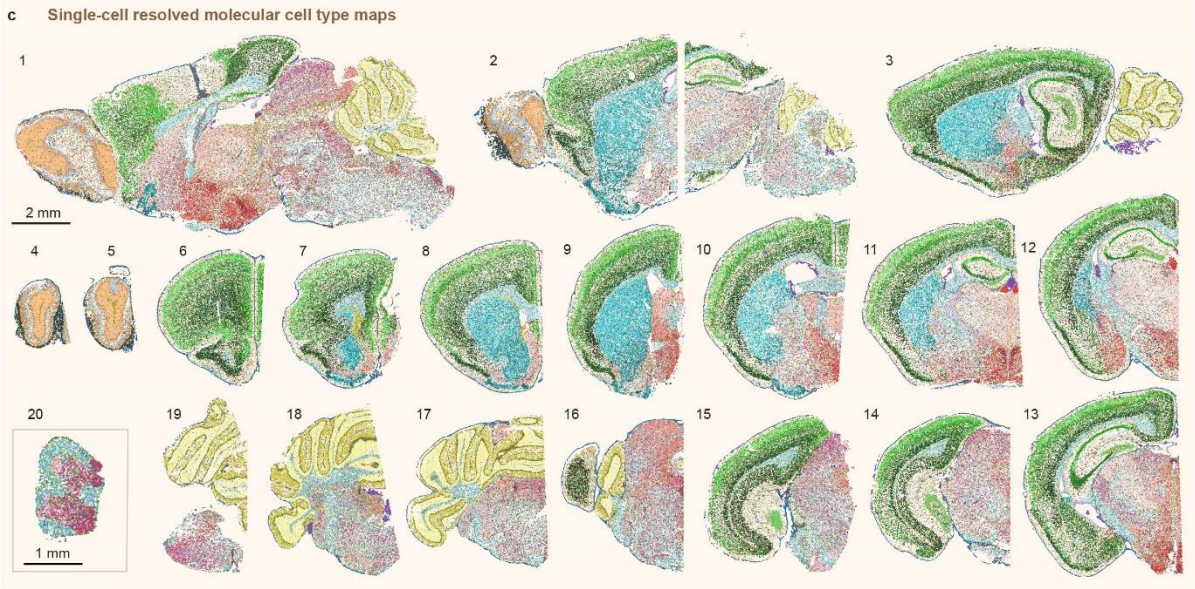
Helen Hay Whitney Foundation Postdoctoral Fellowship

Title: Spatial Atlas of Molecular Cell Types and AAV Accessibility across the Mouse Central Nervous System

Authors: *H. SHI[1,3], Y. HE[1,6], Y. ZHOU[1,3], J. HUANG[1,3], B. WANG[1,4], K. MAHER[1,5], Z. TANG[1,3], S. LUO[1,3], P. TAN[2], M. WU[1], Z. LIN[1,7], J. REN[1,3], Y. THAPA[1,8], X. TANG[1,6], A. LIU[1,3], J. LIU[6], X. WANG[1,3];
[1]Stanley Ctr. for Psychiatric Res., [2]Klarman Cell Observatory, Broad Inst., Cambridge, MA; [3]Dept. of Chem., [4]Computer Sci. and Mol. Biol. Program, [5]Computat. and Systems Biol. PhD Program, MIT, Cambridge, MA; [6]John A. Paulson Sch. of Engin. and Applied Sci., [7]Dept. of Chem. and Chem. Biol., Harvard Univ., Cambridge, MA; [8]Sch. of Natural Sci., Caldwell Univ., Caldwell, NJ

Abstract: Spatially charting molecular cell types at single-cell resolution across the three-dimensional (3D) volume of the brain is critical for illustrating the molecular basis of the brain anatomy and functions. Single-cell RNA sequencing (scRNA-seq) has profiled molecular cell types in the mouse brain, but cannot capture their spatial organization. Here, we employed an *in situ* sequencing technique, STARmap PLUS, to map more than one million high-quality cells across the whole adult mouse brain and the spinal cord, profiling 1,022 genes at subcellular resolution with a voxel size of 194 X 194 X 345 nm in 3D. We developed computational pipelines to segment, cluster, and annotate 231 molecularly defined cell types and 64 tissue regions with single-cell resolution. To create a transcriptome-wide spatial atlas, we further integrated the STARmap PLUS measurements with a published scRNA-seq atlas, imputing 11,844 genes at the single-cell level. Finally, we engineered a highly expressed RNA barcoding system to delineate the tropism of a brain-wide transgene delivery tool, AAV-PHP.eB, revealing its single-cell resolved transduction efficiency across the molecular cell types and tissue regions of the whole mouse brain. Together, our datasets and annotations provide a comprehensive, high-resolution single-cell resource that integrates a spatial molecular atlas, cell taxonomy, brain anatomy, and genetic manipulation accessibility of the mammalian central nervous system (CNS).





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Poster

083. Single Cell Profiling Techniques in Health and Disease

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Program #/Poster #: 083.18

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

Support: NIH 5U19MH114821

Title: Modular and hierarchical cell type organization of cortical areas revealed by in situ sequencing

Authors: *X. CHEN[1], S. FISCHER[2], A. ZHANG[1], J. GILLIS[4], A. M. ZADOR[3];
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[3]Zador Lab., Cold Spring Harbor Lab., Cold Spg Hbr, NY; [4]Univ. of Toronto, Toronto, ON, Canada

Abstract: The cortex is composed of diverse types of excitatory neurons that are organized into specialized cortical areas. Although cortical areas are distinct in their cytoarchitecture, connectivity, and neuronal activity, whether cortical areas contain distinct transcriptomically defined cell types remain unclear. Here we used BARseq, a high-throughput in situ sequencing technique, to interrogate the expression of 107 cell type marker genes in 1.2 million cells over a mouse forebrain hemisphere with high spatial resolution. De novo clustering of gene expression in single neurons revealed transcriptomic types that were consistent with previous single-cell RNAseq studies. Within transcriptomic subclasses, which are shared across all cortical areas, variation in gene expression is dominated by spatial patterns that follow the contours of cortical areas. These spatial patterns in gene expression are recapitulated in the spatial distribution of fine-grained transcriptomic types, which are highly enriched in combinations of cortical areas. The compositions of transcriptomic types are distinct and highly predictive of cortical area identity. Based on the similarity of the composition of transcriptomic types, we identify cortical modules that surprisingly match the cortical hierarchy and modules defined by connectivity. Thus, our findings indicate that the distinct composition of cell types across cortical areas reflects a hierarchical and modular organization of the cortex that is consistent with connectivity.

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Poster

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

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Title: Single-cell transcriptomics of the aging mouse brain

Authors: *K. JIN, C. VAN VELTHOVEN, K. SMITH, J. GOLDY, B. LEVI, N. DEE, K. RONELLENFITCH, Z. YAO, H. ZENG, B. TASIC;
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Abstract: Aging is the leading risk factor for most prevalent chronic diseases, including cardiovascular disease, cancer, and neurodegeneration. The mammalian brain is a highly heterogeneous tissue that is composed of diverse neuronal and non-neuronal cell types that vary in identity and composition across different regions of the brain. Single-cell sequencing methods are rapidly expanding our knowledge of the functions and properties of these different cell types.

However, how these cell types change with age remains to be fully understood. Here we present a large dataset of single-cell transcriptomes of aged (18-month) and young adult (2-month) mouse brain cells from both sexes in the C57BL/6J genetic background. The dataset includes 654,142 aged and 824,304 young adult cells collected using single-cell RNA sequencing on the 10x Genomics v3 platform. This dataset was collected from healthy behaviorally evaluated animals and spans 16 different regions of the brain that were selected due to previously documented age-related vulnerability, including hippocampal formation, prefrontal and medial association cortex, striatum, amygdala, hypothalamus, and parts of midbrain and hindbrain. We find highly congruent gene detection and expression across the two ages, with the strongest age-associated differences observed in non-neuronal cell types. Furthermore, distinctive gene ontology terms were enriched among the differentially expressed genes, such as pro-inflammatory and interferon pathway terms being enriched in aged microglia, and myelin sheath terms enriched among genes that change in aged oligodendrocytes. We further investigated and confirmed some of the newly discovered changes using spatial transcriptomics across several brain areas in the two ages. Together, our analysis demonstrates cell-type specific changes in brain aging and provides the most transcriptomically detailed examination of healthy brain aging across a wide range of brain regions in a well-controlled model organism to date.

Disclosures: **K. Jin:** None. **C. van Velthoven:** None. **K. Smith:** None. **J. Goldy:** None. **B. Levi:** None. **N. Dee:** None. **K. Ronellenfitch:** None. **Z. Yao:** None. **H. Zeng:** None. **B. Tasic:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.20

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

Support: 5U19MH114830

Title: Multiple modality classification of long-range projecting glutamatergic neurons in mouse visual cortex

Authors: *S. SORENSEN[1], N. GOUWENS[1], Y. WANG[4], F. BAFTIZADEH[8], A. BUDZILLO[2], R. DALLEY[8], B. R. LEE[5], M. MALLORY[5], R. MANN[5], C. LEE[9], O. GLIKO[8], A. MUKORA[1], G. WILLIAMS[8], L. ALFILER[6], J. WILSON[6], H. KUO[7], S. YAO[3], P. LESNAR[1], E. SHEN[1], X. KUANG[11], Y. LI[11], L. EL-HIFNAWI[1], U. SÜMBÜL[7], L. NG[8], Z. YAO[1], B. TASIC[10], H. ZENG[8];
[2]Neurobio. and Behavior, [3]Mol. Genet., [1]Allen Inst. for Brain Sci., Seattle, WA; [4]Structure Sci., [5]Electrophysiology, [6]Brain Sci., [7]Allen Inst., Seattle, WA; [9]Modeling Analysis and Theory, [10]Cell and Circuit Genet., [8]Allen Inst. For Brain Sci., Seattle, WA; [11]Wenzhou Med. Univ., Wenzhou, China

Abstract: Excitatory cortical neurons are well-understood to be organized into subclasses defined by layers and interareal projections. However, to resolve excitatory cortical neurons into multi-modal cell types, using Patch-seq we generated a dataset of >1500 neurons sampled across all layers of visual cortex (VIS) with morphological, electrophysiological, and/or transcriptomic information collected from each neuron. Through an integrated analysis (Gouwens 2020), we defined a set of sixteen morpho-electric-transcriptomic (met)-types with congruent properties in each modality in mouse visual cortex. We also mapped neurons to transcriptomic (t)-types based on an existing reference taxonomy (Tasic, 2018). In some cases, met-properties were continuous across t-types, and cells were merged into a single met-type (e.g., L2/3 IT, L6 CT); in other cases, met-properties were distinct and individual t-types correspond to a single met-type (e.g., L5/6 IT Car3, L5 ET Chrna6). Several of this latter group were found to be particularly distinct, yet had not been previously appreciated based on morpho-electric properties alone, highlighting the importance of the transcriptomic identity. To understand the relationship between met-types and long-range axonal projections, we built a random forest classifier based on dendritic features to map a dataset of >300 complete, brain-wide morphologies to our met-type taxonomy. Met-type assignments were validated with multiple methods. For each met-type, we identified specific projection target motifs. Eight different VISp IT met-types each had unique target patterns. Two L5 ET met-types had distinct targeting of subcortical structures, suggesting different functional roles. We also observe clear relationships between met-types and long-range projections in higher order visual areas that begin to reveal the transform for these properties across brain regions. Together these studies reveal an integrated set of excitatory, cortical cell types with unique morphological, electrophysiological, transcriptomic, and long-range axonal projection patterns and establish a flexible method for predicting multi-modal properties from morphology data.

Disclosures: **S. Sorensen:** None. **N. Gouwens:** None. **Y. Wang:** None. **F. Baftizadeh:** None. **A. Budzillo:** None. **R. Dalley:** None. **B.R. Lee:** None. **M. Mallory:** None. **R. Mann:** None. **C. Lee:** None. **O. Gliko:** None. **A. Mukora:** None. **G. Williams:** None. **L. Alfiler:** None. **J. Wilson:** None. **H. Kuo:** None. **S. Yao:** None. **P. Lesnar:** None. **E. Shen:** None. **X. Kuang:** None. **Y. Li:** None. **L. El-Hifnawi:** None. **U. Sümbül:** None. **L. Ng:** None. **Z. Yao:** None. **B. Tasic:** None. **H. Zeng:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.21

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

Title: Characterization of Cocaine-induced Neural Activation and Gene Expression Change in Transcriptomic Cell Types of Mouse Mesocorticolimbic Areas

Authors: *D.-W. KIM, C. LEE, T. CASPER, J. GLOE, M. CLARK, T. PHAM, A. TORKELSON, J. GOLDY, R. FERRER, J. GUZMAN, K. SMITH, N. DEE, Z. YAO, H. ZENG; Allen Inst. for Brain Sci., Seattle, WA

Abstract: Drug addiction is one of major debilitating diseases but its underlying molecular and cellular mechanisms are not yet fully understood. Recent advances of single-cell RNA-sequencing (scRNA-seq) technology have allowed us to capture co-varying gene expression patterns in individual cells in a high-throughput and unbiased manner, and thereby enabling classification of distinct transcriptomic cell types (T-types) in regions of interest. Furthermore, a variant version of scRNA-seq (called "Act-seq") was introduced to identify active neuronal T-types during a particular behavior/manipulation by measuring endogenously induced expression of immediate early genes (IEGs), while unwanted further IEG activation during sample preparation was suppressed. Here we aim for characterizing activated T-types and gene expression changes after systemic injections of cocaine in major mesocorticolimbic areas in mice. We have collected ~858k cells (containing ~330k neurons) dissected from 7 areas (including prefrontal cortex (PFC), ventral striatum (STRv), anterior and medial thalamus (TH:Ant-MM), cortical subplate and amygdalar, midbrain, and hindbrain) using Act-seq (10x Genomics) in 6 different conditions of cocaine administration. Upon mapping QC-passed cells onto our existing whole-brain scRNA-seq taxonomy, we first observe strong IEG activations especially in T-types from PFC, TH:Ant-MM, and STRv in a graded manner (acute cocaine < chronic cocaine + short-term withdrawal < chronic cocaine + long-term withdrawal). Interestingly, some T-types from PL-ILA-ORB (L2/3 IT, L4/5 IT, L6 CT, and Sst GABAergic), TH:Ant-MM (Rxfp1_Epb4), and STRv (MSN D1) are persistently activated only in the chronic cocaine + long-term withdrawal condition. Consistent with previous literatures, we also find differentially expressed (DE) genes relating to epigenetics (e.g. Gadd45b, Kdm6b, Ehmt2) in some neuronal subclasses of PFC and STRv from chronic cocaine conditions. Overall, we have successfully generated large-scale and high-quality scRNA-seq datasets to profile cocaine-induced neural activations and transcriptomic changes at a T-type level. Ongoing work focuses on the following directions: i) how much these cocaine-induced T-type activation patterns are similar to those from other drugs of abuse like morphine, ii) where these activated T-types are spatially located (by mapping it onto our whole-brain multiplexed error-robust fluorescence in situ hybridization (MERFISH) data), and iii) what other interesting DE genes are induced by chronic exposure to cocaine in non-neuronal T-types.

Disclosures: D. Kim: None. **C. Lee:** None. **T. Casper:** None. **J. Gloe:** None. **M. Clark:** None. **T. Pham:** None. **A. Torkelson:** None. **J. Goldy:** None. **R. Ferrer:** None. **J. Guzman:** None. **K. Smith:** None. **N. Dee:** None. **Z. Yao:** None. **H. Zeng:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.22

Topic: I.02. Systems Biology and Bioinformatics

Support: JST ERATO grant number JPMJER2001
the Science and Technology Platform Program for Advanced Biological Medicine
JSPS KAKENHI grant-in-aid for scientific research (S) (grant number JP18H05270)
JSPS KAKENHI grant-in-aid for scientific research (C) (grant number 20K06885)
JSPS KAKENHI grant-in-aid for scientific research (B) (grant number 22H02824)
JSPS KAKENHI grant-in-aid for Early-Career Scientists (grant number 20K16498)
Grant-in-Aid from the Human Frontier Science Program

Title: Whole-brain neuron profiling identifies biological alterations in three-dimensional structure and function at a single-neuron level

Authors: *T. T. MITANI[1,2], S. Y. YOSHIDA[1,2], K. YAMAURA[1,2], K. MATSUMOTO[1,3], E. A. SUSAKI[1,4], H. R. UEDA[1,3];
[1]RIKEN Ctr. for Biosystems Dynamics Res., Osaka, Japan; [2]Grad. Sch. of Med., Osaka university, Osaka, Japan; [3]Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan; [4]Grad. Sch. of Med., Juntendo university, Tokyo, Japan

Abstract: Since the advent of Brodmann's brain map, the cellular architecture, which is the anatomical arrangement of neurons under a microscope, is the basis of brain organization and is closely related to its function. However, its three-dimensional microstructure and function have never been comprehensively studied. Recently, tissue clearing and light-sheet microscopy have facilitated the visualization of three-dimensional macroscale structures, but it has been difficult to distinguish individual cells from densely distributed neurons such as the hippocampus and cerebellum because of the high resolution required. To overcome this problem, we firstly established a pipeline (Cellomics-neurology) to identify the structural information of all neurons in the mouse brain by combining 3D immunostaining, high-resolution light-sheet microscopy, cloud-based whole-brain analysis software, and a single-cell resolution atlas. We clarified changes in the number and arrangement of neurons during development and aging from 1-week-old to 1-year-old mouse brains, and constructed a single-cell resolution neuron atlas to be integrated. In addition, by using drug administration and AAV induction, and transgenic models of neurodegeneration, the areas of neuronal loss were comprehensively identified at the whole-brain scale by using micro-architectural information. Thus, cellomics-neurology opens up a new field of characterizing every neuron within a unique structure. Next, we established a pipeline (Cellomics-activity) to identify the functional information of all neurons in the mouse brain based on an almost similar method. We focused on the interregional circadian coupling on a whole-brain scale, which is not yet well known but is the key to generating circadian-dependent brain physiology. The time-series analysis of the DD state whole-brain samples at each time point ($n \geqq 3$ respectively) revealed oscillations of c-fos immunoreactivity consistent with circadian rhythms in the SCN, as well as alterations in other brain regions. Therefore, cellomics enables a three-dimensional structural and functional evaluation at a single-neuron level that could not be captured by classical histological evaluation. It contributes not only to drug discovery but also has great potential for designing new artificial intelligence models.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.23

Topic: I.02. Systems Biology and Bioinformatics

Support: JST ERATO grant number JPMJER2001
the Science and Technology Platform Program for Advanced Biological Medicine
JSPS KAKENHI grant-in-aid for scientific research (S) (grant number JP18H05270)
JSPS KAKENHI grant-in-aid for scientific research (C) (grant number 20K06885)
JSPS KAKENHI grant-in-aid for scientific research (B) (grant number 22H02824)
JSPS KAKENHI grant-in-aid for Early-Career Scientists (grant number 20K16498)
Grant-in-Aid from the Human Frontier Science Program

Title: Whole organ/body atlas of mouse with a single-cell resolution

Authors: *S. Y. YOSHIDA[1,2], T. T. MITANI[1,2], K. MATSUMOTO[1,3], H. R. UEDA[3,1];
[1]RIKEN Ctr. For Biosystems Dynamics Res., Osaka, Japan; [2]Grad. Sch. of Med., Osaka university, Osaka, Japan; [3]Grad. Sch. of Med., The Univ. of Tokyo, Tokyo, Japan

Abstract: Systemic organs such as the lungs, kidneys, heart, and liver are damaged by a variety of factors, including cancer and drugs. Traditional pathological studies of organs have been done by making sections and partially observing the regions of interest. Observing the whole organ at single-cell resolution will make it possible to quantify cancer cell metastasis or persistence by number and evaluate the damage of organs by drugs with high sensitivity without bias. In recent years, it has become possible to observe whole organs by combining the tissue clearing method and light-sheet microscopy by a method represented by CUBIC (clear, unobstructed brain imaging cocktails and computational analysis) (REF: Susaki et al. Cell 2004). Using this technique, a single-cell resolution atlas of the mouse brain was created (REF: Murakami et al. Nature Neuroscience. 2018, Matsumoto et al. Nature Protocols 2019). This brain atlas has enabled the analysis of perturbations in all regions of the brain (REF: Mano et al Cell Reports Methods 2021). However, a single-cell resolution atlas of organs other than the brain had not been created. First, we created references (atlas) of various organs with a single-cell resolution to lay the foundation for future organ analysis. We performed clearing of 8-week-old mouse organs using CUBIC procedures. The organs such as the lungs, kidneys, heart, liver, bladder, testes, thyroid glands, salivary glands, and pancreas were tissue cleared and Taken with a customized

light-sheet microscope. The cells are counted automatically using CPU and GPU. About 100 million cells were detected in the lungs, 80 million in the kidneys, 35 million in the heart, 350 million in the liver, 6 million in the bladder, 36 million in the testes, and 55 million in the salivary glands, and 79 million in the pancreas. As application examples of whole-cell analysis, a kidney damage model caused by cisplatin and a lung damage model caused by LPS were observed. There was a decrease in cell number in renal damage caused by cisplatin. There was an increase in cell number in lung damage caused by LPS. We performed the cleaning of the whole body of P0 mise using customized CUBIC and Over 300 million cells were detected. These atlantes may also play an important role in a global analysis of organs and the whole body by overlaying various cellular functions.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.24

Topic: I.02. Systems Biology and Bioinformatics

Support: Chan-Zuckerberg Foundation Seed Networks for the Human Cell Atlas program: CZF2019-002457

Title: Single-cell analysis of inter-individual variability in human prefrontal cortex transcriptomes and epigenomes

Authors: J.-F. CHIEN[1], H. LIU[4,5], A. BARTLETT[4,5], R. CASTANON[4,5], N. D. JOHNSON[6,2], B.-A. WANG[4,5], J. R. NERY[4,5], J. OSTEEN[4,5], J. R. ECKER[4,5], M. M. BEHRENS[6,2], E. A. MUKAMEL[3];
[1]Physics, [2]Dept. of Psychiatry, [3]Cognitive Sci., UCSD, La Jolla, CA; [4]Genomic Analysis Lab., [5]Howard Hughes Med. Inst., [6]Computat. Neurobio. Lab., Salk Inst., La Jolla, CA

Abstract: Identifying the conserved and variable epigenomic signatures of the brain's cellular components is critical for understanding the neurobiological basis of individual variation in brain function. Since DNA methylation is dynamic across the lifespan and highlights sex-specific epigenetic regulation (Lister et al., 2013; Keown et al., 2017), single cell DNA methylation profiling can resolve the rich diversity of human brain cell types (Luo et al., 2017; Mulqueen et al., 2018). Recently, a multimodal approach has enabled measurement of DNA methylation and mRNA expression in the same cell (snmCAT-seq) (Luo et al., 2022). While existing datasets have revealed the diversity of brain cell types, the extent of inter-individual difference and conservation of epigenomic regulation is unknown.
To address this, we applied single nucleus DNA methylome and RNA transcriptome sequencing (snmCT-seq) to neurons from the human prefrontal cortex (Broadmann Area BA46). Our data

includes post-mortem tissue from 11 adult donors ranging in age from 23 to 74 years, from both males and females. Based on DNA methylation features, we clustered nuclei into 16 major neuron types. We found that upper layer excitatory neurons have the highest number of age-related differentially methylated regions (DMRs). The DMRs were enriched in genes related to dendrite development and synapse activity. Moreover, upper layer excitatory neurons also had higher interindividual variation. Finally, by analyzing female-specific enrichment of non-CG DNA methylation on X-linked genes, we are able to identify genes that escape from X chromosome inactivation in specific brain cell types. Our multiomic data set from donors with sex and age diversity provides an initial assessment of major dimensions of inter-individual variability for human prefrontal cortical neurons.

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Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.25

Topic: I.02. Systems Biology and Bioinformatics

Title: Single-cell profiling of the zebrafish brain and retina using combinatorial indexing

Authors: *P. BOYD, M. NAKAMOTO, N. PACHECO, L. LIN, J. THOMAS, D. POKHOLOK, F. SCHLESINGER, T. GUETTOUCHE, F. STEEMERS;
Scale Biosci., San Diego, CA

Abstract: Single-cell sequencing technologies have allowed for in-depth analyses of neuronal cell types and demonstrated the vast heterogeneity within these tissues. However, these studies have often been limited in their scope due to low cell throughput and prohibitive costs associated with performing these experiments on large numbers of samples. In this study, we show that combinatorial indexing can be effectively utilized to drastically increase cell output from a single experiment, while allowing multiple tissues from multiple individuals to be analyzed simultaneously, significantly reducing bench time and cost per cell.
Utilizing the ScaleBio ATACseq pre-indexing kit, nuclei from both retina and brain samples from 3 zebrafish were split across 24 wells on the ScaleBio tagmentation plate, allowing unique indexing to identify each tissue and individual. Indexed nuclei were then pooled and superloaded with up to 100,000 nuclei per lane of a single-cell capture system with addition of a second barcode enabling a low effective doublet rate and identification of multiplets. Analysis of these data shows high barcode and mapping rates and high recovery of nuclei from all samples and tissues. Following standard QC filtering we show that all major cell types from both retina and brain can be identified using this system, allowing for direct comparison of cell types between individuals with reduced risk of experiment-induced artefacts. These data show that

combinatorial barcoding can be effectively applied to neuronal tissues, and due to the high number of nuclei low-abundance or sample-specific cell types can be identified, allowing for a greater understanding of sample heterogeneity. In summary, combinatorial indexing allows for a significant increase in sample size, while reducing bench time and cost, without compromising data quality.

Disclosures: **P. Boyd:** A. Employment/Salary (full or part-time):; Scale Biosciences. **M. Nakamoto:** A. Employment/Salary (full or part-time):; Scale Biosciences. **N. Pacheco:** A. Employment/Salary (full or part-time):; Scale Biosciences. **L. Lin:** A. Employment/Salary (full or part-time):; Scale Biosciences. **J. Thomas:** A. Employment/Salary (full or part-time):; Scale Biosciences. **D. Pokholok:** A. Employment/Salary (full or part-time):; Scale Biosciences. **F. Schlesinger:** A. Employment/Salary (full or part-time):; Scale Biosciences. **T. Guettouche:** A. Employment/Salary (full or part-time):; Scale Biosciences. **F. Steemers:** A. Employment/Salary (full or part-time):; Scale Biosciences.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.26

Topic: I.08. Methods to Modulate Neural Activity

Support: Stanford University School of Medicine Dean's Postdoctoral Fellowship
Stanford University Walter V. and Idun Berry Postdoctoral Fellowship
Stanford University Bio-X Undergraduate Summer Research Program
Stanford University Chemistry Undergraduate Summer Research Fellowship
NIH K99/R00 Pathway to Independence Award (1K99NS119784)
Gatsby Foundation Grant

Title: Mapping the Postnatal Dynamics of Single-cell 3D Genome, Transcriptome, and Epigenome in the Developing Mouse and Human Cerebellum

Authors: *L. TAN[1], J. SHI[1,2], R. CHEN[1], I. COBOS[3], L. DUNCAN[4], K. DEISSEROTH[1,4,5]; [1]Bioengineering, [2]Chem., [3]Pathology, [4]Psychiatry and Behavioral Sci., [5]Howard Hughes Med. Inst., Stanford Univ., Palo Alto, CA

Abstract: Throughout neurodevelopment, spatiotemporal expression patterns of thousands of genes are precisely orchestrated, in ways that presumably guide the formation of massive interconnected neural and glial networks. How does complex regulation of gene expression occur at the cellular level? An emerging mechanism involves 3D genome architecture, whose alteration is implicated in multiple disorders including autism spectrum disorder (ASD). However, our understanding of genome architecture in the developing brain is severely limited by a lack of high-resolution in vivo technology for comprehensive single-cell datastreams. To bridge this longstanding gap, we previously developed a high-resolution single-cell chromatin

conformation capture (3C/Hi-C) method—Dip-C—and solved the first genome structure of a single human cell. We applied Dip-C to various developing nervous systems including the mouse eye, nose, cerebral cortex, and hippocampus. We found 3D genome "structure types" to underly functional cell types, and discovered multi-scale 3D transformation after birth.
Here we turn to the cerebellum, which harbors 80% of neurons in our brain and develops unusually late. The cerebellum is also the most consistently affected brain region in ASD. However, little is known about its 3D genome dynamics. We solved the first genome structure of single cerebellar cells, and created a single-cell 3D genome atlas of 10,476 cells from the mouse (12 time points from birth to 2 years) and human cerebellum (24 donors from birth to 86 years) of both sexes—the largest high-resolution 3D genome atlas of any kind. To understand concurrent changes in gene expression and chromatin accessibility, we created a single-cell multi-ome atlas (simultaneous transcriptome and epigenome) of 63,767 cells (7 donors from birth to 38 years). We observed profound changes in all 3 modalities. Most notably, 3D genome of granule cells undergoes highly cell type-specific rewiring throughout life, more extensive than our previous observations in the forebrain. Both species were born with a structure type similar to that of the forebrain, but transitioned into a new structure type with extensive long-range intra-chromosomal interactions—suggesting strong "phase separation". In addition, unlike the cerebral cortex—where most neurons change structure types at the same time, the cerebellum transiently consisted of 2 granule cell structure types, around postnatal day 14 in mice and year 0.5 in humans. Our findings provide new molecular insight into the cerebellum's unique mode of development by creating first-of-its-kind genomic resources, and may point to critical processes relevant to ASD.

Disclosures: **L. Tan:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); L.T. is an inventor on a patent application US16/615,872 filed by Harvard University that covers Dip-C.. **J. Shi:** None. **R. Chen:** None. **I. Cobos:** None. **L. Duncan:** None. **K. Deisseroth:** None.

Poster

083. Single Cell Profiling Techniques in Health and Disease

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 083.27

Topic: H.03. Decision Making

Support: UG3MH120094 (WRS)
DP2MH113095 (WRS)

Title: Genetic element screening for targeting neuron types in the primate cortex and basal ganglia

Authors: ***J. HE**[1], B. N. PHAN[3], O. WIRFEL[1], M. SEDOROVITZ[2], W. G. KERKHOFF[1], E. OZTURK[2], J. CHEN[1], B. M. HOOKS[1], L. BYRNE[2], A. R. PFENNING[3], W. R. STAUFFER[1];

[1]Neurobio., [2]Ophthalmology, Univ. of Pittsburgh, Pittsburgh, PA; [3]Computat. Biol., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Cell type-specific neural signals, such as dopamine reward prediction error responses, are a fundamental component of behavior and cognition. Despite the importance of cell types for understanding neurobiological functions, there remain few avenues to achieve cell type-specific manipulations in Rhesus macaque monkeys, or indeed, primates in general. Distal regulatory elements - enhancers - have been successfully paired with adeno-associated viruses (AAVs) to drive cell type-specific transgene expression. Enhancer-driven expression can be targeted to cell types defined at multiple resolutions including major neuronal classes like GABAergic interneurons, well-defined neuronal class subtypes, including PV and SOM interneurons, or specific cortical layer subtypes. Moreover, enhancers derived from conserved genome sequences have been shown to target cell type-specific populations in multiple species from mice to NHP. Our primary goal is to enable and expand effective experimental cell type-specific manipulations in NHP brain structures related to reward, decision-making, and other cognitive functions. Therefore, we used single nucleus RNA-Seq to identify and distinguish cell types and subtypes within and between cortical and basal ganglia regions. These transcriptionally defined molecular phenotypes are the targets for cell type-specific vectors. We mapped the enhancer landscape of these neuron types and subtypes using single nucleus ATAC sequencing and developed a state-of-the-art machine learning pipeline that integrates genomic and epigenetic conservation across mouse, human, and NHP to learn the underlying sequence sufficient for cell type-specific gene expression. We have computationally screened tens of thousands of enhancers from the dorsolateral prefrontal cortex and striatum and selected the top 5 candidates for each cell type. We paired each candidate with a unique DNA barcode and cloned candidate enhancer-DNA barcode pairs into AAV constructs. We packaged the constructs into an NHP neural tropic AAV9 serotype and injected them into rhesus monkey and mouse brains. Preliminary results indicate cell type-specific expression in the striatum, but also a strong effect of a minimal promoter. These results lay the foundation for broad screening of cell type-specific AAVs in the NHP brain.

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Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

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Program #/Poster #: 084.01

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

Support: Samsung Research Funding & Incubation Center for Future Technology (SRFC-IT1702-09)

Title: Analyzing heterogeneity of the thalamus of a mouse brain through PICASSO

Authors: *J.-B. CHANG[1], J. SEO[1], Y. SIM[2], J. KIM[1], H. KIM[1], I. C. CHO[1], H. NAM[1], Y.-G. YOON[1];
[1]Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of; [2]Sungkyunkwan Univ., Suwon, Korea, Republic of

Abstract: Multiplexed imaging is essential in revealing a range of neuronal cell types, neural connections, and heterogeneity in the brain, by visualizing the distribution, expression, and interaction of various biomolecules in situ. Recently, a cutting-edge ultra-multiplexed imaging technique termed PICASSO has been developed, which can provide more than 15-color imaging without the use of additional equipment (e.g., spectral detector) in a single staining and imaging cycle. In this study, we analyzed neuronal cell types and heterogeneity of the thalamus of the mouse brain through PICASSO. Using PICASSO, we were able to acquire images of 11 proteins (i.e., three cell type markers; NeuN (neuron marker), S100B (astrocyte marker), and G protein-coupled receptor 17 (GPR17, oligodendrocyte marker) and eight proteins; necab2, ZNF3, sox2, PV, calretinin, calbindin, rap1gap, and 4-aminobutyrate aminotransferase (ABAT)) and DAPI in the thalamus of a mouse brain. Then, based on the expression levels of the 11 proteins, 7,759 cells in the thalamus were classified into their own neuronal cell subtypes. As a result, each subregion of the thalamus showed various neuronal cell compositions, indicating that multiplexed imaging can uncover new neuronal cell subtypes and the brain's heterogeneity. We envision that by mapping protein spatial distribution and neuronal heterogeneity in the brain, PICASSO will enhance spatial proteomics research and become a versatile tool for a wide spectrum of researchers.

Disclosures: J. Chang: None. **J. Seo:** None. **Y. Sim:** None. **J. Kim:** None. **H. Kim:** None. **I.C. Cho:** None. **H. Nam:** None. **Y. Yoon:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.02

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

Support: National Research Foundation of Korea (NRF) (NRF-2021M3A9I4026318)

Title: Simple and fast multiplexed cyclic staining method for visualizing three-dimensional spatial protein distribution

Authors: *H. KIM, S. BAE, H. NAM, J. SEO, Y.-G. YOON, J.-B. CHANG;
Korea Advanced Inst. of Sci. and Technol., Daejeon, Korea, Republic of

Abstract: Multiplexed fluorescent imaging, which allows the observation of multiple targets in the same specimen has recently received a huge attention for implementation of spatial

proteomics and its potential in biomedical fields. Cyclic staining is a representative method to visualize multiple target proteins by iteratively eliminating the signals between adjacent imaging rounds. Such iterative signal removal step is mainly carried out through either antibody stripping or fluorophore inactivation. Both approaches require chemical exposure to the sample, which can cause sample distortion and incomplete signal removal along consecutive imaging rounds. Repeated chemical exposure may create sample distortion issues such as physical damage and cell loss, whilst residual signal issues are caused by binding affinity of antibodies and bleaching property of fluorophores. While it comes to signal elimination, all of the concerns must be taken into account when optimizing chemical composition and process time. However, because they are in a trade-off relationship, obtaining both entire signal elimination and sample distortion minimization is incongruent. Here we develop a simple and fast multiplexed cyclic imaging method, named IMPASTO. 37-plex fluorescent imaging *via* IMPASTO is successfully demonstrated within 12 staining rounds. Additionally, we show 12-plex 3D IMPASTO imaging to visualize 3D protein distribution and also confirm the potential of utilizing IMPASTO in combination with conventional antibody stripping method to perform more robust multiplexed cyclic imaging.

Disclosures: **H. Kim:** None. **S. Bae:** None. **H. Nam:** None. **J. Seo:** None. **Y. Yoon:** None. **J. Chang:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.03

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

Support: NIH RF1MH123237
NIH R24EY027283
NIH K08EY027093
NIH R01EY033103
NIH EY025585

Title: Cell-specific regulation of gene expression using splicing-dependent frameshifting

Authors: *J. LING, A. BYGRAVE, C. SANTIAGO, R. CARMEN-OROZCO, V. TRINH, Y. LI, B. LANGMEAD, S. SUN, K. NIELSEN, M. SINGH, W. DALTON, F. RAJAII, R. L. HUGANIR, S. BLACKSHAW;
Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Precise and reliable cell-specific gene delivery remains technically challenging. Here we report a splicing-based approach for controlling gene expression whereby separate translational reading frames are coupled to the inclusion or exclusion of cell-specific alternative exons. Candidate exons are identified by analyzing thousands of publicly available RNA

sequencing datasets and filtering by cell specificity, sequence conservation, and local intron length. This method, which we denote splicing-linked expression design (SLED), can be combined in a Boolean manner with existing techniques such as minipromoters and viral capsids. SLED vectors can leverage the strong expression of constitutive promoters, without sacrificing precision, by decoupling the tradeoff between promoter strength and selectivity. We generated SLED vectors to selectively target all neurons, photoreceptors, or excitatory neurons, and demonstrated that specificity was retained in vivo when delivered using AAVs. We further demonstrated the utility of SLED by creating what would otherwise be unobtainable research tools, specifically a GluA2 flip/flop reporter and a dual excitatory/inhibitory neuronal calcium indicator. Finally, we show the translational potential of SLED by rescuing photoreceptor degeneration in Prph2(rds/rds) mice and by developing an oncolytic vector that can selectively induce apoptosis in SF3B1 mutant cancer cells. The flexibility of SLED technology enables new avenues for basic and translational research.

Disclosures: **J. Ling:** None. **A. Bygrave:** None. **C. Santiago:** None. **R. Carmen-Orozco:** None. **V. Trinh:** None. **Y. Li:** None. **B. Langmead:** None. **S. Sun:** None. **K. Nielsen:** None. **M. Singh:** None. **W. Dalton:** None. **F. Rajaii:** None. **R.L. Huganir:** None. **S. Blackshaw:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.04

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

Support: will be disclosed at **poster**

Title: Effect of vaporized THC chronic self-administration in the expression of peripheral cannabinoid receptors and changes in the gut microbiome of rats

Authors: *J. ROSADO-FRANCO[1], C. F. MOORE[2], E. M. WEERTS[2], D. W. WILLIAMS[3]; [2]Psychiatry and Behavioral Sci., [3]Mol. and Comparative Pathobiology, [1]Johns Hopkins Univ., Baltimore, MD

Abstract: Vaping of cannabis and constituents such as Δ9-tetrahydrocanabinol (THC) is on the rise. Vaping has been promoted as a safer alternative to smoked cannabis, however data on health benefits and risks of chronic vaping of cannabinoids is lacking. This project intends to study the effect of chronic THC vapor self-administration on changes in peripheral endocannabinoid system receptor expression and in the microbiome using female Sprague-Dawley rats. Rats were assigned to either vehicle vapor (100% propylene glycol [PG], N=24) or THC vapor (N=24) groups. Rats were allowed to self-administer PG or THC (50 mg/ml) vapor puffs in operant chambers for one hour on intermittent days (e.g. Mon, Wed, Fri). Rats initially self-administered vapor puffs (PG or 50 mg/ml THC) under a fixed ratio (FR) 1 schedule, after which the FR or the THC concentration (5-200 mg/ml) was systematically manipulated. The

total duration of vapor self-administration was 9 months. At the end of chronic vapor self-administration, a subset of rats (N=12 PG vapor; N=12 THC vapor) were tested for anxiety-like behavior using the open field test. Rats were later euthanized with isoflurane overdose and rapid decapitation. Pancreas, heart, lung, kidney, liver, muscle, colon, mesenteric lymph node, spleen and feces were individually collected in a subset of rats (N=6 PG vapor; N=5 THC vapor). Blood was washed using fresh 1X PBS, then the sample was flash frozen on dry ice and stored at -80°C. For gene analysis, RNA was extracted using RNeasy mini kit (QIAGEN), cDNA was synthesized using iScript™ cDNA synthesis kit (BIORAD) and relative gene expression of *cnr1, cnr2, gpr55, gpr119, gpr18, adenosine 2a, ppara, pparg, trpv2, trpm8, faah* and *naaa* was determined using quantitative Polymerase Chain Reaction (qPCR) using TaqMan gene expression analysis primers (ThermoFisher). Relative expression was calculated using the $2^{-\Delta CT}$ method and subtracting the expression of 18S as endogenous control of expression. For microbiome analysis, DNA was extracted using QIAmp PowerFecal Pro DNA kit (QIAGEN) and relative bacterial gut abundance was determined using 16S sequencing and QIIME2. Average number of puffs obtained under the FR5 schedule was 4.7 (±0.6) puffs of 50 mg/ml THC or 3.8 (±0.7) puffs of PG vapor. Female rats who self-administered THC vapor showed greater anxiety-like behavior than rats administering PG vapor ($t(1,18)=2.99$; $p<0.01$). Full pharmacological and behavioral results from THC vapor self-administration in these rats will be discussed elsewhere. This project will characterize changes to peripheral endocannabinoid system receptor expression and in the microbiome produced by voluntary THC vapor exposure.

Disclosures: J. Rosado-Franco: None. **C.F. Moore:** None. **E.M. Weerts:** None. **D.W. Williams:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

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Program #/Poster #: 084.05

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

Support: NIH/NINDS R21NS116760
Whitehall Foundation (2017-05-35)

Title: Identification of Neuronal E3 ubiquitin ligase substrates for TRIAD3A using Orthogonal Ubiquitin Transfer (OUT)

Authors: *W. WEI[1], R. LIU[2], A. J. GEORGE[1,3], J. YIN[2], A. M. MABB[1,3];
[1]Neurosci. Inst., [2]Dept. of Chemistry, Ctr. for Diagnostics & Therapeut., [3]Ctr. for Behavioral Neurosci., Georgia State Univ., Atlanta, GA

Abstract: Protein ubiquitination (UB) is a posttranslational modification that is mediated by E1-E2-E3 enzymatic cascades. Ubiquitination regulates key pathways of neuronal cell biology that include signal transduction, protein degradation, DNA repair, inflammation, neural development,

and synaptic plasticity. The way in which ubiquitin is transferred to a substrate is dependent on the type of E3 ligase catalytic domain. E3 UB ligases also specify the targets, timing, and subcellular location of protein ubiquitination reactions. Mutations in the E3 Ub ligase *RNF216/TRIAD3* have been identified in Gordon Holmes syndrome (GHS), a rare disorder associated with hypothalamic dysfunction, dementia, and neurodegeneration. Although GHS causative mutations have been shown to decrease ubiquitination of RNF216/TRIAD3 targets, a knowledge gap remains on the molecular mechanisms that drive the development of GHS due to challenges to identify E3 ligase substrates in neurons. Here, we use an innovative platform known as "orthogonal ubiquitin transfer (OUT)" to identify new substrates for RNF216/TRIAD3A, which takes advantage of an exogenous UB transfer cascade constituted by xE1-xE2-xE3 to exclusively deliver an engineered xUB to its substrate proteins. Purifying xUB-conjugated proteins from neural cells and identifying them by proteomics would generate the substrate profile for RNF216/TRIAD3.

Disclosures: W. Wei: None. **R. Liu:** None. **A.J. George:** None. **J. Yin:** None. **A.M. Mabb:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.06

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

Support: NIH 2R01NS044025-15

Title: Microglia and neurons in the developing neonatal mouse cortex show distinct transcriptional and translational signatures following the lipopolysaccharide mediated innate immune challenge.

Authors: *S. GADAGKAR[1], H. BOUTEJ[1], Y. WENG[1], E. DI MARTINO[3], Z. S. VEXLER[4], J. KRIZ[2];
[2]Dept. of Psychiatry and Neurosci., [1]CERVO Brain Res. centre, Laval Univ., Quebec City, QC, Canada; [3]Neurol., Univ. of California San Francisco, San Francisco, CA; [4]Neurol., Univ. California San Francisco, San Francisco, CA

Abstract: Neonatal mouse brain is very dynamic, with activated microglia involved in the processes such as synaptic pruning, immuno-surveillance and homeostasis. An immunological insult during the early developmental stages can lead to aberrant microglial response, ultimately damaging the neurons and potentially leading to neurological disorders. While studies have been focusing on the identification and description of context-dependent microglia immune transcripts, *in vivo* microglial and neuronal proteomics and associated regulatory mechanisms in neonates are less well defined (Beutner et al, *Glia*, 2013) . One of the limiting factors is lack of appropriate animal models to study real-time *in vivo* transcriptional and translational dynamics.

To decipher the microglial-neuronal molecular communication *in vivo*, we created the transgenic CD11brGFPxNFLrRFP mouse line, where the ribosomes of microglia are labelled with GFP and FLAG and of neurons with RFP. We performed a systemic lipopolysaccharide injection on unsexed post-natal day (P) 9 mice to stimulate immune response and analyzed on P10 (n=6, saline as control). Using modified translational ribosome affinity purification, we took a snapshot of the dynamic translational state of microglial and neuronal ribosomes by capturing the real-time transcribed mRNAs and translated peptides. The collected RNAs were subjected to Affymetrix® mouse gene chip array and the peptides to mass spectrometry.
We identified the top mRNA and peptide signatures associated with microglia and neurons. Interestingly, we found that the top upregulated microglial and neuronal transcripts were not translated. Both the microglial and neuronal mRNA signatures suggest an inflammatory profile, but their peptidyl profiles gravitate towards homeostasis. We verified our findings by performing western blot on microglia and neurons which were isolated using CD11b beads and the neuronal isolation kit (Miltenyi Biotec®). Additionally, our results from microglial cytokine array indicate no distinct differences between the expression of inflammatory cytokines between the LPS-treated male and female neonatal mice.
Collectively our results indicate a discrepancy in the mRNA and the peptide profiles of microglia and neurons in neonatal mice post the LPS-challenge. This possibly hints at the existence of a post-transcriptional regulation. Targeting such regulators may normalize the immune profile by aiding microglial phagocytosis and ultimately bringing homeostasis, thus paving a way to novel therapeutic targets.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.07

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

Support: NIH/NIA R01AG044372
R01AG067762
R01NS082730

Title: Production and purification of spermidine-modified recombinant tau protein

Authors: *M. M. ALHADIDY[1,3], J. LAMP[1,2], I. VEGA[1,2,3], N. M. KANAAN[1,3];
[1]Translational Neurosci., [2]Integrated Mass Spectrometry Unit, Col. of Human Medicine, Michigan State Univ., Grand Rapids, MI; [3]Neurosci. Program, Col. of Natural Science, Michigan State Univ., East Lansing, MI

Abstract: Tau protein aggregates in tauopathies and is subject to diverse post-translational modifications (PTMs). Although phosphorylation of tau is extensively studied within the context of disease pathophysiology, less abundant PTMs have not gained the same attention. This limitation stems, in part, from the relative lack of production methods generating tau modified with a diverse profile of PTMs. Polyamination of tau is one understudied PTM where polyamines (e.g., spermidine; SPD) are incorporated onto glutamine residues by the transglutaminase (TG) enzyme. In this work, we describe a method to produce and purify recombinant tau modified with spermidine from *E. Coli*. First, we produce and purify non-modified recombinant tau protein from BL21 (DE3) using multiple chromatography techniques. Second, recombinant tau is modified by an *in vitro* reaction with SPD by TG that requires calcium and DTT to catalyze the polyamination reaction. The products of this polyamination reaction are 1) SPD-modified monomeric tau, 2) SPD-modified dimeric tau, 3) intramolecularly cross-linked monomeric tau, and 4) intermolecularly cross-linked multimeric tau. At this stage, tau is processed through a two-step procedure for further purification. Step 1 is to pass the polyamination reaction through a membrane concentrator with 100kDa molecular weight cutoff. The purpose of this step is to help remove cross-linked, multimeric tau and most polyamine-modified dimeric tau species that cannot pass through the membrane. Step 2 includes a His-tag purification to separate the monomeric tau (the flow-through from step 1) from TG and the other components of the polyamination reaction. Modification of tau by SPD is then validated by western blot (WB) and mass spectrometry (MS). MS results indicate tau modification with SPD at the following glutamine residues: Q6, Q49, Q88, Q165, Q244, Q307, Q351, and Q424. Interestingly, Q6, Q88, Q244, Q351, and Q424 were reported as sites of tau modified by TG in cross-linking reactions (i.e., polyamination reactions conducted with no polyamine in the medium). Ongoing studies are investigating the effects of polyamination on aggregation kinetics, aggregate morphology, formation of pathogenic conformations such as oligomers, and the ability to seed aggregation in a tau biosensor cell line. This work highlights a novel method to generate highly purified polyaminated recombinant tau proteins and the impact of these modifications on tau aggregation.

Disclosures: **M.M. Alhadidy:** None. **J. Lamp:** None. **I. Vega:** None. **N.M. Kanaan:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.08

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

Support: HHMI

Title: Brain-wide measurement of synaptic protein turnover with high spatial and temporal resolution

Authors: *B. MOHAR[1], J. B. GRIMM[1], P. W. TILLBERG[1], L. LAVIS[1], N. SPRUSTON[1], K. SVOBODA[2];
[1]HHMI Janelia Res. Campus, Ashburn, VA; [2]Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: Cells regulate function by modulating the synthesis of new proteins. Protein synthesis is countered by protein degradation. Together, these active processes result in protein turnover. Protein turnover varies across different proteins, tissues, cell types, and cellular compartments. In the brain, protein lifetimes range from tens of minutes (e.g., immediate early gene proteins) to months (e.g., synaptic structural proteins). Behavioral experiences modify the brain's proteome in specific subsets of synapses across widely distributed brain circuits. However, previous methods for tracking protein turnover lack the spatial and temporal resolutions needed to investigate these processes. Moreover, the blood-brain barrier presents special challenges for studying protein turnover *in vivo*. Analysis of the spatial and temporal pattern of synaptic proteins could help identify the brain regions and pathways involved in plasticity mechanisms that support learning. Here, we describe a pulse-chase method for measuring protein turnover with high spatial and temporal resolution across the body, including the brain. We fused specific proteins with the self-labeling enzyme HaloTag (HT), enabling the covalent capture of multiple, spectrally separable HT ligand dyes. These are delivered systemically to the entire body, including the brain, to estimate the ratio of proteins labeled with a pulse and a chase. Longer lived proteins have a higher ratio of pulse dye out of the total pulse and chase dyes. Using calibration measurements and simulations, we assessed the sensitivity and accuracy of our approach. We find that turnover of proteins with moderate to long lifetime (> 5 h) can be accurately measured, provided that protein-HT can be saturated with ligand dye. We characterized HT ligand Janelia Fluor (JF) dyes for bioavailability *in vivo*, identifying three dyes that saturate abundant brain proteins (JFX673, JF669, JF552; Grimm et al., 2017, 2020, 2021). We used our pulse-chase method to measure protein lifetimes of the nuclear transcriptional repressor protein MECP2 and the synaptic scaffold PSD-95. Turnover of neuronal MECP2-HT in knock-in mice (Piccolo et al., 2019) varied across brain regions, with particularly high stability in the cerebellum and hypothalamus. We also measured turnover of PSD-95-HT at synaptic resolution using another knock-in mouse line (Masch et al., 2018). PSD-95-HT was relatively stable in layer 1 of neocortex and CA1 of the hippocampus compared to other cortical layers and CA3. Experience in an enriched environment influenced PSD-95-HT turnover in the neocortex, especially in layer 1.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.09

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

Title: Novel recombinant antibodies for neuroscience research

Authors: A. BALL, Y. HSIEH, P. HSIEH, C. HUANG, S. HUANG, F. SU, S. WU, Y. CHANG, J. LIU, *C. LIN;
GeneTex, Irvine, CA

Abstract: Recombinant monoclonal antibodies (rAbs) are essential tools for basic neuroscience research as they offer numerous advantages over standard polyclonal and monoclonal antibody reagents. Recombinant technology generates antibodies that can be strictly defined at the sequence level and have consistent performance and supply. These features make rAbs distinctive when compared to traditional polyclonal and hybridoma-generated monoclonal reagents, whose lack of dependability has been widely documented. To address this challenge, GeneTex has developed a recombinant antibody production platform with enhanced validation standards. The goal is to manufacture reliable recombinant rabbit antibodies for reproducible performance in various experimental applications commonly employed by neuroscientists. GeneTex's rAb production protocol is based on the approach described by Starkie *et al.* (2016). This leverages a multi-parameter fluorescence-activated single cell sorting (FACS)-based methodology to select antigen-specific IgG+ memory B cells from an immunized rabbit, with cloning of the antibody variable region genes from selected cells into an IgG backbone and subsequent introduction into and expression by mammalian cells. This technique is particularly beneficial as the heavy and light chains from the same B cell are cloned, thereby maintaining natural pairing. The manufacturing sequence also allows application-specific testing (e.g., for western blot (WB), immunohistochemistry (IHC), and immunocytochemistry (ICC/IF), etc.) of clones during production. Antibody validation is performed inhouse and, where feasible, in relationships with academic or industry/pharma institutions where ideal samples and reagents are often more accessible, including specific tissue samples. This platform has created well-characterized antibodies for a series of protein targets important for neuroscience research. This includes reagents against the Dopamine D2 receptor, AIF-1/IBA1, GFAP, Acetylcholinesterase, Retinoid isomerohydrolase, alpha-Synuclein, and Somatostatin, among many others. Extensive validation for multiple applications was performed. For example, for the Dopamine D2 receptor, validation by WB using cell lines with differential protein expression and comparable antibodies was complemented by both IHC and ICC/IF staining. For the AIF-1/IBA1 antibody, similar characterization was done and further confirmed using orthogonal validation correlating protein and mRNA levels. In summary, GeneTex is utilizing a recombinant production mechanism to produce and validate antibody reagents to facilitate neuroscience research.

Disclosures: **A. Ball:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **Y. Hsieh:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **P. Hsieh:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **C. Huang:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **S. Huang:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **F. Su:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **S. Wu:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **Y. Chang:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **J. Liu:** A. Employment/Salary (full or part-time):; GeneTex, Inc. **C. Lin:** A. Employment/Salary (full or part-time):; GeneTex, Inc..

Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

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Program #/Poster #: 084.10

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

Support: 22-BR-02-01
NRF-2015M3C7A1029037
NRF-2017M3C7A1048092

Title: Brain region resolved proteomic approaches to identify the underlying pathophysiology in Fmr1 knockout mouse

Authors: *B. HA[1], J. CHOI[1], T.-S. PARK[2], J.-Y. HEO[2], Y.-J. JANG[2], S. CHAE[2], S.-J. JEONG[2];
[1]Korea Brain Res. Inst., [2]Korea Brain Res. Inst., Daegu, Korea, Republic of

Abstract: Brain transcriptome, connectome, and proteome provide a framework for a system-level understanding of tissue- or cell-type diversity in the CNS and serve as a resource for brain development and function analysis. In this study, we performed liquid chromatography-mass spectrometry-based proteomics for the analysis of the protein profiling in brain regions such as the posterior parietal cortex (PPC), prefrontal cortex (PFC), and amygdala (AMYG) of Fmr1 KO mice with hyperactivity, autism spectrum disorder (ASD) like behaviors and mental retardation. The 1,256 different proteins are identified, and 544 proteins with significance are clustered by molecular function, biological process, and signal pathway using DB analysis like STRING, PANTHER, and KEGG pathway. Comparisons of 544 proteins in wild-type and Fmr1 KO mice showed diverse alterations in protein expressions; PPC (up 244, down 96), PFC (up 91, down 292), AMYG (up 141, down 170). Many proteins related to glutamatergic-, GABAergic-, cholinergic-, serotonergic-, and dopaminergic synapse, as well as long-term potentiation and long-term depression, showed opposite patterns in protein expression profiles of PPC compared to PFC. From the viewpoint of signal pathways, Ras-. cAMP-, cGMP-PKG-, and PI3K-Akt signaling pathway-related to protein expressions are altered. In conclusion, our findings provide a clue for a better understanding of developmental disease and the future strategy of therapeutics targets.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.11

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

Support: NSF SBIR #2033921

Title: Self-assembled monolayer surface chemistry-conjugated ligands as a novel standardized method for interrogating astrocyte and neuron behavior and morphology.

Authors: E. G. THOMPSON[1], ***P. J. CALHOUN**[2], M. C. ROBITAILLE[3], J. A. CHRISTODOULIDES[3], J. M. BYERS[3], M. P. RAPHAEL[3], J. D. ROTHSTEIN[4];
[1]Neurol., Johns Hopkins Med. Institutions, Baltimore, MD; [2]Nanocrine, Inc., Frederick, MD; [3]Materials Sci. and Technol. Div., Naval Res. Lab., DC, DC; [4]Brain Sci. Inst., Johns Hopkins Univ., Baltimore, MD

Abstract: Ligand availability is known to impact cell behavior, morphology, adhesion, intracellular signaling, and cell-cell coupling. Surface ligand availability, and it's measured activity, is underappreciated during *in vitro* assays with highly variable coating methods and assumptions leading to unaligned protocols and ambiguous results. To address this, we have developed a standardized surface utilizing self-assembled monolayer surface chemistry to conjugate cyclized Arg-Gly-Asp (cRGD) ligands at known densities and derived mean spacing in parallel with a gold sensor chip to enable measurements of cRGD ligand activity. This approach enables tuning of ligand availability which we show affects cell morphology and behavior. Furthermore, we have demonstrated this technology is uniquely capable of phenotypically differentiating cellular insults such as viral infection. Finally, we carried out a direct comparison study between our substrates (Nanocrine Surface Chemistry Biochips), Matrigel, poly-D-lysine, and poly-D-lysine plus laminin, to define key benefits when used with primary human astrocytes, primary mouse astrocytes, and primary mouse neurons.

Disclosures: E.G. Thompson: None. **P.J. Calhoun:** A. Employment/Salary (full or part-time):; Nanocrine, Inc.. **M.C. Robitaille:** None. **J.A. Christodoulides:** 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.; Nanocrine, Inc. **J.M. Byers:** 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.; Nanocrine, Inc. **M.P. Raphael:** 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.; Nanocrine, Inc.. **J.D. Rothstein:** None.

Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.12

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

Title: Evaluating Genetic Targets for Parkinson's Disease Using a High Content Imaging Approach

Authors: *C. ATKINS[1], A. N. KAR[2];
[1]GlaxoSmithKline (GSK), Collegeville, PA; [2]Novel Human Genet., GlaxoSmithKline, Royersford, PA

Abstract: Evaluating Genetic Targets for Parkinson's Disease Using a High Content Imaging ApproachAmar Kar[1], Betsy Valoret[1,] Hoang Tran[2], Christian Watson[1] Jean-Louis Klein[1], Chun-Fang Xu[3], John Eicher[3], Alastair Reith[1], Chrissa Dwyer[1], and Charity Atkins[1]
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Parkinson's disease (PD) is the second most prevalent neurodegenerative disease in individuals 65 years or older. A major unmet need for PD patients is the availability of a disease modifying therapy resulting from a gap in understanding the causal genetic and environmental factors. Genome-wide association studies (GWAS) have provided novel insights into the complex genetic associations of this devastating disease. Using our variant to gene mapping tools, we identified a prioritized list of PD risk associated candidate genes implicated in mitochondrial homeostasis and/or lysosomal function, cellular processes that have been shown to contribute to the pathophysiology observed in Parkinson's disease . To evaluate the effects of candidate gene expression on mitochondrial and lysosomal phenotypic endpoints, we developed an unbiased phenotypic profiling strategy employing high-content imaging-based cell paint assay coupled to a robust lentiviral shRNA delivery system in human iPSC-derived astrocytes. We evaluated 95 mitochondrial and lysosome associated cellular features to generate models to identify candidate gene-specific phenotypic fingerprints. Consistent with existing literature, our results showed that shRNA-mediated knockdown of a subset of the PD-GWAS associated candidate genes resulted in statistically significant changes in mitochondrial and lysosomal phenotypes, suggesting possible dysregulation in mitochondrial activity and lysosomal function in this cellular system. We also observed that modulation of expression of certain target genes resulted in unique gene-specific cell paint fingerprints. These results highlight the possibility that specific candidate genes may employ unique pathways which affect the function of mitochondria and lysosomes. Current studies are focused on identifying the functional consequences of perturbations in candidate gene expression on these pathways. Future experiments will extend these studies into more translationally relevant models to identify specific molecular mechanisms linking these genes to underlying disease pathology.

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Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.13

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

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Title: Development of new tools to study lipidated mammalian ATG8

Authors: *P. JEON[1], S.-W. PARK[2], A. YAMASAKI[3], H. CHOI[1], H.-E. LEE[4], J.-Y. MUN[4], Y.-W. JUN[2], J.-H. PARK[2], S.-H. LEE[2], S.-K. LEE[1], H.-K. SONG[5], M. LAZAROU[6], D.-H. CHO[7], K. MASAAKI[9], N. N. NODA[10], D.-J. JANG[8], J.-A. LEE[1];
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Abstract: Mammals conserve multiple mammalian ATG8 proteins (mATG8s) consisting of γ-aminobutyric acid receptor-associated protein (GABARAP) and microtubule-associated protein 1 light-chain 3 (LC3) subfamilies that tightly bind to the autophagic membranes in a lipidated form. They are crucial in selective autophagy and recruit proteins bearing LC3-interacting region (LIR) motifs. However, because limited research tools are available, information about the specific roles of each lipidated mATG8 in selective autophagy is scarce. Here, we identified LIR motifs specific to the lipidated form of each mATG8 and characterized the residues critical for their selective interaction using cell-based assays and structural analyses. Then, we used these selective LIR motifs to develop probes and irreversible deconjugases that targeted selective lipidated mATG8s in the autophagic membrane, revealing that lipidated GABARAP subfamily proteins regulate aggrephagy of amyotrophic lateral sclerosis-linked protein aggregates. Our tools will be useful in elucidating the functional significance of each mATG8 protein in autophagy research.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.14

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

Title: Optimization of immunoblotting for high efficiency transfer and detection of neuronal proteins

Authors: *B. STEER, B. DWORECKI, S. IYER, R. PANDEY, P. HANEY, M. THACKER, K. CHONG, S. SCHLEZINGER, S. PARAMESWARAN;
Thermo Fisher Scientific, Carlsbad, CA

Abstract: Neuroscience is a multidisciplinary science involving study of various proteins and their interaction/s in neurological, psychiatric, neurodevelopmental and in pathophysiological conditions. Several key proteins are a major research focus in the field of neuroscience, the preferred method of investigation of which is via immunoblotting. The characteristics of these proteins in neuronal cells vary greatly, and their detection and quantification can be technically challenging, requiring modified immunoblot assay conditions. We demonstrate that a combination of specialized reagents and processing/assay conditions can be used to this end. We present the case of Ryanodine receptor (homo-tetramers, each subunit of 565 kDa) and Huntingtin (347 kDa) proteins, where we made use of NuPAGE Tris-Acetate gel and buffer system, ethanol equilibration process, and optimized high-voltage dry transfer to facilitate transfer and detection on nitrocellulose membranes. For low molecular weight proteins such as VAMP1/ VAMP2 (~12 kDa) we demonstrate detection using NuPAGE Tricine gel and buffer system, a variant transfer protocol, along with smaller pore nitrocellulose membrane to retain protein for immunoblotting. A unique case of Alpha-synuclein, which binds loosely to surface of the nitrocellulose/PVDF membrane, is also presented, where post-transfer cross-linking with paraformaldehyde retains the protein onto the membrane and thereby enables detection. We demonstrate that certain proteins (e.g., Nestin) which are otherwise difficult to be detected with the use of standard ECL reagent due to extremely low abundance, can be detected using an ultra-high sensitivity detection system (SuperSignal™ West Atto). It also highlights an instance where despite optimal transfer, choice of detection reagent can become critical for detection. Finally, we demonstrate using ABCC1 (an ABC transporter with 17 trans-membrane domains), as an example, how the choice of methods for sample processing may significantly affect the presence, and therefore detection, of the antigen of interest on the blot. We propose that use of integrated protein detection and quantification workflows, and advanced specificity-validated antibodies can address many of the existing issues around detection and characterization of difficult neuronal targets and facilitate advanced neuroscience research.

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Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.15

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

Title: Comprehensive, Single-molecule Proteomics and Detection of Isoform-Specific Tau Phosphorylation

Authors: *G. KAPP[1], J. D. EGERTSON[1], S. TAN[1], C. STAWICKI[1], T. RINKER[1], D. PARK[1], B. NORTMAN[1], J. ROBINSON[1], R. WANG[1], C. INMAN[1], L. ROUGE[2], D. ARNOTT[2], N. PANDYA[2], T. GILLIES[1], J. JOLY[1], N. STEINER[1], M.-Y. BRUSNIAK[1], K. CARDENAS[1], J. MCGINNIS[1], J. LEANO[1], K. CHEN[1], S. WATTS[1], H. BOYCE[1], D. DIPASQUO[1], A. KILLEEN[1], V. LOBANOV[1], D. S. KIRKPATRICK[3], A. ROHOU[2], P. MALLICK[1], **S. GUHA**[1]; [1]Nautilus Biotech., San Carlos, CA; [2]Genentech, South San Francisco, CA; [3]Interline Therapeut., South San Francisco, CA

Abstract: Biology is largely driven by the complex mixture of proteins in a sample. The activity of proteins and their interactions with other proteins and biomolecules are further modified by a range of post-translational modifications (PTMs). Specific PTM patterns, known as proteoforms, could be responsible for, or early biomarkers of, health outcomes. Tau is often highly modified and plays a central role in normal and diseased brain function. There is evidence that changes in Tau proteoforms are linked with Alzheimer's Disease (AD) and may be used as biomarkers for early detection of AD and other tauopathies.
We are developing a new, single-molecule platform for proteome analysis, including proteoforms. Single-molecule analysis of intact proteins, instead of peptides, allows for the localization and patterning of multiple PTMs on a per-molecule basis. Proteoform composition is determined by performing this analysis massively in parallel, with billions of individual protein molecules analyzed simultaneously in a single experiment. First, we validate the platform by measuring defined mixtures of recombinant tau proteins. Next, we examine tau protein enriched from human iPSC-derived neurons and Tau-expressing cell lines.Proteoforms operate in the context of the complex milieu of other proteins in a sample. Using the same single-molecule platform, Protein Identification by Short-epitope Mapping (PrISM) can be used for broadscale proteome analysis. PrISM is a single-molecule, affinity-based method in which full-length proteins are immobilized and repeatedly analyzed by a unique type of high affinity but low specificity affinity reagents. The combination of information from multiple rounds of detection of short epitopes should enable the identification of more than 95% of the human proteome with just 300 reagents. We successfully identified a set of model proteins using a smaller number of affinity reagents, exemplifying the approach of using short-epitope binding reagents to identify proteins.Coupled together, the ability to analyze nearly the entire proteome and probe specific proteins, like Tau, to further investigate the heterogeneity of the proteoform landscape provides thorough analysis of complex biological samples. This proteoform and proteomics data should

help advance the understanding, prediction, and treatment of Alzheimer's and other neurological diseases.

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Poster

084. Methods for Gene and Protein Expression in the Brain

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.16

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

Support: NIH Grant CA255979

Title: In situ imaging of nuclear RNA exosome activity in brain cancer

Authors: *V. V. DIDENKO, C. L. MINCHEW;
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Abstract: We describe a new molecular technology for *in situ* assessment of the RNA degrading activity of nuclear RNA exosome. RNA exosome (not to be confused with the unrelated vesicular exosomes) is the major enzymatic complex controlling RNA metabolism in cells. Its fundamental function is to keep cells in the proliferating state. An overactive exosome complex leads to higher rates of cellular proliferation and is implicated in cancer progression. It is also a molecular target of anticancer therapies. Nuclear RNA exosome activity is useful in assessments of tumor cell stress and cell death propensity, and in evaluating cancer response to therapies. Our approach labels activity of nuclear RNA exosome in the fixed tissue section format. We demonstrate its functional capabilities in brain cancer assessment by using tissue sections from low-grade astrocytomas and glioblastoma.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.17

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

Support: Max-Planck-Gesellschaft

Title: Tools for investigating the subcellular distribution of receptors and channels in motion-sensing neurons of Drosophila.

Authors: *R. M. VIEIRA[1], S. FENDL[1,2], E. SAMARA[1,2], A. BORST[1,2];
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Abstract: Neurotransmitter receptors and voltage-gated ion channels are important neural circuit components that shape the biophysical properties of neurons. Neurotransmitter receptors determine the effect of a presynaptically released transmitter on the postsynaptic neuron, while voltage-gated ion channels shape the electrical activity of the neuron. Importantly, the subcellular distribution of these molecules determines how signals are integrated. Knowing the localizations and types of receptors and ion channels is therefore fundamental to our understanding of neural computation. Though the fruit fly *Drosophila melanogaster* has unparalleled genetic accessibility, tools for endogenously labelling proteins in a cell-type-specific manner were missing. To this avail, we developed FlpTag, a tool for endogenous,

conditional labelling of proteins using a flippase-dependent, invertible GFP cassette integrated into the gene of interest. FlpTag is generalizable and can be easily integrated into genes containing ΦC31 attP sites such as those of the vast MiMIC library, or specifically targeted to a gene locus of interest using the CRISPR-Cas9 gene editing system. This permits the tagging of virtually any protein within the fly using FlpTag. To expand this system, we developed SingleFlp, a new addition to the FlpTag technique which permits cell type specificity with single cell resolution. SingleFlp uses a multi-recombinase approach to endogenously tag proteins in single cells of a genetically defined type. Using these methods, we investigated the subcellular distribution of specific subunits of glutamate, acetylcholine, and GABA receptors in individual motion-sensing T4/T5 neurons. Within the dendrite, receptor subunits localize to different regions in a spatial order that exactly matches the synapse numbers and distributions of the different input neurons described in previous electron microscopic reconstructions. Further, we discovered a strictly segregated subcellular distribution of voltage-gated ion channels within different compartments of T4/T5 neurons. These findings lay the foundation for future functional investigations of receptors and ion channels within these neuronal cell types. Additionally, these strategies can be expanded to different circuits or even different species in the future.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Program #/Poster #: 084.18

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

Support: NIH R01 Brain 441406-ZL-29425
HHMI

Title: Mapping endogenous neurotransmitter receptors in the Drosophila brain at single cell and single synapse resolution

Authors: ***P. SANFILIPPO**[1], J. YOO[1], A. KIM[1], H. BEVIR[1], A. YUEN[1], P. MIRSHAHIDI[1], A. BHUKEL[2], Y. ASO[2], S. L. ZIPURSKY[1];
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Abstract: The connectome of the *Drosophila* brain has revealed extraordinary complexity of brain wiring, with each neuron receiving hundreds to thousands of inputs from diverse neuron types. Synapses are molecularly diverse, in part, by the neurotransmitter receptors localized to them. The *Drosophila* genome encodes over 50 neurotransmitter receptor subunits. To map receptors to the connectome, we have modified endogenous receptor gene loci to simultaneously introduce tags and sparsely label processes of identified cell-types. Here we report the generation of conditional knock-in alleles of 11 cys-loop ionotropic receptor subunits belonging to a large family of structurally related proteins, including nAChRβ1, Rdl, and GluClα receptors for

acetylcholine, GABA and glutamate, respectively.
We characterize diverse patterns of receptor localization and the spatiotemporal dynamics of localization during development in multiple optic lobe neurons. Of particular interest, we observe differential localization of Rdl and GluClα to respectively the base and the tip of motion sensitive T4 dendrites. This pattern of receptor distribution agrees with the select wiring of GABA and glutamate inputs at either the base or the tips of T4 dendrites as defined from EM connectome data. Strikingly, mapping of nicotinic acetylcholine receptor subunits in T4 dendrites shows differential distribution of nAChRα5 subunits to the middle of the dendritic arbor and distal localization of nAChRβ1 subunits to the tips of the dendrites. The middle of T4 dendrites is innervated by two major cholinergic inputs while the dendritic tips receive inputs from adjacent cholinergic T4 neurons. This data raises the possibility that different cholinergic inputs wire into T4 dendrites by using distinct acetylcholine receptors. In addition, differential neurotransmitter receptor type distribution could play a role in the dendritic computation of motion detection in T4 dendrites.
These reagents provide tools for studying synapse development, the role of specific receptors in circuit function and behavior, and uncovering mechanisms of protein localization in an array of morphologically diverse neuron types.

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Poster

084. Methods for Gene and Protein Expression in the Brain

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Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 084.19

Topic: H.08. Learning and Memory

Support: NSF

Title: Signal molecules in nerveless animals (Trichoplax adhaerens, Placozoa)

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Abstract: The origin of signaling mechanisms in animals and their neural systems is one of the greatest unresolved mysteries in biology. Nerveless placozoans such as *Trichoplax* are the simplest free-living animals apparently preserved ancestral modes of chemical signaling in the form of volume transmission. Despite just 6-10 morphological cell types, placozoans exhibit

complex feeding and locomotory patterns, including social-type behavior. However, little is known about their signal molecules and cellular bases of behavior in general. Here, using scRNA-sea and analytical microchemical approaches, we identified more than 180 candidates for signal molecules and characterized their distributions and potential functions. The majority of identified molecules are novel lineage-specific short peptides plus several low molecular weight transmitters (including NO, ATP, Glutamate, Glycine, and GABA as the most ancient metazoan intercellular messengers). Expression patterns indicated that at least a half of predicted pro-hormones had cell-specific distribution with multiple peptides co-localized within the same cell types (e.g., fiber cells, lipophil, and crystal cells, plus various subpopulations of secretory epithelial cells). Furthermore, by employing transmission and scanning electron microscopy, we have revealed complex exosome-type vesicular organization across the entire animal's body with distinct micro-cavities as potential sites for behavioral integration. The observed diversity of signal molecules combined with the dynamic microanatomical organization of chemoconnectomes in placozoans provides molecular and structural bases for versatile chemical computation in nerveless animals. These models can serve as prototypes of early neuroid-type non-synaptic transmission and even designs of alternative neural systems without synapses.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH: Rehabilitation Research Resource to Enhance Clinical Trials Center Grant
Division of Nutritional Sciences University of Illinois

Title: Effects of Lutein Consumption on Carotenoid Status and Cognition among Persons with Multiple Sclerosis

Authors: *S. MARTELL[1], J. KIM[2], T. MEHTA[2], L. ROSOK[1], J. ERDMAN, Jr.[3,4], B. ADAMSON[6,5], R. MOTL[7], N. KHAN[3,1,2,5];
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Abstract: Multiple sclerosis (MS) is an immune-mediated, demyelinating disease of the central nervous system often accompanied by visual and cognitive impairment. Carotenoids have anti-inflammatory properties and may support cognitive function. The xanthophyll carotenoid lutein preferentially accumulates in both macular and brain tissue, and higher macular pigment optical

density (MPOD), a non-invasive measure of macular xanthophyll status, is associated with greater cognitive and visual processing speed in healthy adults. The effects of lutein consumption on changes in carotenoid status and cognitive function have not been examined among persons with MS.
We conducted a parallel group randomized control trial design that examined the effects of 4-months of lutein supplementation on carotenoid status and cognitive function in persons with relapse-remitting multiple sclerosis (RRMS, n = 21). Participants were randomly assigned to the placebo (safflower oil without lutein, n = 9) or treatment (20 mg/day lutein with safflower oil, n = 12) group. Carotenoid status was assessed in the macula (MPOD) and skin using heterochromatic flicker photometry and reflection spectroscopy at the fingertip, respectively. There was a significant group by time interaction whereby the treatment group exhibited a significant increase in MPOD ($\Delta 0.20 \pm 0.33$, $p=0.029$) and skin carotenoids ($\Delta 139.4 \pm 81.55$, $p<0.001$). There was no significant interaction effect for flanker accuracy ($p=0.441$) or reaction time ($p=0.468$). However, there was a significant positive association between change in MPOD and change in flanker incongruent accuracy in the treatment group (rho = 0.70, $p = 0.008$) but not the placebo group (rho = -0.34, $p = 0.207$). Further, no significant associations were observed for changes in skin carotenoids and cognitive function in either the placebo (all p's>0.062) or treatment (all p's>0.285) group.
4-month daily lutein supplementation improved carotenoid status both in the skin and the macula. Additionally, improvement in macular carotenoids, and not skin carotenoids, was associated with increased attentional inhibition, providing support for targeting neural carotenoids for cognitive benefits in persons with MS. Although larger prospective intervention trials are necessary, the present study provides novel evidence demonstrating improvement in carotenoid status following lutein supplementation and the potential to support cognitive function in persons with MS.
This research was funded by the University of Illinois Division of Nutritional Sciences and the National Institutes of Health Rehabilitation Research Resource to Enhance Clinical Trials Center.

Disclosures: **S. Martell:** None. **J. Kim:** None. **T. Mehta:** None. **L. Rosok:** None. **J. Erdman:** None. **B. Adamson:** None. **R. Motl:** None. **N. Khan:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSERC Discovery Grant
MS Society of Canada Research Grant
CIHR Canada Graduate Scholarships - Master's

Title: Correlation between microstructural and volumetric MRI measures in multiple sclerosis white matter lesions, normal appearing white matter, and deep gray matter regions

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Abstract: Multiple sclerosis (MS) is one of the most well-known demyelinating diseases. However, individual differences in neurodegeneration, responses to treatment, and complex clinical outcomes make accurate prognosis in MS extremely difficult. While MRI plays a key role in diagnosis, and certain biomarkers based on advanced MRI sequences could potentially play a role in MS prognosis, identifying which biomarker(s) to use, or even the relationships between different candidate biomarkers, has not been well established. The current study aimed to tackle this problem by examining correlations between 11 advanced MRI metrics (i.e., diffusion tensor imaging axial diffusivity [AD], radial diffusivity [RD], mean diffusivity [MD] and fractional anisotropy [FA]; T2-relaxation myelin water imaging myelin water fraction [MWF], intra- and extra-cellular water fraction [IEWF] and geometric mean T2 [gT2]; quantitative susceptibility mapping [QSM] and R2* mapping; T1-weighted by T2-weighted ratio [T1w/T2w]; and volumetric measures) in each of three tissue classes (i.e., white matter lesions [WML], normal appearing white matter [NAWM], and deep gray matter [DGM]) - a total of 33 different MRI measures from a cohort of 79 MS participants. As expected, the WML class exhibited significantly lower microstructural integrity compared to NAWM for all metrics. Receiver operating characteristics were also computed to investigate each MRI metric's ability to distinguish WML from NAWM. Diffusion-based AD, RD and MD exhibited perfect classification accuracy, while QSM (68.23%) and IEWF (69.24%) are the two least favourable candidates. Moreover, 230 of the 528 pairwise Spearman rank correlations (across all metrics and tissue classes) were statistically significant with a Benjamini-Horgberg corrected threshold of $p<0.05$. The significant correlations were evenly distributed across metrics, except for: 1) QSM measures, which had the fewest correlations (9, 3.91% of significant pairs); and 2) volumetric measures, which had the most correlations (60, 26.09% of significant pairs). The present work therefore provided both an objective assessment of different MRI measures' ability to characterize white matter damage, and assessed the intra- and inter-class correlations to identify MRI measures that provide similar information versus those that provide novel information. This can hopefully be used to guide future work by shortening MRI scanning protocols (i.e., not collecting redundant data), and informing the selection of MRI measures to include in multi-parametric statistical models for MRI-based MS prognoses.

Disclosures: K. Wong: None. **C.R. Figley:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.03

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Myelin mapping helps assess pain in trigeminal neuralgia secondary to multiple sclerosis

Authors: *R. YAKUBOV[1,2], T. LATYPOV[3,1], M. R. WALKER[1], P. S.-P. HUNG[1], W. WANG[1], P. TSAI[1,3], M. HODAIE[4,1];
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Abstract: Objectives: Patients with trigeminal neuralgia secondary to multiple sclerosis (MS-TN) often present with multiple bilateral plaques. It is unclear how bilateral plaques contribute to the expression of unilateral MS-TN pain. An advanced assessment of white matter may reveal new insights on MS-TN pathogenesis. The "myelin mapping" (MM) technique permits the visualization and quantification of myelin content from a ratio of co-registered T1-w/T2-w images. We aim to validate the MM method by evaluating its cross-scanner generalizability and ability to distinguish MS-TN patients from healthy controls (HCs) based on supratentorial white matter (WM) changes. We also explore MM differences associated with left- (MS-LTN) versus right-symptomatic MS-TN (MS-RTN) to assess pain-related WM signatures.
Methods: This study was conducted in two steps. First, to evaluate MM generalizability, MMs were constructed for 52 local HCs and 52 age- and sex-matched external HCs. Second, to assess MM differences between MS-TN patients and HCs, MMs were constructed for 64 MS-TN patients and 64 age- and sex-matched HCs. MMs were segmented to 48 regions according to the Johns Hopkins University WM atlas. Regional radiomics were extracted for statistical analyses corrected for multiple comparisons.
Results: Two-one-sided t-tests demonstrated equivalence between MMs of local and external HCs (all regions $p<0.05$), confirming cross-scanner generalizability. Significant MM differences between MS-TN patients and HCs demonstrated demyelination in the superior fronto-occipital fasciculus, superior longitudinal fasciculus, cingulum, corpus callosum, middle cerebellar peduncle, corticospinal tract, corona radiata, sagittal stratum, posterior thalamic radiation, and the retro-lenticular part of the internal capsule ($p<0.0001$) in MS-TN patients. A univariate analysis comparing the hemispheres according to pain laterality in MS-LTN patients demonstrated reduced myelination in the contralateral fornix-stria terminalis ($p<0.05$). In MS-RTN patients, reduced myelination was observed in the ipsilateral fornix-stria terminalis and contralateral tapetum ($p<0.05$). These results indicate that MS-TN patients consistently exhibit demyelination in the right fornix-stria terminalis.
Conclusions: This study confirms the cross-scanner robustness of the MM technique and its ability to identify demyelination in MS-TN. WM signatures of MS-LTN and MS-RTN in the fornix-stria terminalis support previously reported TN-related hippocampal changes, encouraging further investigation in this area. Overall, the MM technique may serve as an advanced MS-TN WM assessment tool.

Disclosures: R. Yakubov: None. **T. Latypov:** None. **M.R. Walker:** None. **P.S. Hung:** None. **W. Wang:** None. **P. Tsai:** None. **M. Hodaie:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Abx-002, a novel thyroid receptor beta-selective agonist, demonstrates functional brain delivery in vivo and enhances efficacy in an eae model in combination with immune-modifying therapy

Authors: *M. B. WOERNER, J. P. LEONG, I. S. STEIN, B. A. STEARNS, D. A. MACKENNA;
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Abstract: Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). MS pathogenesis is complex and involves multiple mechanisms both central and peripheral. The use of immune-modifying therapies has shown benefit for patients, but disability progression continues, in part due to limited ability of these treatments to directly access the brain, where primary pathogenesis drives disease progression.
ABX-002 is a prodrug that delivers a highly potent and selective agonist for the thyroid hormone receptor beta (TRβ). ABX-002 demonstrates enhanced brain delivery and induction of thyroid-responsive genes in vivo in the CNS.
Thyroid hormone is known to regulate many systems in vivo, including induction of oligodendrocyte precursor cell (OPC) differentiation and activity of microglia in the CNS. Thus, brain-directed thyromimetics have potential to enhance both remyelination and immune responses at the site of action of neurodegenerative diseases such as multiple sclerosis. Studies to profile ABX-002 were performed in cellular (OPC differentiation) and rodent models of MS (experimental autoimmune encephalitis (EAE)), both alone and in combination with fingolimod, an immune-modifying treatment approved for MS.
The active metabolite of the ABX-002 prodrug enhanced OPC differentiation in vitro with an EC50 value of 2 nM.
In an EAE mouse model, ABX-002 administered in a prophylactic paradigm dose-dependently improved disease onset and maximum disease severity. Fingolimod alone showed a dose response relationship to clinical scoring and EAE incidence, with ~80% incidence at 0.03 mg/kg and ~60% at 1 mg/kg. When ABX-002 at multiple doses and FTY720 at 0.03 mg/kg were co-administered, the incidence of EAE decreased to 50% or less - lower than the effect of either compound alone. Histological analysis of spinal cord 28 days after immunization demonstrated a reduction of inflammatory foci, apoptotic cell count, and area of demyelination with ABX-002 or FTY720 dosed alone and an even larger decrease when in combination. Analysis of gene expression in the brains of these animals demonstrated robust induction of thyroid-responsive genes, demonstrating engagement of the TRβ in the CNS.
In summary, thyromimetic prodrugs demonstrate enhanced brain delivery in vivo and have efficacy in models of MS, both independently and when used in combination with existing MS therapies.

Disclosures: **M.B. Woerner:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **J.P. Leong:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **I.S. Stein:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **B.A. Stearns:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics. **D.A. MacKenna:** A. Employment/Salary (full or part-time):; Autobahn Therapeutics. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Autobahn Therapeutics.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Genentech

Title: Remyelination PET probe biomarker discovery for multiple sclerosis

Authors: *C. CHO, S. PANDEY, J. W. HOFMANN, J. KOEPPEN, J. MARIK, M. SIU, R. M. WEIMER, A. A. NUGENT;
Genentech Res. and Early Develop., Genentech Inc., South San Francisco, CA

Abstract: Multiple sclerosis (MS) is a neurologic disease characterized by chronic inflammation and demyelination, leading to axon degeneration. It causes CNS-related disability, such as loss of vision, weakness of muscle movements, and limb paralysis. Promoting remyelination is a key therapeutic approach to enable neuroprotection in MS, potentially restoring function lost during disease progression. Currently, there are few biomarker approaches to measure the efficacy of remyelinating therapeutics. We used a bioinformatic approach to identify oligodendrocyte lineage-enriched candidate factors that are amenable as PET targets to selectively image remyelination. We utilized existing mouse scRNAseq datasets to identify genes with enriched expression in the oligodendrocyte lineage, then cross referenced candidates with published control and MS human post-mortem brain snRNA-seq as well as LPC and cuprizone mouse model scRNAseq datasets to assess expression during de- and remyelination. Top candidates were then filtered for those with available chemical matter for further protein based validation using human post-mortem brain. We identified sphingosine-1-phosphate receptor 5 (S1PR5) as

highly enriched in human and mouse oligodendrocytes and as a top candidate for further exploration as a target for PET probe development to image remyelination.

Disclosures: C. Cho: None. **S. Pandey:** None. **J.W. Hofmann:** None. **J. Koeppen:** None. **J. Marik:** None. **M. Siu:** None. **R.M. Weimer:** None. **A.A. Nugent:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Identification of functional biomarkers of demyelination in two animal models of multiple sclerosis with functional ultrasound imaging.

Authors: *B. BELIARD[1], L. DELAY[1], C. ISSAAD[1], Y. TRAVERT-JOUANNEAU[1], T. DEFFIEUX[1], M. TANTER[1], D. P. BRADLEY[2], S. PEZET[1];
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Abstract: Multiple Sclerosis (MS) is a chronically evolving inflammatory disease of the central nervous system resulting in focal and diffuse demyelination. To develop new treatments and examine the multiple systems impacted by MS, new imaging technologies are needed at the preclinical stage. Functional ultrasound imaging (fUSI) has recently been demonstrated to robustly measure brain cerebral blood volume (CBV) dynamics as an indirect measure of neural activity.
Aim: We postulated that fUSI could allow the identification of biomarkers of de- and re-myelination in animal models of MS. The aim of this study was to assess and improve the reproducibility of the experimental conditions and signal processing, in order to use it in preclinical studies involving model systems and pharmacology.
Methods: We studied the changes of CBV induced by whiskers stimulation in naïve, cuprizone or lysolecithin injected C57Bl6 mice (N=8 animals/ group), imaged at 5-10 Weeks and 7-14-21 days, respectively.
Results: First we developed a protocol to trigger the whiskers stimulations and reduce user-dependent variability. The parameters of the stimulation (frequency, duration of stimulation) were optimized, along with the plane of imaging through a fully-automatic ultrasound-based neuro-navigation approach. Finally, this protocol was used in two animal models of MS and showed contrasting results (increase CBV in Cuprizone at 5W and10 W and no difference of CBV in Lysolecithin-induced models at D1, D7, D14, and D21).
Conclusion: The measure of evoked hemodynamic response is brand new technique that depends on a very complex set of process involving not only demyelination but also inflammation, neuronal loss and neurovascular coupling. It might highlight differences in the demyelination models, but the mechanisms are not completely understood yet.

Disclosures: **B. Beliard:** A. Employment/Salary (full or part-time):; Biogen. **L. Delay:** None. **C. Issaad:** None. **Y. Travert-Jouanneau:** None. **T. Deffieux:** None. **M. Tanter:** None. **D.P. Bradley:** A. Employment/Salary (full or part-time):; Biogen. **S. Pezet:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: U54 AG065187

Title: The Emory-Sage-SGC TREAT-AD Center: Diversifying the Alzheimer's Disease Target Discovery Pipeline Through Open Science

Authors: *K. LEAL[1], A. AXTMAN[2], R. BETARBET[3], P. BRENNAN[5], G. W. CARTER[6], Y. DU[4], H. FU[4], A. K. GREENWOOD[1], F. M. LONGO[7], K. PEARCE[2], A. EDWARDS[8], A. I. LEVEY[4];
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Abstract: The Emory-Sage-SGC TREAT-AD Center was established with the aim of developing reagents for a diverse portfolio of promising Alzheimer's Disease (AD) target hypotheses and to use open science practices to accelerate drug development for new therapies. With repeated failures and a lack of portfolio diversity in the AD drug development pipeline, it is becoming evident that clinical care for treatment of AD will be possible only when the landscape of drug development expands and we have multiple drugs available that operate across multiple disease-causing biological pathways in order to effectively reduce the burden of AD.
The Emory-Sage-SGC TREAT-AD Center has identified a diverse set of novel AD targets derived from systems biology studies within the Accelerating Medicines Partnership in AD (AMP-AD) consortium and further evaluated using data from multiple AD consortia and existing literature. By using a systems biology and bioinformatic analyses approach, we can search for multiple ideal targets to perturb for potential AD drug development versus one target at a time. The Center is committed to accelerating this process and is generating and openly distributing experimental tools for use in target validation to advance or reject proposed therapeutic hypotheses in AD. Target Enabling Packages and all associated data including expression constructs, knockout cell lines, assays, antibody validation data, and crystal structures for prioritized targets are publicly shared through standard repositories and cataloged on the AD Knowledge Portal (https://adknowledgeportal.org) under Target Enabling Resources. The goal of TREAT-AD (https://treatad.org/) is to de-risk prioritized AD therapeutics by promoting robust

and independent evaluation of a diverse portfolio of promising yet untested AD therapeutic hypotheses.

Disclosures: K. Leal: None. **A. Axtman:** None. **R. Betarbet:** None. **P. Brennan:** None. **G.W. Carter:** None. **Y. Du:** None. **H. Fu:** None. **A.K. Greenwood:** None. **F.M. Longo:** None. **K. Pearce:** None. **A. Edwards:** None. **A.I. Levey:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.09

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Effect of neostigmine on donepezil-induced extracellular acetylcholine levels in the prefrontal cortex and hippocampus: analysis by LC-MS/MS highlights the need for caution when making pharmacological interventions

Authors: V. SIMMONDS, R. S. KULKARNI, C. TAYLOR, H. FLINT, J. DAVIES, S. BEST, H. L. ROWLEY, *S. C. CHEETHAM;
Sygnature Discovery, Nottingham, United Kingdom

Abstract: Up until recently the majority of microdialysis studies measuring extracellular levels of acetylcholine (ACh) have been performed using HPLC with electrochemical detection (HPLC-ECD) which requires the inclusion of the acetylcholinesterase inhibitor neostigmine in the microdialysis perfusate to artificially elevate levels of ACh[1]. HPLC coupled to mass spectrometry (LC-MS/MS) has greater specificity and sensitivity over HPLC-ECD as an analytical method and subsequently has been shown to successfully measure ACh without the need for inclusion of neostigmine (in-house data). In order to determine whether the presence of neostigmine alters the donepezil-induced ACh levels, the effect of intraperitoneal administration of donepezil (1 mg/kg) was examined in both the presence and absence of neostigmine (1 µM) with samples analysed using LC-MS/MS. Dual-probe dialysis was carried out in male Sprague-Dawley rats (250-350g) whereby probes were stereotaxically implanted into the prefrontal cortex (PFC) (AP +3.2 mm, ML ±2.5 mm, DV 4.0 mm, 2 mm) and hippocampus (HIPP) (AP -4.8 mm, ML ±4.8 mm, DV -7.8 mm, 4 mm; DV coordinates relative to the skull surface) of each rat. Following surgery, the rats were placed in dialysis bowls with their microdialysis probes connected to a swivel and a counter-balance arm. The following day dialysate samples were collected at 20 min intervals for 80 min prior to administration of vehicle or donepezil and for 4 h after using a flow rate of 1.2 µl/min. Data are given in fmol/5µl and are adjusted means and SEMs. In the presence of neostigmine donepezil significantly decreased extracellular ACh levels in the PFC and HIPP with maximal effect at 80 min post dose. PFC: 20.74 ± 2.47 compared to vehicle 54.28 ± 9.85 ($p<0.001$) and HIPP: 21.94 ± 1.11 compared to vehicle 46.95 ± 5.41 ($p<0.001$). However in the absence of neostigmine donepezil resulted in a significant increase in extracellular ACh in the PFC and HIPP with maximal effect observed at 40 min post dose. PFC:

2.63 ± 0.23 compared to vehicle 0.29 ± 0.08 ($p<0.001$) and HIPP: 2.82 ± 0.20 compared to vehicle 0.59 ± 0.05 ($p<0.001$). These results confirm that in the absence of neostigmine ACh levels in microdialysates were detectable by LC-MS in this study. The data also show that neostigmine has a dramatic effect on donepezil-induced changes in ACh levels and highlight the need for caution when interpreting data from microdialysis studies where the cholinergic system has been pharmacologically manipulated.[1]König M, Thinnes A & Klein J (2018) Microdialysis and its use in behavioural studies: Focus on acetylcholine. *Journal of Neuroscience Methods*, 300, pp 206-215.

Disclosures: V. Simmonds: None. **R.S. Kulkarni:** None. **C. Taylor:** None. **H. Flint:** None. **J. Davies:** None. **S. Best:** None. **H.L. Rowley:** None. **S.C. Cheetham:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of an assay for compounds modulating tyrosination and de-tyrosination in mouse cortical cultures using microfluidic co-culture plates

Authors: *L. STRID ORRHULT[1], N. ARBEZ[2], P. DELAGRANGE[3], J. PIHL[1], M. KARLSSON[1];
[1]Cellectricon AB, Cellectricon, Mölndal, Sweden; [2]Cell. Sci. Dept., [3]Neurol. and Inflammation Therapeut. Area, Inst. de Recherches Servier, Croissy sur Seine, France

Abstract: Defects in axonal transport have been reported as a common feature in different neurodegenerative diseases. In addition, several neurological disease-causing genes are associated with axonal transport systems. Microtubules (MTs), made of α- and β-tubulin heterodimers, extend along the axon and function as cytoskeletal tracks in transport processes, and the MT system is constantly polymerized and depolymerized in a dynamic manner. These processes are under control of post-translational modifications (PTMs) including detyrosination, phosphorylation and acetylation. Axonal MTs, which are very stable, are highly enriched in detyrosinated (detyr) tubulin whereas more unstable MTs present in growth cones and the distal regions of neurites are predominantly tyrosinated (tyr). The detyr/tyr cycle is controlled by the enzymatic removal of the C-terminal tyrosine of α-tubulin by tubulin tyrosine carboxypeptidase (TCP) and its re-addition by tubulin tyrosine ligase (TTL). Since abnormal tubulin PTMs can lead to neuronal defects, the identification of small molecules modulating the activities of tubulin-altering enzymes is instrumental in the development of treatments for neurodegenerative diseases. The aim of this study was to develop a high content analysis (HCA) assay for axonal detyr/tyr levels in microfluidic co-culture (MC) plates. Mouse primary cortical cells were plated in MC-plates allowing axons to grow into a cell-free interconnecting well. At 7 days in vitro, a TCP inhibitor, alkyne-epoY, was added to the cellular side. 48 h after compound addition, axonal

levels of tyr and detyr α-tubulin and cell health were quantified and compound effects were evaluated as ratio of tyr/detyr α-tubulin. A total of five experiments were performed to evaluate repeatability and to technically validate the assay. The axonal growth into the interconnecting well was sufficient to enable development of a quantitative analysis workflow. Treatment with alkyne-epoY, up to 30 µM, did not affect cell health and significantly increased the ratio of tyr/de-tyr α-tubulin in a concentration-dependent manner compared to DMSO control in all experiments.
By using MC-plates, it is possible to repeatedly detect changes in the ratio between tyr and detyr of α-tubulin in axons after treatment with alkyne-epoY and the quality of the assay is sufficient for concentration-response evaluation of small molecules. We consider that we have demonstrated a viable assay concept, and that it will be feasible to use the assay for determination of compound effects and rank-ordering of compounds on modulation of tyr and de-tyr in mouse cortical cultures.

Disclosures: **L. Strid Orrhult:** A. Employment/Salary (full or part-time):; Cellectricon AB. **N. Arbez:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **P. Delagrange:** A. Employment/Salary (full or part-time):; Institut de Recherches Servier. **J. Pihl:** A. Employment/Salary (full or part-time):; Cellectricon AB. **M. Karlsson:** A. Employment/Salary (full or part-time):; Cellectricon AB.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.11

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: U54 AG065187

Title: Methodology To Objectively Prioritize Therapeutic Hypotheses In Alzheimer's Disease Via Target Risk Scoring and Biological Domain Annotation

Authors: *S. KEEGAN[1], J. WILEY[2], G. CARY[1], J. GOCKLEY[2], J. LIU[3], L. HEATH[2], A. GREENWOOD[2], L. MANGRAVITE[2], B. LOGSDON[2], R. BUTLER[4], J. ZHANG[3], H. WU[3], K. HUANG[3], F. LONGO[4], A. LEVEY[5], G. W. CARTER[1];
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Abstract: Objective: Integrate large datasets to identify and rank hypotheses and subordinate targets for Alzheimer's Disease therapeutics.
Rationale: Current therapeutics for Alzheimer's Disease (AD) are not disease altering. Given the heterogeneity of the disease, large datasets are being generated to identify molecular signatures associated with disease. Efforts are needed to integrate these data in order to prioritize hypothesis areas and candidate targets for further resource development. Target Enablement to Accelerate

Therapy Development for Alzheimer's Disease (TREAT-AD) is an National Institute of Aging funded consortium that exists to help diversify resources available for potential therapeutic areas by objectively nominating focal genes based on disease risk and biological domain of impact. We developed two resources to aid this effort: the TREAT-AD Target Risk Score (TTRS) and the AD Biological Domains (BD).
Method: We identify BDs as sets of disease-linked endophenotypes defined sets of gene ontology (GO) terms. BDs provide an objective rubric to assess the biological context of each gene's contribution to disease processes. The TTRS is a composite of genetic risk, genomic risk, and literature recency. Evidence supporting the genetic component is derived largely from genetic association studies of large AD cohorts. The multi-omic component combines evidence from transcriptomic and proteomic studies of human post-mortem brain tissue in the Accelerating Medicine Partnership for Alzheimer's Disease (AMP-AD) cohorts. The literature recency score utilizes PubMed text mining to identify trends in emerging and recent AD literature. The sum of these scores (max 7 points) is the final TTRS which is used for target prioritization and hypothesis evaluation. Gene set enrichment analyses (GSEA) of BD annotated GO terms identifies aspects of AD biology enriched in risk.
Result: We delineate 16 BDs composed of 4,788 unique and largely non-overlapping GO terms. The TTRS has been computed for 24,000+ genes. GSEA of the TTRS and component scores identifies enriched risk in terms annotated to the Synapse, Structural Stabilization, Proteostasis, and Mitochondrial Metabolism BDs.
Conclusion: Prioritizing and organizing targets using these resources provides an evidence-based metric for assessing therapeutic targets and hypotheses. High-priority targets will be considered for further assay development, structural biology, and medicinal chemistry studies in the TREAT-AD consortium. These resources will be openly shared with the research community to facilitate the development of AD therapeutics.
treatad.org/data-tools/

Disclosures: S. Keegan: None. **J. Wiley:** None. **G. Cary:** None. **J. Gockley:** None. **J. Liu:** None. **L. Heath:** None. **A. Greenwood:** None. **L. Mangravite:** None. **B. Logsdon:** None. **R. Butler:** None. **J. Zhang:** None. **H. Wu:** None. **K. Huang:** None. **F. Longo:** None. **A. Levey:** None. **G.W. Carter:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.12

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Research Association Autonomy in old Age (AiA): European Union (ERDF-European Regional Development Fund) & State of Saxony-Anhalt, Germany; ID: ZS/2016/05/78611

Title: Investigating the interplay of physical and cognitive performance in a dual-task training regime in AD patients by means of hierarchical continuous-time dynamic modeling

Authors: *S. SCHWARCK[1,2], M. C. VOELKLE[3], A. BECKE[1,2], N. BUSSE[1,2], W. GLANZ[1,2], E. DÜZEL[1,2], G. ZIEGLER[1,2];
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Abstract: Physical inactivity is one common risk factor for the development of Alzheimer's disease (AD). Some cardiovascular training studies report improvements or maintenance of cognitive performance of AD patients. However, the majority of studies using repeated measurements show only undirected associations rather than analyzing the dynamic change and interplay of physical and cognitive states. To overcome problems of conventional longitudinal modeling approaches, such as biased parameter estimates and unclear directionality, we used a hierarchical continuous-time dynamic modelling approach to analyze extensive longitudinal data of a 24-week physical exercise and memory recognition performance training in 17 older adults with AD (age: 73.33 ± 3.43; MMSE: 23.50 ± 3.45, m: 9). The dynamic model was specified with two fully connected state variables, each measured by a single manifest indicator, enabling bidirectional interactions between physical and cognitive performance over 72 measurement occasions. The model also incorporates stochastic state noise enabling session-to-session fluctuations due to unexplained intraindividual variability. The auto-effects (or self-connections) and cross-effects of the drift matrix parametrize state-dynamics (and interplay) and were estimated both on an individual- and group level. The model results in 49 parameters and was estimated using MCMC sampling methods. The results show a reliable continuous-time cross-effect suggesting that physical performance is dynamically linked to cognitive performance. Thus, higher physical performance predicted improved memory recognition performance in terms of faster reaction times in subsequent sessions. In addition, a transformation into discrete-time parameters shows that a change in physical performance improves prediction of cognitive performance for up to four days after training session after which the stochastic elements dominate. Furthermore, the dynamic cross-domain interaction was found to be stronger in the second half of the training regime, which might suggest that training affects the interplay. Finally, another model with a clinical score as covariate supported the validity of our observed effects, since higher MMSE baseline scores were associated with lower persistence in cognitive performance. In summary, using a state of the art hierarchical continuous-time dynamic modelling approach these findings support the short-term positive effect of physical exercise training on the cognitive performance in patients with AD. Thus, physical exercise seems to be a promising non-drug treatment in terms of counteracting AD symptoms.

Disclosures: S. Schwarck: None. **M.C. Voelkle:** None. **A. Becke:** None. **N. Busse:** None. **W. Glanz:** None. **E. Düzel:** None. **G. Ziegler:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.13

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: R01 AG066750

Title: Drug Associations with Alzheimer's Disease Incidence and Severity through Insurance Claim Data.

Authors: *E. Y. HU, N. WINEINGER, A. SU;
Scripps Inst., La Jolla, CA

Abstract: In recent years, a significant number of drugs, both novel and repurposed, have been proposed for the treatment of Alzheimer's Disease (AD). Our group looks to contribute to efforts of repurposed drug discovery using a multi-faceted approach through computational methods: knowledge graph data mining, differentially expressed gene complementarity, and insurance claim records correlation analysis. Here, we present the claim records portion of the project. In this study, we utilized claims from Blue Cross Blue Shield members and analyzed them to determine correlation between the use of various drugs on AD incidence and claim frequency. Drug use for up to 200 drugs was observed within AD-diagnosed and non-AD diagnosed members of the insurance group; filters and propensity matching was applied to provide proper comparison between user and non-user groups. Survival analysis was conducted on both AD and non-AD population to observe AD incidence, while the paired t-test was used for an AD only population to observe claim differences. Results reveal that psychotic drug use is associated with higher AD incidence and claim count, while antibiotic and anti-inflammatory medication is mildly correlated with reduced AD incidence. In conclusion, the insurance data analysis provides insight to overall trends between various drug types and AD rate, which may prompt further experimental investigation between the correlated drug-disease relationships.

Disclosures: E.Y. Hu: None. **N. Wineinger:** None. **A. Su:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.14

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant #R01AG062006-04 from the NIA to CM
Acknowledgements: gratefully acknowledge the UCSD ADRC and especially Dr.s Douglas Galasko and Christina Gigliotti

Title: Effects of theApoEε4 allele and CSF total tau on BOLD response during incorrect odor identification in older adults at risk for Alzheimer's disease

Authors: *A. ALBERTAZZI[1], C. FRANK[1], A. DE LA CRUZ[1], E. ROBINSON[1], C. MURPHY[1,2];
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Abstract: Background: Essential olfactory processing brain regions are notably affected by early stages of Alzheimer's Disease (AD) pathology, a devastating neurodegenerative disease that affects about 1 in 9 adults ages 65 years or older. Previous studies have shown older adults genetically at risk for AD (ApoE ε4 carriers) demonstrate poor olfactory performance and differential event-related brain activity, but less is known about the relationship between AD risk factors and neural processes underlying olfactory dysfunction in preclinical AD-risk populations. **Objective:** To investigate early effects of the ApoE ε4 allele and hallmark AD biomarkers (beta amyloid [Aβ] and tau) on blood-oxygen-level-dependent neural responses during odor identification (OID) errors and the brain regions associated with each effect. **Participants:** A de-indentified sample of non-demented older adults (age 68-87; M_{age}= 77.63) was used in analysis. The current sample (n=27; $n_{Females}$=15) includes older adults with normal cognition (n=23) and MCI (n=4), that are either ApoE ε4+ or ε4-, and have various CSF levels of $A\beta_{42}$(range: 349-1616 pg/mL) and total tau (t-tau; range: 120-1185 pg/mL). Additionally, as part of a larger study, all participants have neuroimaging derived data reflecting blood-oxygen-level-dependent (BOLD) neural responses during odor identification. **Methods:** The effect of ApoE ε4 status and Aβ and tau levels on OID is being investigated using a direct odor-stimulus delivery event-related fMRI design of 8 common odors. Subjects are presented each odor, one-at-a-time, in the scanner and identify the smell by selecting one of four odor names on a screen, via a button box. BOLD activation during incorrect OID responses was processed using the Analysis of Functional Neuro Imaging software package. The effects of ApoE ε4 status, age, gender, both CSF measures, and the interaction effect of ε4 status and CSF tau on incorrect OID BOLD activation was investigated in a multiple regression. **Results:** There is a main effect of ApoE ε4 status and an interaction effect of ApoE ε4 status with CSF tau levels on BOLD activations during incorrect OID responses. Possession of the ε4 (ε4+) allele demonstrated less activation (p=.005), compared to no ε4 allele (ε4-), in the precuneus, orbitofrontal cortex (OFC), and middle temporal gyrus. The ApoE ε4*CSF tau interaction demonstrated less activation in bilateral cerebellum and regions of the inferior temporal lobe (p=.005) for ε4+ older adults with greater CSF tau levels than ε4- older adults. Further fMRI research of olfactory task BOLD responses in older adults at risk for AD is needed to better understand preclinical AD manifestations.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.15

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: VA Nerit Review Grant# I01BX005742

Title: Developing nanobodies to target MSUT2 MSUT2:poly(A) RNA binding for tauopathy disorders.

Authors: *R. L. UHRICH[1], M. BAUM[1], A. SAXTON[1], J. D. BAKER[1,2], B. C. KRAEMER[1,2,3,4];
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Abstract: Tauopathies are neurodegenerative diseases characterized by pathological aggregation of the protein tau (MAPT). This includes but is not limited to Alzheimer's disease, corticobasal degeneration, and frontotemporal dementia. Mammalian suppressor of Tauopathy 2 (MSUT2), an RNA binding protein that controls poly(A) tail length, has previously been shown to suppress tauopathy when knocked out in mice. This includes reduced atrophy, reduced tau burden, an overall improvement to cognition, and decreased inflammation. Loss of the MSUT2 CCCH poly(A) RNA binding domain suppresses tauopathy in both *Caenorhabditis elegans* and mice. Therefore, inhibition of the MSUT2 CCCH domain binding poly(A) RNA should be investigated as a potential therapeutic idea for tauopathy. One strategy for inhibiting MSUT2 capitalizes on antibody derived molecules. To target MSUT2 with a biological agent, we raised heavy chain only antibodies (nanobodies) against MSUT2 CCCH RNA binding domain in alpacas. Nanobodies are comprised of a single heavy chain, making them one tenth the size of antibodies while still containing the therapeutic advantages of antibodies including high affinity and exquisite specificity. This allows for easier BBB penetrance (Li et al., 2012) and ability to add functionalized peptide and small molecule tags. MSUT2 nanobodies raised thus far have been prioritized based on their ability to inhibit MSUT2:poly(A) RNA binding through AlphaAssay, a bead based high throughput screen. Their specificity is determined via counter screening against an unrelated poly(A) RNA binding protein, PABPN1. To date we have identified and expressed five distinct MSUT2:poly(A) RNA inhibiting nanobodies with IC50's in the nanomolar range. With this work flow in place, we will optimize novel MSUT2 nanobodies for their translational potential and explore new functionalization approaches potentially leading to validated new therapeutic approaches for tauopathy disorders.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.16

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: JST PRESTO JPMJPR178C
JSPS KAKENHI JP21K09153
JSPS KAKENHI JP20H04053
AMED JP21dm0207070

Title: Potential of optogenetic neuromodulation treatment for stroke

Authors: *F. YOSHIDA[1], T. KURIHARA[2], T. KITANO[4], T. HIROHATA[3], K. BABA[4,5]; [1]Dept. of morphology and function for nursing, Saga Univ., Saga, Japan; [2]Dept. of Pharmacology, Grad. Sch. of Med. and Dent. Sci., [3]Dept. of Rehabil. and Physical Medicine, Kagoshima Univ. Grad. Sch. of Med., Kagoshima Univ., Kagoshima, Japan; [4]Dept. of Neurology, Osaka Univ. Grad. Sch. of Med., Osaka Univ., Osaka, Japan; [5]Dept. of Neurology, Fac. of Medicine, Academic Res. Div., Univ. of Toyama, Toyama, Japan

Abstract: Cerebral stroke is clinically characterized as loss of brain function. one of the possible symptom, motor impairment is severely affect the patients, which not only interfere with the ability to perform activities of daily living, but also significantly reduce quality-of-life. Functional recovery occurs mainly within the first two weeks after onset of stroke, but the process continues for several months. Rehabilitation play an important role in post-stroke states. Various rehabilitatio methods have been developed to improve functional recovery in patients with hemiplegia due to stroke, including the brain stimulation. In order to enhance the rehabilitation after stroke, we have started to test a brain stimulation method using optogenetics—a new method for the manipulation of neurons. Optogenetics is based on genetically modified ion channels that respond directly to light. These light-gated ion channels, such as Channelrhodopsin-2 (ChR2), allow precise, millisecond control of specific neurons.Here we report data from primary experiment in non-human primate. We show that optogenetic stimulation of the motor cortex reduces deterioration of the median speed after stroke. The initial results suggest that the potential of the optogenetic stimulation for therapeutic use with the non-human primate brain.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.17

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Novo Nordisk Foundation Grant NNF15OC0016674
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Sino-Danish Center for Education and Research

Title: Exploring possible routes by which remote ischemic conditioning alleviate brain damage following stroke - implications of extracellular vesicles and miRNAs

Authors: K. T. STENZ[1,5], J. JUST[1,6], Y. YAN[2], Z. HUANG[7], K. VISSING[3], X. WANG[7], J. KJEMS[2,4], *K. R. DRASBEK[1,5];
[1]Ctr. of Functionally Integrative Neuroscience, Dept Clin. Med., [2]Interdisciplinary Nanoscience Ctr., [3]Exercise Biology, Dept of Publ. Hlth., [4]Dept Mol. Biol. and Genet., Aarhus Univ., Aarhus, Denmark; [5]Sino-Danish Col., Univ. of Chinese Acad. of Sci., Beijing, China; [6]Dept Mol. Med., Aarhus Univ. Hosp., Aarhus, Denmark; [7]Inst. of Genet. and Developmental Biology, Chinese Acad. of Sci., Beijing, China

Abstract: Stroke is one of the leading causes of death and long-term disability. In the search for protective treatments, remote ischemic conditioning (RIC) has shown promising effects. However, the mode of action of this non-invasive treatment is not well understood. We have studied plasma extracellular vesicles (EVs) released after RIC and their protective potential in different models of stroke pathophysiology. In addition, as EVs contain large amounts of miRNAs, we have studied miRNA changes following RIC and their possible roles in cell protection. The study was designed in accordance with the Declaration of Helsinki and included 12 young healthy males randomized to either RIC or a non-intervention control group undergoing identical sampling without RIC. Plasma samples were obtained before and after RIC (5 min, 30 min) and as well as after 6 weeks of 3 times per week RIC. EVs were purified using size exclusion chromatography and characterized using tunable resistive pulse sensing and nanoparticle tracking analysis for concentration and size changes. Even though individual fluctuations in EV concentration were observed, no significant changes were seen following RIC or in the non-intervention control group at any time point. In contrast, testing biological effects of EVs in an oxygen and glucose deprivation assay using human brain microvascular endothelial cells mimicking acute hypoxia showed a robust protecting effect when post-RIC EVs were added compared to pre-RIC EVs and non-intervention control EVs. In an effort to study RIC induced miRNA changes, we used next generation sequencing to quantify differentially expressed miRNAs after RIC. Of these, we studied the mRNA targets using bioinformatics of miR-16-5p, miR-144-3p, miR-182-5p, and miR451a, and validated miRNA-mRNA interactions using a luciferase assay and cellular stroke models. In conclusion, even though RIC did not result in changes in EV concentration, we found a clear protective effect when administering post-RIC EVs suggesting that EVs are part of the protective response induced by RIC. Furthermore, several of the post-RIC miRNAs showed relevant mRNA targets that are involved in molecular pathways, which could protect cells and tissue following a stroke.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

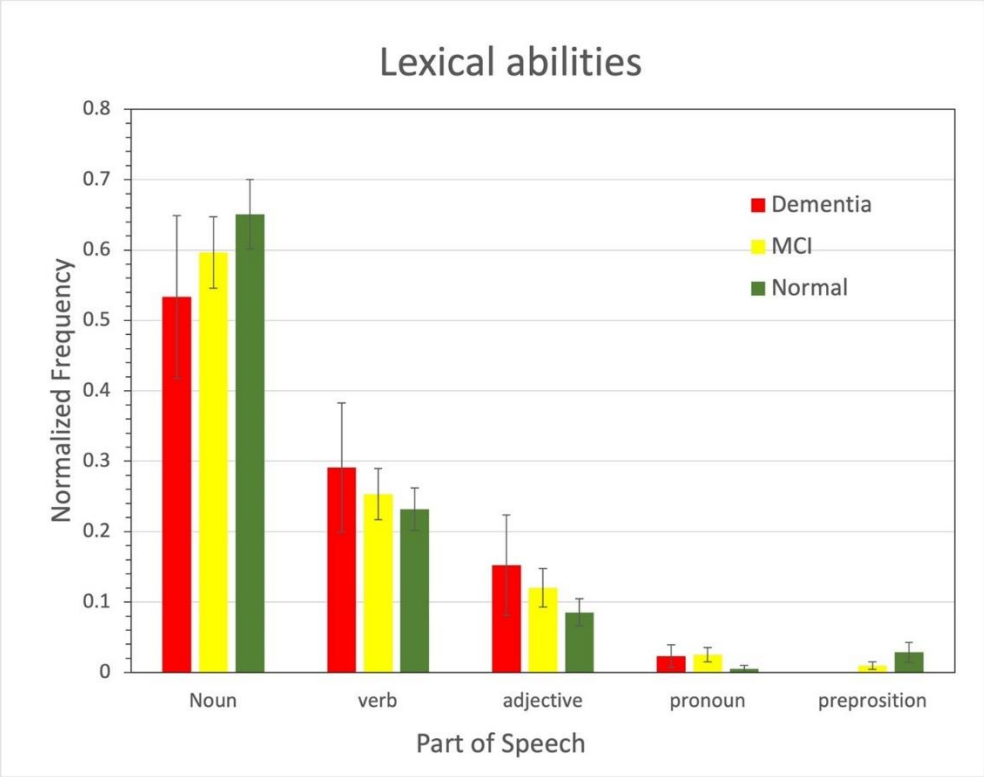
Program #/Poster #: 085.18

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Differences In Proportion Of Lexical Categories In Verbal Fluency Task Amongst Dementia, Mild Cognitive Impairment And Healthy Populations

Authors: *N. KETPRAPAKORN[1], C. POUNGTUBTIM[1], S. CHUNAMCHAI[1], N. CHANCHAOCHAI[1], S. CHANTAVARIN[1], A. PETCHLORLIAN[2], C. CHUNHARAS[2]; [2]Fac. of Med., [1]Chulalongkorn Univ., Bangkok, Thailand

Abstract: Dementia is currently the seventh leading cause of death and has tremendous physical, psychological, social and economic impacts. Early detection of cognitive changes in at-risk populations is crucial in order to prevent and hasten progression to dementia. Verbal fluency test (VFT) is a well-known neuropsychological assessment which is a part of a standard cognitive screening tool for detecting a mild cognitive impairment (MCI). The traditional scoring focuses on the number of any words. A total of 54 subjects (18 dementia patients, 18 MCIs and 18 healthy controls, median age of 78, range from 60 to 90 years old, 70.4% female) were included in this study. We investigate changes in the production of different types of words. Specifically, we categorize word types only when it is backed up by neuroscience empirical findings that different types of words correspond to the activities in different brain areas or by demonstrating that patients with brain lesions exhibited double dissociation. We first classified the words by its lexical categories (noun, verb, adjective, pronoun). For noun words, we further classified it as animate vs inanimate objects. The result depicted in this study suggests that using a combination of numbers and types of words may improve the effectiveness of VFT as a screening test in modern day clinical practice. We believe that a similar approach can be applied to other neuropsychological tasks especially when the measurements are more complex than the traditional scoring. More broadly, this study represents potential benefits of cross-talks between psychology, neuroscience, psychiatry and neurology.



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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.19

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Development of a fast-kinetic high-content imaging assay and analysis method to rapidly quantify mitochondrial dynamics in hiPSC-derived neurons

Authors: S. LETZSCH[1], M. WASCHOW[1], K. BOETTCHER[1], N. GARBOW[1], ***K. KUSHIRO**[2];
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Abstract: Mitochondria are essential organelles in neurons, playing a role in energy metabolism, homeostasis, and synaptic processes. Mitochondrial imbalances contribute to human diseases, including neurodegenerative disorders. Mitochondria undergo dynamic and coordinated cycles of fission, fusion, and disintegration (mitophagy); their physical transport and localization are critical to neuron survival and energy conversion. Tools that quantitatively assess mitochondria

dynamics would be valuable for disease-relevant phenotype studies and drug discovery purposes. In addition, with challenges around CNS sampling, the field has developed hiPSC-derived neuronal cells as a surrogate to primary human cells. An hiPSC-based fast-kinetic imaging assay was developed as a high-throughput screen for dysregulation of mitochondria dynamics. hiPSC-derived neurons were purchased, cultured, and imaged in real time to monitor growing axon networks (DIV 0-6). These replicate wells (n=8) were stained for mitochondria and imaged before and after a 5 min and 30 min treatment incubation with 20µM Glutamate, 1µM FCCP or 2.5µM FCCP against an untreated control on a high-content imager with meander-line sampling. FCCP abolishes mitochondrial membrane potential, and abundance of the neurotransmitter glutamate leads to neurotoxicity. An analysis pipeline was developed for time-lapsed series imaging using two channel acquisitions to find cell bodies and the neural networks prior to image segmentation and incorporation of time-series measurements to calculate a quantitative sum area of remaining pixels as dynamic mitochondrial fraction. The final readout is presented as an area ratio between this dynamic fraction and the full mitochondrial population at each sampled timepoint. Our results show a significant reduction of mitochondria dynamics with pharmacological perturbation by addition of FCCP was captured after 5 and 30 min of treatment, which was not observed with glutamate addition. Further, the fast kinetic imaging of 32 wells (50 time points per field with 2 fps for the fluorescent channel group) collectively took a total of 16 min and did not impact the dynamic fraction when compared within the same treatment groups, indicating the stability of the assay and imaging specification for a rapid assessment. Live cell analysis coupled with fast-kinetic high-content imaging strategies would allow the addition of a relatively simple phenotypic screen to complement functional genomic and pathway mapping studies, as well as better curate potential mitochondria-based therapeutics in a quantitative, real-time, and scalable manner early in research and discovery.

Disclosures: **S. Letzsch:** A. Employment/Salary (full or part-time):; PerkinElmer. **M. Waschow:** A. Employment/Salary (full or part-time):; PerkinElmer. **K. Boettcher:** A. Employment/Salary (full or part-time):; PerkinElmer. **N. Garbow:** A. Employment/Salary (full or part-time):; PerkinElmer. **K. Kushiro:** A. Employment/Salary (full or part-time):; PerkinElmer.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.20

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Energipole (M. Mallart), M.Villain and Société Française de Médecine Esthétique (M. Legrand) for unrestricted support for Research on Parkinson’s disease
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Fondation Saint-Michel

Title: Magnetic Resonance Imaging Biomarker Using Deep Learning to Assess Neuromelanin Depigmentation in Patients with Early-Stage Parkinson's Disease

Authors: *R. GAURAV[1], R. VALABRÈGUE[1], L. YAHIA-CHÉRIF[1], G. MANGONE[1], N. PYATIGORSKAYA[1], I. ARNULF[2], J.-C. CORVOL[3], M. VIDAILHET[3], S. LEHÉRICY[4]; [1]Paris Brain Inst. (ICM), Paris, France; [2]Sleep Disorders Unit, [3]Dept. of Neurol., [4]Dept. of Neuroradiology, Pitié-Salpêtrière Hospital, AP-HP, Paris, France

Abstract: Parkinson's disease (PD) demonstrates neurodegeneration of substantia nigra (SN) using neuromelanin (NM)-sensitive MRI technique. As SN manual segmentation is time-taking and prone to inter-individual variability across raters, there is a critical need for the development of a robust automatic framework to investigate nigral NM. We investigated such changes automatically by prospectively including 144 early-stage idiopathic PD patients (disease duration = 1.5 ± 1.0 years) and 55 healthy volunteers (HV) scanned at 3 Tesla MRI using NM-MRI. The regions of interest (ROI) were delineated automatically using deep learning, and compared to manual segmentations performed by two experts. The SN volumes (Vol), volumes corrected by total intracranial volume (C_{vol}), normalized signal intensity (NSI), and contrast-to-noise ratio (CNR) were computed. One-way general linear model - analysis of covariance was performed while adjusting for age and sex. Diagnostic performance was assessed using the receiver operating characteristic (ROC) analysis. The agreements between methods were estimated using Dice and tested using intraclass correlation coefficient (ICC) based on a mean-rating, two-way, mixed-effects model estimates for absolute agreement. Cronbach's α was estimated to assess inter-method consistency. Automatic segmentation took less than a second for a subject. Using both methods, all SN measures differed between PD and HV with an effect of sex for C_{vol} and CNR. ICC values between the methods demonstrated optimal agreement for C_{vol} and CNR (ICC > 0.9) with high reproducibility (Dice: 0.80). The SN measures also showed good to excellent consistency (Cronbach's alpha > 0.87). Bland-Altman plots of agreement demonstrated no association of measurement differences between the methods and ROI average measurements while confirming that 95% of the data points were ranging between the limits of the mean difference. Percentage changes between PD and HV were -27.4% and -17.7% for Vol, -30.0% and -22.2% for C_{vol}, -15.8% and -14.4% for NSI, -17.1% and -16.0% for CNR for automatic and manual measurements respectively. Using the automatic method, areas under the ROC curve were 0.83 for Vol, 0.85 for C_{vol}, 0.79 for NSI, and 0.77 for CNR. Disease duration correlated negatively with NSI of the patients using both methods.
Taken together, the NM-MRI, coupled with our fully automatic non-rater dependent method, might allow a noninvasive assessment of NM depigmentation in patients with PD with increased precision. Furthermore, this could be a useful biomarker for clinical trials of disease-modifying therapies, and in large-scale neuroimaging studies.

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Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.21

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Campbell Foundation
GVSU OURS

Title: Refining SH-SY5Y cell culture conditions to study microRNAs in Parkinson's disease

Authors: *Z. WALTERS[1], S. KHOO[2];
[1]Grand Valley State Univ., Grand Valley State Univ., Allendale, MI; [2]Grand Valley State Univ., Grand Rapids, MI

Abstract: Zane Walters and Sok Kean KhooRefining SH-SY5Y cell culture conditions to study microRNAs in Parkinson’s diseaseParkinson’s disease (PD) is the second most common neurodegenerative disease, affecting over ten million people worldwide. PD is characterized by dopaminergic neurons deterioration and overexpression/buildup of alpha-synuclein protein, especially in the midbrain. SH-SY5Y is a human neuroblastoma cell line that is commonly used to study PD. It can be differentiated and treated with various chemical to mimic PD cells. However, there is no standard protocol to study microRNA transfection in these cells. Our objective is to refine SH-SY5Y cell culture conditions for microRNA transfection studies in PD. In 6-well plate, we treated the cells with 2 µL of 10 mM retinoic acid to stimulate neurite formation on Day 1, followed by 5 µL of 25 ng/µL brain-derived neurotropic factor (BDNF) to promotes overall neuronal health on Day 4. Finally, the cells were treated with the neurotoxin rotenone on Day 7 for neuron degradation similar to PD. To determine the optimal concentration of rotenone, we cultured cells in 0 nM, 50 nM, 250 nM, 500 nM, 1 mM, and 2 mM rotenone for 9 days and determined cell death by trypan blue staining. While alpha-synuclein gene expression with quantitative real-time PCR did not show any differences within concentration, we found 50 nM rotenone at day 3 produced approximately 50% cell death which is optimal to perform cell studies. We then used this rotenone concentration to perform an optimization study of transfection efficiency for miR-34c mimic, an alpha-synuclein related miRNA, in the concentrations of 12.5 pmol, 25 pmol, 50 pmol, and 100 pmol. We found 12.5 pmol showed lower miR-34c expression (QRT-PCR CT value 9.69) than the rest of the transfection concentration (CT value 8.34-8.76). This suggests 25 pmol of miRNA
mimics may be sufficient to transfect and express miRNA in SH-SH5Y cells. Future direction

includes validation of our refinement with other alpha-synuclein related miRNAs such as miR-7 and miR-153.

Disclosures: Z. Walters: None. **S. Khoo:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.22

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: In Silico Phase-Targeted Stimulation Effect in the Subthalamic Nucleus on Neurophysiology of Parkinson's Disease

Authors: *M. KIM[1], Y. SALIMPOUR[2], P. KUDELA[2], W. S. ANDERSON[2], K. A. MILLS[1]; [1]Neurol., [2]Neurosurg., Johns Hopkins Sch. of Med., Baltimore, MD

Abstract: Deep Brain Stimulation (DBS) provides a promising therapeutic effect against refractory motor complications in advanced Parkinson's Disease (PD). DBS applies continuous, high-frequency electrical stimulation pulses, which may normalize pathological neurophysiology in PD. However, conventional DBS may disturb physiological activity during movement and induce side effects such as dyskinesia and dysarthria. More parsimonious delivery of electrical pulses, timed to specific features of pathological neurophysiologic markers, could treat symptoms with a lower risk of side effects associated with high-frequency stimulation. To explore the effects of phase-targeted stimulation (PTS), which delivers stimulation pulses at specific phases of a neural waveform, on a model cortico-basal ganglia-thalamic circuit, we hypothesized that stimulation of the trough phase of the β-band waveform of the subthalamic nucleus (STN) may significantly suppress pathological neurophysiological activity. In response to PTS, we studied three neurophysiological properties observed in baseline PD conditions: β-band (13-35 Hz) frequency power, (2) β-band waveform asymmetry, and (3) phase-amplitude coupling (PAC) between the phase of β-band phase and amplitude of high-frequency oscillation (HFO). We adopted a computational model representing the pathological cortico-basal ganglia-thalamus network in PD. In the model, we delivered rectangular pulses (amplitude = 3 mA/cm2, pulse width = 50 μsec) at time points at which each of the 12 phase directions (0° - 330°, 30° increments) occurred in the β-band of recorded STN signals. For STN mean β-band power, it was significantly suppressed for all phasic stimulation compared to baseline PD conditions. Within the PTS conditions, mean β-band power was maximally suppressed targeting 210° - 240°. For STN β-band waveform asymmetry, it was significantly attenuated during PTS compared to baseline condition and maximally reduced during 270° stimulation. For β-HFO PAC, it was significantly suppressed following stimulation delivery, and 180° PTS resulted in its greatest suppression. Our results suggest that delivering stimulation at a narrow phase range (180° - 270°) of the β-band waveform of STN, corresponding from trough to the rising phase, effectively suppressed pathological neurophysiological signals observed in PD. In this study, we have

identified and proposed a unified phase range for stimulation after surveying several domains of neurophysiology, which may result in fewer side effects compared to conventional DBS systems and allow engagement of other network nodes where high frequency stimulation is not tolerated.

Disclosures: M. Kim: None. **Y. Salimpour:** None. **P. Kudela:** None. **W.S. Anderson:** F. Consulting Fees (e.g., advisory boards); Longeviti Neuro Solutions, Globus Medical. **K.A. Mills:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.23

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Symmetry-violated metasurfaces: a potential amyloid beta detection technique

Authors: *Z. HADDADIN, D. KIM, L. V. POULIKAKOS;
UCSD, La Jolla, CA

Abstract: Metasurfaces are on-chip imaging platforms composed of periodically repeating sub-wavelength geometries. The controlled symmetry violations of these geometries lead to unique, selective interactions with electromagnetic waves. These light-matter interactions can be exploited for quantitative, colorimetric, and non-invasive detection of amyloid beta (Aβ) plaque deposition in the human eye. Like metasurfaces, Aβ plaques - a hallmark of Alzheimer's Disease - consist of sub-wavelength-scale fibrils that lead to polarisation-dependent interactions with electromagnetic waves. Through the proper engineering of the symmetries present in the sub-wavelength geometries of metasurfaces, it becomes possible to detect the polarised light output from Aβ. This work quantifies the relationship between specific symmetries in metasurface geometry and their respective electromagnetic response. Drawing inspiration from the fields of machine learning and information security, we combine genetic algorithms and hash visualisations in the form of identicons to generate optimised metasurface geometries. Combined with a mathematical quantification of the degree of rotational and reflective symmetries present in these geometries, we correlate the extent to which each of those symmetries (and their respective violations) affect the output polarisation of light by measuring the structural colour readout. Doing so presents insights on how symmetry violations can be quantitatively engineered to control the arising electromagnetic interactions from metasurfaces. This paves the way towards future applications wherein the strategic incorporation of these appropriately-engineered metasurfaces into modern and future ophthalmoscopes can be used for the non-invasive detection of Aβ deposits in the eye.

Disclosures: Z. Haddadin: None. **D. Kim:** None. **L.V. Poulikakos:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.24

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: R01 NS072446
R01 NS082
European Leukodystrophy Association

Title: The role of basal forebrain neurons in Adrenomyeloneuropathy

Authors: *Y. GONG[1], F. LAHEJI[2], A. BERENSON[2], A. MOSER[3], A. QIAN[4], C. MAGUIRE[5], F. EICHLER[2];
[1]Massachusetts Gen. Hosp., Massachusetts Gen. Hosp., Boston, MA; [2]Massachusetts general hospital, Boston, MA; [3]Hugo W Moser Res. Inst., Baltimore, MD; [4]Massachusetts Gen. hospital, Boston, MA; [5]Massachusetts Genral hospital, Boston, MA

Abstract: X-linked Adrenoleukodystrophy (X-ALD) is caused by mutations in the peroxisomal half-transporter *ABCD1*. The most common manifestation is adrenomyeloneuropathy (AMN), a hereditary spastic paraplegia of adulthood. We report that in mice high expression of ABCD1 is found in periaqueductal gray matter, basal forebrain and hypothalamus. In X-ALD mice deficient in ABCD1 (Abcd1-/y), these structures reveal alpha synuclein and other signs of neurodegeneration. Similarly, human brain shows high expression of ABCD1 in deep gray nuclei, while in autopsy specimen of human X-ALD patients these structures show high levels of phosphorylated tau, gliosis and complement activation. This suggests that basal ganglia pathology results from a loss of peroxisomal transport activity and could potentially contribute to motor impairment. As cholinergic neurons in basal forebrain express high levels of ABCD1, we investigated ABCD1 silencing in SHSY5Y neurons and found impaired functional proteins as well as a decrease in acetylcholine levels similar to that seen in plasma of the X-ALD mouse. We also provide evidence that hind limb clasping exhibited in the X-ALD mouse can be corrected by intraventricular adeno-associated virus (AAV)-vector-mediated delivery of *ABCD1*. A comparison to lumbar intrathecal delivery of vector identifies basal forebrain neurons as critical to behavioral rescue. The data support the contention that rescuing peroxisomal transport activity in basal forebrain neurons might represent a therapeutic strategy for X-ALD.

Disclosures: Y. Gong: None. **F. Laheji:** None. **A. Berenson:** None. **A. Moser:** None. **A. Qian:** None. **C. Maguire:** None. **F. Eichler:** None.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.25

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Sangamo Therapeutics

Title: Engineered zinc finger protein transcription factors enable robust and reliable gene regulation in neurons

Authors: J. LEE, K. MARLEN, Q. YU, M. JALAN, H.-O. NGUYEN, T. ROTH, S. HINKLEY, J. ESHLEMAN, D. PASCHON, A. HATAMI, M. SAMIE, A. POOLER, B. ZEITLER, *P. DUNN;
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Abstract: Therapeutic gene modulation has the potential to treat numerous neurodegenerative and neurodevelopmental diseases. We are creating genomic medicines for an array of neurological disorders using zinc finger protein transcription factors (ZF-TFs). ZF-TFs are derived from naturally occurring human proteins and can be engineered to recognize specific genomic DNA sequences. Since uncovering novel regulatory sites for highly-specific gene repression or activation can require extensive empirical testing, we leveraged our proprietary design algorithm to assemble hundreds of candidate ZF-TFs against multiple neurological disease targets to efficiently identify hot spots for gene regulation in both mouse and human genomes. We then developed an iterative and automated *in vitro* screening platform to test them in cell lines, primary neurons, and iPSC-derived neurons for on- and off-target activity. Automated transfection protocols were optimized to transiently and efficiently deliver multiple doses of ZF-TF mRNA to human (SKNMC) or mouse (N2a) neuroblastoma cell lines using a Tecan Fluent 720. We also developed automated cDNA synthesis and RT-qPCR reaction set-up on a Tecan Fluent 1080 to assess gene expression changes 24 hours post-transfection. Using these methods, we screened approximately 384 candidate ZF-TFs per target per species at multiple mRNA doses for their ability to regulate target gene expression. For each target, we identified unique genomic regions amenable to regulation by a selection of active ZF-TFs. From these candidates, we selected dozens per target per species and manufactured AAV-ZF-TF vectors to examine their activity in primary (mouse) or iPSC-derived (human) neurons. ZF-TF performance in neurons was highly correlated with performance in neuroblastoma lines with a large fraction of the ZF-TFs screened in neurons demonstrating potent and dose-dependent target gene regulation. Next, to identify ZF-TFs with favorable whole-transcriptome profiles, we evaluated AAV-ZF-TF specificity by examining the number of differentially expressed genes between ZF-TF and control-treated neurons using high-throughput Affymetrix peg-based microarrays. Using this approach, we identified several active ZF-TFs per target with few to no detectable off-targets. Our ability to identify potent and selective AAV-delivered ZF-TFs that target novel regulatory sites for multiple targets and species highlights the potential of this platform to identify genomic medicines for additional nervous system diseases.

Disclosures: **J. Lee:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **K. Marlen:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **Q. Yu:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **M. Jalan:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **H. Nguyen:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **T. Roth:** A. Employment/Salary

(full or part-time):; Sangamo Therapeutics. **S. Hinkley:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **J. Eshleman:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **D. Paschon:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **A. Hatami:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **M. Samie:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **A. Pooler:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **B. Zeitler:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics. **P. Dunn:** A. Employment/Salary (full or part-time):; Sangamo Therapeutics.

Poster

085. Neurodegenerative Diseases: Tools, Biomarkers, and Treatments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 085.26

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Pioneer Award
Reversal Solutions

Title: Photobiomodulation is anti-inflammatory and prevents neuroinflammation in mouse model of LPS induced systemic inflammation

Authors: *S. SHAMLOO[1], E. B. DEFENSOR[2], J. A. FORTKORT[2], G. OGAWA[2], L. M. VIDANO[2], J. S. LIN[2], M. SHAMLOO[2], A. E. BARRON[2];
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Abstract: Neuroinflammation is associated with various neurological disorders and disease states. While the causal relationship of the neuroinflammatory processes in neurodegeneration is still under investigation, there are currently multiple explorations into therapeutic approaches to block or modulate these inflammatory processes to prevent, delay, and alleviate neuronal degeneration. Photobiomodulation (PBM), the therapeutic use of specific wavelengths of light, has already shown anti-inflammatory properties in a wide range of applications. The current study was aimed at evaluating the effects of PBM on Lipopolysaccharide (LPS)-induced peripheral and central inflammation in mice as an experimental model for neuronal degeneration and neurodegenerative diseases. Group housed mice were administered PBM for 30-minute daily sessions for 12 days, 5 days at a time. Mice received either no light therapy, only red/NIR Light at 640 and 880 nm (RL), or red/NIR light plus 40 Hz gamma frequency flicker (RLG). On day 11, mice have dosed IP with either vehicle or LPS (1 mg/kg). Brain tissue and plasma were collected 24 hours after LPS/vehicle injection after the final PBM treatment. The samples collected were investigated for the inflammatory response at the mRNA level with qPCR. Tissue protein expression was evaluated by Western Blot and Luminex assay, while plasma was analyzed by Luminex. We found that RL and RLG differentially altered the expression of some of the cytokines in both the brain and the plasma. Ultimately, we observed that RL and RLG modulate this LPS-induced inflammatory response. At the protein level, RL and RLG prevented

the induction of IL33R, IFN-α, SRANKL, IL15, and IL7RA after the LPS challenge. Further, we observed an upregulation of IL10 and CCL4. At the mRNA level, there was downregulation of cytokines, including IL18, CD68, and IL6, following the LPS challenge and with light treatments. Our results demonstrate that PBM with RL or RLG has potential local and systemic anti-inflammatory properties in naïve mice following LPS. In future studies, implementing a longer period between LPS administration and tissue collection will be useful to allow for the investigation of the immune pathways modulated by light stimulation.

Disclosures: S. Shamloo: None. **E.B. Defensor:** None. **J.A. Fortkort:** None. **G. Ogawa:** None. **L.M. Vidano:** None. **J.S. Lin:** None. **M. Shamloo:** None. **A.E. Barron:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.01

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: John G. Kulhavi Professorship
Central Michigan University Program of Neuroscience
Central Michigan University College of Medicine
Central Michigan University Department of Chemistry and Biochemistry
E. Malcolm Field and Gary Leo Dunbar Endowed Chair in Neuroscience at Central Michigan University
Student Research & Creative Endeavors Grant received by Johnathon E.S. from the Office of Research and Graduate Studies at Central Michigan University

Title: The Efficacy of Dendrimer-encapsulated Nocodazole on Attenuating Glioblastoma Metabolism, Migration and Proliferation in vitro

Authors: *J. EVERS SMITH[1,2], B. SRINAGESHWAR[1,2,5], A. POUDEL[1,2], J. SWIONTEK[1,2], O. SMITH[1,2], D. SWANSON[3], G. DUNBAR[1,2,4,6], A. SHARMA[3], J. ROSSIGNOL[1,2,5]; [1]Field Neurosciences Inst. Lab. for Restorative Neurol., Mount Pleasant, MI; [2]Program in Neurosci., [3]Chem. & Biol., [4]Psychology, Central Michigan Univ., Mt. Pleasant, MI; [5]Central Michigan Univ. Col. of Med., Mt. Pleasant, MI; [6]Field Neurosciences Inst., Saginaw, MI

Abstract: Glioblastoma (GB) is the deadliest known central nervous system tumor. Using the current standard of treatment, the overall median survival for GB patients is only 12-14 months subsequent diagnosis, and this has remained virtually unchanged since the emergence of the Stupp Protocol in 2005. Thus, a new approach to GB treatment is needed. Nocodazole is a promising antineoplastic treatment, as it reversibly inhibits microtubule polymerization and mitosis by binding to beta-tubulin. This induces apoptosis, thereby inhibiting tumor progression. In this study, we encapsulated nocodazole in the novel G4 70/30-cystamine Poly(amidoamine) dendrimer, which increases the solubility and bioavailability of the drug. We then investigated

the impact of this treatment on U87 human GB cell metabolism and proliferation *in-vitro*. We quantified the metabolic impact of dendrimer encapsulated nocodazole (DN) using an MTT assay, where U87 cells were treated with different concentrations of DN for 72 hours. We treated cells with nocodazole alone as a treatment control, and used HEK293 cells as control cells to determine the impact of DN on a non-cancerous cell line. Following DN treatment, U87 cells were less viable than HEK293 cells at an optimal DN concentration of 200 mM. DN also reduced U87 cell metabolism to a greater degree than nocodazole alone did, demonstrating that the encapsulation of nocodazole increases the efficacy of the treatment. Additionally, we used a scratch assay to quantify the migration and proliferation of U87 cells subsequent treatment with 200 mM of DN for 72 hours. HEK293 cells were used as a non-cancerous cell line control. The scratch assay results indicate that DN significantly inhibits U87 cell growth ($p<0.001$). Additionally, no significant discrepancy was found between untreated HEK293 cell growth and the growth of HEK293 cells treated with DN, suggesting DN may confer a U87-specific treatment effect. Confirmation *in vivo* on animal models is ongoing.

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Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.02

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Egf-coupled gold nanoparticles increase the expression of cnpase and the myelin-associated proteins mag, mog and mbp in the septal nucleus demyelinated by cuprizone

Authors: *E. LIRA-DIAZ[1,2], J. MONROY-RODRIGUEZ[3], J. GUZMAN-MUÑIZ[1], N. MOY-LOPEZ[1], J. J. ACEVEDO-FERNANDEZ[2], L. CASTRO-SANCHEZ[3], O. GONZALEZ-PEREZ[1]; [1]Sch. of Psychology/University of Colima, Colima, Mexico; [2]Sch. of Medicine/UAEM, Cuernavaca, Morelos, Mexico; [3]Univ. Ctr. for Biomed. Research/University of Colima, Colima, Mexico

Abstract: Background: Current pharmacological therapies against demyelinating diseases are not quite satisfactory to promote remyelination. Epidermal growth factor (EGF) can expand the population of oligodendrocyte precursor cells (OPCs) that may help with the remyelination process, but its delivery into the injured tissue is still a biomedical challenge. Gold nanoparticles (GNPs) have unique physical and chemical properties that allow them to function as a drug-delivery system for several tissues, including the brain. Objective: To evaluate remyelination in the septal nucleus after intracerebral GNPs coupled with EGF (EGF-GNPs). Methods: we administered intracerebral EGF-GNPs in C57BL6/J mice that were previously demyelinated with 0.4% cuprizone (CPZ). The animals were divided in groups: Sham, Ctrl, GNPs, EGF, and EGF-

GNPs. We evaluated the remyelination process at two time-points: 2 weeks and 3 weeks post-injection (WPI) of each treatment. We used the rotarod for evaluating motor coordination. Then, we did a Western blot analysis for myelin-associated proteins. Results: EGF-GNPs increase the expression of CNPase, MAG, and MOG at 2 WPI. At 3 WPI, we found that the EGF-GNPs treatment improves motor coordination and increases myelin proteins CNPase, MAG, MOG, and MBP. Conclusion: EGF-GNPs enhance the expression of myelin-associated proteins and improve the motor coordination in mice.

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Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.03

Title: WITHDRAWN

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.04

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: FRM
ANR
Northwestern University

Title: Ionic doped peptide amphiphiles (IDPAs) dynamically enhance development and intrinsic excitability of neurons enhancing neuro-regenerative properties

Authors: M. MUSELLA[1], M. ALVAREZ-SAAVEDRA[2], J. EXTREMET[1], S. STUPP[2], D. DEBANNE[3], Z. SYRGIANNIS[2], ***S. INCONTRO**[3];
[1]UNIS 1072, Aix-Marseille Univ., Marseille, France; [2]Simpson Querrey Inst. for Nanobiotechnology, Northwestern Univ., Chicago, IL; [3]UNIS 1072, Aix-Marseille Univ. - INSERM, Marseille, France

Abstract: The activation or inhibition of neuronal responses is mainly due to the strong binding of small organic molecules (neurotransmitters, lipids, and neuropeptides) to proteins (receptors

and channels). The use of scaffold synthetic molecules at the nanoscale, is emerging as a strategy to deliver a therapeutic cargo or materials functioning as bioactive scaffold to specific cells. Peptide amphiphiles (PAs) are novel engineered biomaterials that self-assemble into supramolecular systems candidates as very promising in drug delivery across the cell membrane in the field of brain diseases, regenerative medicine, and cancer. The intensity of molecular motions within the bioactive fibrils, correlates with enhanced axonal regrowth, neuronal survival, blood vessel regeneration, and functional recovery from spinal cord injury according to a recent work studying the regenerative properties of PAs. Here we introduce a novel technology called ion doped peptide amphiphiles (IDPAs) which adds an ionic cloud to the PAs backbone showing neuro-regenerative properties. IDPAs were loaded with either KCl or NaCl for examining their effect on neuronal development through controlled release of the ions. These ions' reservoirs are biocompatible, biodegradable, and physio-compatible synthetic molecules which dynamically interact with neurons through a novel ionic exchange mechanism, enhancing development and intrinsic excitability of mammalian neuronal networks.

Disclosures: **M. Musella:** None. **M. Alvarez-Saavedra:** None. **J. Extremet:** None. **S. Stupp:** None. **D. Debanne:** None. **Z. Syrgiannis:** None. **S. Incontro:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.05

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: SNF grant 310030_185298 / 1

Title: Development of folate receptor α-functionalized gene-therapy vectors for targeted brain delivery

Authors: *C. BELLOTTI[1,2], A. STÄUBLE[1,2], R. STEINFELD[2];
[1]Neurol., Children's Hosp. Zurich, Zurich, Switzerland; [2]Univ. of Zurich, Zurich, Switzerland

Abstract: One of the major obstacles to the treatment of disorders of the central nervous system (CNS) is the presence of biological barriers like the blood-brain barrier (BBB) or the blood-cerebrospinal fluid barrier (BCSFB), which prevent therapeutics from reaching their target cells. In order to elude these barriers, we can try to mimic endogenous processes for cargo transport to the CNS. For example, extracellular vesicles (EVs) act as natural transporters of proteins and nucleic acids and have thus become a research focus for their potential as therapeutic vehicles. Folate receptor α (FRα) is the main transporter of folates to the CNS and can be found on the surface of EVs in the CSF. Consequently, our research focused on designing FRα-functionalized EV-like vectors for selective targeting to the CNS. Specifically, we wanted to design a gene-therapy vector for treatment of monogenic disorders of the CNS. Firstly, we generated HEK 293 cell lines that overexpressed FRα. Using functionalized magnetic beads for EVs isolation and

flow cytometry, we demonstrated the presence of FRα on the majority of CD9- and CD63-positive EVs. This confirmed the coexpression of FRα with major EVs marker. Since it was previously reported that overexpression of some EVs markers could have a positive effect on lentiviral transduction efficiency, we tested FRα-functionalized lentiviruses *in vitro*. Transduction efficiency in HEK 293 T cells was measured by flow cytometry and visualized by fluorescent microscopy. However, FRα did not increase transduction efficiency. Therefore, we decided to investigate alternative types of gene-therapy vectors. Lipid nanoparticles (LNPs) have recently become an extremely popular drug delivery system and can resemble EVs in their structure. We are currently establishing a protocol for integration of purified FRα in the LNPs formulation in order to selectively target them to the CNS. Using nanoparticle tracking analysis, we can visualize FRα on the LNP surface. Transfection efficiency of our functionalized vectors is tested on differentiated SH-SY5Y cells expressing typical neuronal markers β-tubulin III and MAP2. The design of a LNP-based gene-therapy vector capable of bypassing the BCSFB could have an important impact on advancement of non-invasive treatment of CNS disorders, especially considering the low-immunogenicity of LNPs compared to traditional virus-based gene-therapy vectors.

Disclosures: **C. Bellotti:** None. **A. Stäuble:** None. **R. Steinfeld:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.06

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSF Graduate Research Fellowship (2036197)
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NIH Grant DE026806
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Title: Tackling PAR_2-mediated Oral Cancer Pain with Nanocarriers

Authors: *D. BHANSALI[1,2], T. NGUYEN[2], C. PEACH[2], T. LI[1], K. INOUE[2], D. D. JENSEN[2], N. W. BUNNETT[2], B. L. SCHMIDT[2], K. W. LEONG[1];
[1]Columbia Univ., New York, NY; [2]New York Univ., New York, NY

Abstract: Background: Seventy percent of oral cancer patients experience high levels of pain that affects their day-to-day activity and quality of life. This pain worsens with disease progression and responds poorly to opioids. Protease-activated receptor-2 (PAR_2) found in the endosome has been implicated in oral cancer pain. Here we explore the use of nanoparticles to deliver PAR2 receptor antagonists to the endosome as a therapeutic strategy to manage cancer

pain.
Methods: Third generation poly(amidoamine) was functionalized with cholesterol to form an amphiphilic molecule that self-assembled into a nanoparticulate drug delivery system. Nanoparticles were characterized by dynamic light scattering and transmission electron microscopy, and tagged with a Cy5 N-Hydroxysuccinimide-dye for fluorescent imaging. The uptake of fluorescent nanoparticles was studied using confocal microscopy. Nanoparticles were loaded with a PAR_2 antagonist, AZ3451, via probe sonication and rotary evaporation. Loading and release were quantified using high performance liquid chromatography. Drug-loaded particles were evaluated with fluorescence resonance energy transfer analysis, calcium transients, and luciferase gene assays. A xenograft mouse model of oral cancer pain was generated by inoculating *Foxn1^{nu}* mice with human oral cancer cell lines, HSC3 and OSC20, into the paw. Biodistribution and behavioral analysis, von Frey withdrawal and Hargreaves, were conducted in the *in vivo* models of cancer.
Results: The nanoparticles were less than 200 nm in size and over +30 mV in zeta potential in PBS, both with and without drug loading. The PAR_2 inhibitor, AZ3451, was loaded (40% by mass with 99% efficiency) and exhibited extended release over 24 hours. Confocal imaging revealed nanoparticle uptake and localization to early endosomes, the site of PAR_2, within 1 hour and retained for over 4 hours (N=5). The AZ3451-loaded nanoparticles modulated downstream signaling of PAR_2, including calcium, RhoA, and ERK (N=5, $P<0.05$). Drug-loaded nanoparticles reduced cancer nociception more than free drugs for extended periods of time (N=5, $P<0.05$).
Conclusions: This study reports promising evidence that an endosome-targeted nanomedicine may provide superior pain relief from oral cancer pain and other forms of PAR_2-mediated pain.

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Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.07

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NS102432
NS104769

Title: Intrathecal catheter to enhance neuraxial redistribution

Authors: M. A. HUNT[1], K. A. EDDINGER[2], *T. L. YAKSH[3];
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Abstract: Intrathecal therapeutics, small molecules, toxins and transfection platforms, are viable interventions for efficacious targeting of pain, spasticity, neuraxial cancer and neurodegenerative disorders. Given the absence of cerebrospinal fluid flow, a fixed intrathecal volume and the need to distribute drugs along the rostro-caudal spinal axis, adequate drug distribution has posed a problem. Common approaches (increased injectate volume or continuous infusion) are not feasible or fail to achieve adequate distribution along the spinal axis. We addressed these issues by designing a new catheter composed of micro-valves. Micro-valves increase solute exit resistance, which addresses distribution issues in two ways. 1) Micro-valves distributed along the catheter are opened simultaneously by local transmural pressures. This transmural pressure, produced by injection, is evenly distributed along the catheter fluid column. 2) Buildup of transmural pressure (prior to microvalve opening) results in increased solute exit velocity. High speed imaging of micro-valve catheters in planar diffusion chambers showed for a given volume and delivery rate, fluid exit velocities are significantly increased versus typical open-ended catheters. Increased exit velocity prevents pooling of injectate proximal to opening. When 11 cm catheters (PE-08, 0.36 mm OD), containing 10 paired microvalves, arranged at 120°-140° to one another, were placed intrathecally in rats, small injection volumes (7.5µL) of dye or AAV9-RFP (1xe10 or 1xe12 total viral genomes), resulted in an even rostro-caudal distribution along the spinal axis and robust transfection of neurons from cervical to lumbar dorsal root ganglia (DRG). In contrast, such equivalent injections with a typical open-ended catheter resulted in localized distribution and transfection proximal to the lumbar delivery site. The paired multivalve catheter design resulted in equivalent transfection rates of cervical DRG neurons as lumbar DRG, using 100x lower titer. Finally, unlike an open-ended catheter, microvalves are closed when not in use. Accordingly, unlike open port catheters, no debris accumulation occurs in the implanted un-injected microvalve catheter over 14 days after injection, showing potential for long-term intermittent use. This catheter platform, suitable for small models, is scalable and addresses many problems observed with common catheter systems.

Disclosures: **M.A. Hunt:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Has Patent though UCSD on catheter system, University of California San Diego. **K.A. Eddinger:** None. **T.L. Yaksh:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Has patent though UCSD for catheter system.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.08

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Extracellular fluid and cerebrospinal fluid measurements following acute or chronic administration of different DREADD actuators

Authors: *L. N. BELOATE[1], N. ZHANG[2];
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Abstract: Chemogenetics, specifically the use of Designer Receptors Exclusively Activated by Designer Drugs (DREADD), is a widely used tool in neuroscience research. Historically, the preferred DREADD agonist has been clozapine n-oxide (CNO). However, in recent years, compelling evidence has been published showing that CNO is back metabolized to clozapine (CLZ), does not easily cross the blood-brain-barrier and has off-target effects in naïve rodents. This has led to the push for alternative DREADD agonists, including microdoses of CLZ and, most recently, deschloroclozapine (DCZ). However, very little brain drug availability data is available for the systemic use of these alternatives and even less so for oral administration, such would be used in chronic DREADD manipulations. In one experiment, young adult male Long Evans rats were chronically implanted with a unilateral guide cannula directed towards the dorsal anterior cingulate area (dACC; n=1-3) or lateral ventricle (LV; n=1-3). Following recovery, extracellular fluid (ECF) or cerebrospinal fluid (CSF) samples were acquired using microdialysis. Briefly, animals were lightly anesthetized, and a microdialysis probe (20k Da CO PAES) was inserted into the guide cannula, and eCSF was pumped into the inlet (30 ul; 2 ul/min) while a sample was taken from the outlet. ECF or CSF samples were taken at different target timepoints (0, 30, 60, 90 min) following an acute i.p. injection (within-subject, counterbalanced design) of different DREADD agonists (1 & 10 mg/kg CNO; 0.1 & 1 mg/kg CLZ; 0.1 & 1 DCZ). In another experiment, rats were given chronic access to drinking water with CLZ (~1 mg/kg) or DCZ (~1 mg/kg), and microdialysis samples (60 ul) were taken from dACC under light anesthesia at different timepoints over 1 m (ZT2 or ZT8; Days 1, 7, 14, 21 & 28). Relative levels of CNO, CLZ and DCZ were quantified in all samples for both experiments using liquid chromatography-mass spectrometry (LC-MS). Preliminary results suggest that both CLZ and CNO are detected in CSF following an acute injection of CNO. Furthermore, following an acute injection of 1 mg/kg DCZ, there are detectable levels in ECF up to 90 min following injection. Together, these results not only support previous claims that CNO is back metabolized to CLZ and could be the primary actuator for DREADD in the brain but, to our knowledge, this is the first information of its kind to exist for this relatively new DREADD agonist, DCZ. This set of studies could provide useful information for future studies utilizing microdoses of CLZ and DCZ in both acute and chronic chemogenetics studies.

Disclosures: L.N. Beloate: None. **N. Zhang:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.09

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Cervical Intrathecal Catheter Placement Leads to Improved Rostral Distribution of Radiolabeled 18F-Baclofen Analog in Cynomolgus Monkeys

Authors: *C. S. ROEGGE[1], B. A. DUCLOS[1], H. DOBSON[3], S. HALLER[4], J. P. BODNER[5], L. PAGE[2], S. PANNEM[8], J. GESAMAN[4], A. NOURMOHAMMADI[6], E. A. FEHRMANN[7]; [1]Targeted Drug Delivery Res., [2]Targeted Drug Delivery, Medtronic, Minneapolis, MN; [3]CanCog Technologies Inc., Toronto, ON, Canada; [4]Translational Imaging Ctr., Charles River Labs., Mattawan, MI; [5]Core Technologies, [6]Neuromodulation Res. and Core Technol., [7]Targeted Drug Delivery Res., MEDTRONIC, Minneapolis, MN; [8]Discovery and Preclinical Services, Invicro, Invicro, MA

Abstract: Cervical Intrathecal Catheter Placement Leads to Improved Rostral Distribution of Radiolabeled 18F-Baclofen Analog in Cynomolgus Monkeys
Introduction: Intrathecal (IT) catheter delivery of baclofen via continuous infusion using an implantable pump is an important means of treating patients with severe spasticity. We evaluated the impact of IT catheter placement (upper vs. lower) on brain and spine distribution of a radioactive tracer molecule using positron emission tomography (PET) and CT imaging.
Methods: Three young adult male cynomolgus monkeys were implanted with an Ascenda™ IT catheter, with the distal tip first located at C1 and later revised to a T10 location and attached to an implanted SynchroMed™ II continuous infusion pump. A radioactive tracer molecule, an ^{18}F-baclofen analog, and PET/CT imaging were utilized to observe tracer distribution and quantitate levels of tracer for the first 6 hours of infusion in both the brain and spine according to catheter tip location. This non-human primate study was fully approved by the Medtronic Global Animal Use Policy and was conducted in full compliance with USDA IACUC regulations at a testing facility accredited by AAALAC International with protocol reviewed and approved by IACUC before any investigational work began.
Results: It was consistently determined that a high cervical (C1) catheter tip placement resulted in both more rapid distribution and higher concentrations of radiotracer in the brain and upper spine compared with lower thoracic (T10) placement. With the catheter tip at C1, radioactivity was identified in portions of the brain within the first 5 minutes of imaging compared to nearly 90 minutes at T10. However, for very small and deeper areas, such as the anterior putamen, no significant activity was detected for at least 2 hours even at C1. The highest tracer exposure was in the hindbrain and the periphery where the brain parenchyma is immediately adjacent to cerebrospinal fluid.**Conclusion:** These results indicate that delivery of ^{18}F-baclofen by IT catheter results in distribution within regions of the brain and spine. The data also suggest that the greatest exposure to the brain and cervical spinal cord occurs when the catheter tip is located at C1. Although the ability to manage spasticity is excellent with current clinical practices, it is important to understand the implications of changing this surgical implant parameter. In addition to spasticity, there are a variety of indications where enhanced distribution to the brain would be of value and potentially offer an alternative and less invasive solution to brain catheterization.

Disclosures: C.S. Roegge: A. Employment/Salary (full or part-time):; MEDTRONIC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MEDTRONIC. **B.A. Duclos:** A. Employment/Salary (full or part-time):; MEDTRONIC. **H. Dobson:** A. Employment/Salary (full or part-time):; Invicro. 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.; Invicro. **S. Haller:** A. Employment/Salary (full or part-time):; Charles River Laboratories. 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.; Charles River Laboratories. **J.P. Bodner:** A. Employment/Salary (full or part-time):; MEDTRONIC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MEDTRONIC. **L. Page:** A. Employment/Salary (full or part-time):; MEDTRONIC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MEDTRONIC. **S. Pannem:** A. Employment/Salary (full or part-time):; Invicro. 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.; Invicro. **J. Gesaman:** A. Employment/Salary (full or part-time):; Charles River Laboratories. 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.; Charles River Laboratories. **A. Nourmohammadi:** A. Employment/Salary (full or part-time):; MEDTRONIC. **E.A. Fehrmann:** A. Employment/Salary (full or part-time):; MEDTRONIC. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); MEDTRONIC.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.10

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: W. Garfield Weston Foundation
Beamish Foundation
Temerty Chair in Focused Ultrasound Research at SRI
National Institute of Biomedical Imaging and Bioengineering (NIH)
Canadian Institutes of Health Research

Title: Cnn-based classification of ultrasound-stimulated bubble activity for control of brain therapies

Authors: *D. MCMAHON, R. M. JONES, D. LEAVITT, R. RAMDOYAL, K. LEE, W. KAN, S. YANG, Y.-S. CHEN, C. ADAMS, K. HYNYNEN;
Sunnybrook Res. Inst., Toronto, ON, Canada

Abstract: Background: Focused ultrasound and microbubble (FUS+MB)-mediated blood-brain barrier (BBB) permeability enhancement can achieve targeted, non-invasive drug delivery to the

brain. Control of FUS exposures via 3D passive cavitation imaging (PCI) of coherent MB activity can be prone to missing low levels of MB activity, leading to treatment variability. Objective: Compare classification methods for detecting spatially coherent ultraharmonic MB activity used for FUS+MB exposure control. Methods: FUS exposures for BBB permeability enhancement were performed in rabbits (~3 kg) using an in-house developed 3840-element sparse hemispherical phased array. Following MB injection (4µl/kg Definity), burst-mode FUS (258 kHz, 10 ms bursts every 1s) was electronically steered to 8-16 targets, with whole-burst temporal-average volumetric PCI (128 receiver elements) data reconstructed. The applied acoustic pressure was ramped to levels at or beyond those required for generating ultraharmonic emissions at each target. Classification of spatially coherent MB activity was assessed using fixed imaging parameters (peak sidelobe ratio, localization error), a logistic regression model, and a convolutional neural network (CNN), and compared to manual classification. Results: Using 8 rabbits (93 targets; 4650 bursts), fixed imaging parameter thresholds were optimized, and logistic regression/CNN models were trained to detect manually classified, spatially coherent MB activity. The CNN demonstrated the best performance during cross-validation, with significantly higher recall (0.96±0.02) compared to both fixed imaging parameters (0.64±0.03; $p<0.01$) and the logistic regression model (0.43±0.07; $p<0.01$). On validation data (16 rabbits, 212 targets, 2860 bursts), the CNN maintained high precision (0.99) and recall (0.86) compared to the logistic regression model (precision=1.0, recall=0.37) or fixed imaging parameters (precision=1.0, recall=0.56). Conclusions: CNN-based classification can detect spatially coherent ultraharmonic MB activity with a high degree of precision and recall. These methods may be incorporated within acoustic imaging-based control algorithms designed to calibrate exposure levels for FUS+MB-mediated BBB permeability enhancement, and are expected to result in finer drug delivery control and improved safety.

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Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.11

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant T32EB019940-05
The G. Harold and Leila Y. Mathers Foundation

Title: Wide-field delivery and detection of exogenous molecules in the rodent brain

Authors: ***M. DAWSON**[1], S. BRICAULT[1], P. HARVEY[2,1], H. WEI[1], A. WIśNIOWSKA[1], A. JASANOFF[1];
[1]MIT, Cambridge, MA; [2]Univ. of Nottingham, Nottingham, United Kingdom

Abstract: Delivery of therapeutic and diagnostic agents to the central nervous system is hindered by the blood-brain barrier (BBB), a system of tightly juxtaposed endothelial and perivascular cells that prevents extravasation of most blood-borne molecules. Brain-wide administration of exogenous molecules past the BBB could address many purposes, but achieving systematic delivery remains a challenge. Here we quantitatively evaluate and compare three strategies for wide-field brain delivery using molecular magnetic resonance imaging (MRI) in rats. We examine MRI contrast enhancement profiles spatiotemporally following intravenous injection of the contrast agent gadoteridol following chemically mediated or unfocused ultrasound-based BBB disruption, or after infusion of gadoteridol into the cerebrospinal fluid (CSF). We find that the three techniques produce spatially differentiated labeling patterns, with the most homogeneous delivery across both cortical and subcortical regions obtained using chemically mediated or ultrasound-based BBB manipulation methods. Contrast enhancement distributions are similar following chemical and ultrasound procedures, suggesting that BBB structures in different brain areas display consistent susceptibility to disruption. Furthermore, regional washout rates apparently track susceptibility to disruption and display selective similarly to kinetics observed following intra-CSF delivery. Our results thus document the systematic spatial variation of BBB properties while offering guidance about brain-wide application of molecular technologies in neuroscience and neuromedicine.

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Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.12

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Procedural Overview of Common Dose Routes and Recent Refinements for Targeted Administration of Cell and Gene Therapies to the Central Nervous System

Authors: J. GESAMAN, B. GUNTER, J. OGLE, R. PIELEMEIER, J. VEENSTRA, ***D. LINIHAN**;
Surgical Services, Charles River Labs., Mattawan, MI

Abstract: The development of gene and cell therapies are a promising approach for treatment of central nervous system (CNS) disorders, but clinical success has been limited due to the physiological challenges of transport across the blood brain barrier. There is the additional concern of off target gene expression and possible immunological response when these treatments are delivered systemically. Therefore, it is necessary to develop and refine surgical procedures that will deliver these therapies safely into the target tissue of interest.
At Charles River Mattawan, we have specialized in a variety of CNS targeted delivery techniques in large and small animal models. These include MRI-guided and stereotactic brain

infusions (intracerebroventricular and intraparenchymal), intrathecal (intracisternal magna and lumbar cistern), epidural, and peri-DRG/intra-DRG administration.
Recent refinements to epidural and intra-DRG administration in large animals (canines and non-human primates, respectively) will be highlighted in this procedural overview. Improvements to the surgical procedures to make them as minimally invasive as possible has decreased anesthesia time and improved animal recovery (reduced post-surgery clinical signs and need for veterinary intervention). The implementation of the hemilaminectomy (with partial removal of the facet joint) procedure in place of the more invasive laminectomy procedure for intra-DRG dosing is one such example. These improvements have in turn increased the number of animals that can be dosed in one day, thus limiting the variability that can observed across animals/dosing groups when utilizing multiple dosing days, while still maintaining effective and safe delivery of the therapy.

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Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.13

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NSF Graduate Research Fellowship Program #1762114
NIH Grant NS107609
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R01 NS119395
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University of Washington Mary Gates Research Scholarship
Center for Neurotechnology EEC-1028725

Title: Improving the efficacy and accessibility of intracranial viral vector delivery in non-human primates

Authors: *W. AU[1], D. J. GRIGGS[2], A. D. GARCIA[3], W. K. S. OJEMANN[1], A. G. JOHNSON[5], J. T. TING[6], E. A. BUFFALO[3], A. YAZDAN-SHAHMORAD[4];
[1]Bioengineering, [2]Electrical and Computer Engin., [3]Physiol. and Biophysics, [4]Bioengineering and Electrical Engin., Univ. of Washington, Seattle, WA; [5]Bellevue Sch. District, Bellevue, WA; [6]Human Cell Types, Allen Inst. For Brain Sci., Seattle, WA

Abstract: Non-human primates (NHPs) are precious resources for cutting edge neuroscientific research, including large-scale viral vector-based experimentation such as optogenetics. We propose to improve surgical outcomes by enhancing surgical preparation practices of convection-enhanced delivery (CED), which is an efficient viral vector infusion technique for large brains

such as NHPs'. Here we present both real-time and next-day MRI data of CED in the brains of ten NHPs, and we present a quantitative, inexpensive, and practical bench-side model of the *in vivo* CED data. Our bench-side model is composed of food coloring infused into a transparent agar phantom, and the spread of infusion is optically monitored over time. Our proposed method approximates CED infusions into the cortex, thalamus, medial temporal lobe and caudate nucleus of NHPs, confirmed by MRI data acquired with either gadolinium-based or manganese-based contrast agents co-infused with optogenetic viral vectors. These methods and data serve to guide researchers and surgical team members in key surgical preparations for intracranial viral delivery using CED in NHPs, and thus improve expression targeting and efficacy and, as a result, reduce surgical risks.

Disclosures: W. Au: None. **D.J. Griggs:** None. **A.D. Garcia:** None. **W.K.S. Ojemann:** None. **A.G. Johnson:** None. **J.T. Ting:** None. **E.A. Buffalo:** None. **A. Yazdan-Shahmorad:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.14

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Title: Installation and Refinements of the ClearPoint Navigation System for Targeted Brain Delivery of Therapeutics in Preclinical Animal Models

Authors: S. WOODS[1], D. LINIHAN[1], R. PIELEMEIER[1], J. DEVRIES[1], J. HORVATH[1], D. CHOI[2], N. CLAYCOMB[2], S. KORSZEN[2], ***B. GUNTER**[1];
[1]Charles River Labs. - Mattawan, Charles River, Mattawan, MI; [2]ClearPoint Neuro, Solana Beach, CA

Abstract: Delivery of therapeutics with central nervous system (CNS) indications that cannot readily cross the blood brain barrier require a surgical approach. Assessment of efficacy and safety of these therapeutics in preclinical animal models will utilize stereotactic surgery to ensure successful targeting. While the technique has a long history of success, refinements such as the use of magnetic resonance imaging (MRI) can improve accuracy of delivery and limit possible adverse effects due to subtle anatomical differences and diffusion characteristics. This is especially true for studies that require a large animal model. Recently, our site implemented the ClearPoint Navigation System to enable the use of real-time intraoperative MRI in large animal models such as non-human primates (NHPs) and canines. The commercially available ClearPoint Array system was used for unilateral injections and a novel head fixation frame (0rchestra) was installed for concurrent bilateral injections with the SmartFlow cannula. This was the first use of the Orchestra frame in an *in vivo* study. A gadolinium-containing solution, infused into the targeted brain structure at rates of 1-5 µL/min, was used as a contrast agent which allowed for real-time MRI scanning confirmation of dose site accuracy and dose diffusion monitoring.

Several non-survival studies and a single survival study were conducted for training purposes and to assess accuracy, safety, and throughput of the Navigation system. The results of these studies confirm the use of ClearPoint Navigation system is a clinically relevant preclinical method for targeted brain delivery with high level of accuracy. Furthermore, refinements executed during these installation sessions, in particular the use of the Orchestra device, will be implemented in future preclinical studies for translational continuity when performing safety assessment of therapeutics with CNS indications.

Disclosures: S. Woods: None. **D. Linihan:** None. **R. Pielemeier:** None. **J. Devries:** None. **J. Horvath:** None. **D. Choi:** None. **N. Claycomb:** None. **S. Korszen:** None. **B. Gunter:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.15

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: SNF 185298

Title: Folate receptor α positive hybridosomes as a vector for non-invasive brain-targeted gene therapy

Authors: *A. STÄUBLE[1,2], C. BELLOTTI[1,2], R. STEINFELD[1];
[1]Neurol., Univ. of Zurich, Zurich, Switzerland; [2]Neurol., Children's Hosp. Zurich, Zurich, Switzerland

Abstract: Lipid nanoparticles (LNPs) carrying nucleotides gain popularity for gene-therapies and vaccines, as most of us were already introduced up close and received a treatment. They allow tissue-specific transduction with low immunogenicity suitable for repeated applications. In this study, we exploit this method for brain targeted gene therapy. We are optimizing the LNP formulation, with the focus on different helper and cationic lipids and nucleotides. Additional to mRNA, we use DNA-nanoplasmids equipped with a nuclear targeting sequence. Membrane anchored receptors can be attached to LNPs leading to the development of hybridosomes. This modification improves cell-type specific targeting and the passage of barriers such as the blood-CSF-brain barrier (BCSFB). Previously, our group was able to demonstrate the passage of GPI-anchored folate receptor α positive (FRα+) extracellular vesicles through BCSFB. For our hybridosomes, we use purified polyhistidine-tagged FRα.
For our in vitro experiments, EGFP coding nanoplasmid was delivered over LNPs and hybridosomes to HEK293 and SH-SY5Y cells. Specific helper lipids increase EGFP expression in different cell types. DOPC is best suited for HEK293 whereas DSPG and DOPG works best for SH-SY5Y neurons. Cationic lipids impact the encapsulation efficiency of nucleotides. MC3 increased the encapsulation of mRNA and DNA whereas DODMA is best suited for nanoplasmids. Our preliminary data showed that the addition and attachment of FRα decreased

the encapsulation efficiency but lead to similar EGFP expression levels in SH-SY5Y neurons. Our next plans include in vivo experiment in a reporter mouse line. Because of the ability of FRα to pass the BCSFB barrier, we can non-invasively apply our hybridosomes over intranasal inhalation and track expression of target cells over time. This will allow newly LNP-based gene therapies for neurodegenerative diseases.

Disclosures: **A. Stäuble:** None. **C. Bellotti:** None. **R. Steinfeld:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

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Program #/Poster #: 086.16

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: Ratchadaphiseksomphot Endowment Fund
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IBRO-SfN Travel Grant award

Title: Cns safety profiles of curcumin diethyl γ-aminobutyrate, a carbamate ester prodrug of curcumin

Authors: *H. HASRIADI, P. DASUNI WASANA, O. VAJRAGUPTA, P. ROJSITTHISAK, P. TOWIWAT;
Chulalongkorn Univ., Bangkok, Thailand

Abstract: Curcumin prodrugs have been widely introduced as a better form of curcumin with improved pharmacokinetic and pharmacodynamic properties. We have currently synthesized curcumin diethyl γ-aminobutyrate (CUR-2GE), a novel curcumin prodrug using gamma-aminobutyric acid (GABA) ethyl ester as a promoiety, with improved physicochemical properties and anti-neuroinflammatory activities compared to the parent curcumin. CUR-2GE demonstrated increased stability under an acidic environment and enhanced penetration into the systemic circulation. However, the presence of GABA ethyl ester as a promoiety in CUR-2GE may induce CNS depressive effects if accumulated in a high amount in the CNS. Therefore, in this study, potential CNS effects of CUR-2GE were investigated using the LABORAS automated behavioral analysis to provide insights into the CNS safety profile of CUR-2GE. Male ICR mice (6-8 weeks) were orally administered with the vehicle (0.5% carboxymethylcellulose), chlorpromazine (5 mg/kg), curcumin (100 mg/kg), or its equimolar dose of Cur-2GE (8 mice/group). The mice were placed in the LABORAS home cages immediately after drug administration, and the long-term locomotor activity (day and night cycle) was recorded for 24 h post-compound administration. The results indicate that the mice treated with the vehicle were more mobile during the nighttime compared to the daytime owing to their nocturnal characteristics. Accordingly, the mice treated with either curcumin or CUR-2GE showed similar locomotive behaviors to the vehicle-treated mice at each 2 h behavioral analysis. However,

chlorpromazine-treated mice showed impaired locomotive behavior indicating significantly lower mobility (rearing, locomotion, and climbing) and increased immobility. The comparable home-cage behavior observed between the curcumin- or CUR-2GE-treated groups and vehicle-treated group in all locomotive behaviors signifies no potential sedative or CNS stimulative effects of curcumin and CUR-2GE. Moreover, CUR-2GE had no effects on the weight loss, food, and water intake of mice that underwent LABORAS behavioral assessment for 24 h. Overall, our study provides preclinical evidence supporting the CNS safety profile of CUR-2GE, which can further be evaluated in clinical trials.

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Poster

086. Advances in Drug and Gene Delivery Systems

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Support: NIH/NINDS (U01NS117284)
AstraZeneca supplied SAR.

Title: Advantages of diet-incorporated saracatinib, a disease-modifier, versus oral gavage- its relevance in chronic epilepsy model: Pharmacokinetics and Welfare

Authors: *S. SUNDARA VASANTHI[1], N. MASSEY[1], L. SHOWMAN[2], T. THIPPESWAMY[1];
[1]Dept. of Biomed. Sci., [2]W.M. Keck Metabolomics Res. Lab., Iowa State Univ., Ames, IA

Abstract: Post-seizure activation of Fyn/Src mediates neuroinflammation and neurodegeneration in experimental models of epilepsy. Saracatinib (SAR, also known as AZD0530) is a Src tyrosine kinase inhibitor and has been shown to mitigate SE-induced effects in epilepsy models upon oral gavaging (PMIDs: 34087381; 34720886). Though oral gavaging of SAR is effective, but long-term repeated dosing can be stressful as indicated by weight loss in early days and challenging in aggressive epileptic rats. Thus, a novel approach for administration of SAR is warranted in chronic epilepsy models. In this study, we incorporated SAR in rat chow for adult male Sprague-Dawley rats to achieve anticipated dose range (10-16mg/kg). Further, for comparing oral SAR gavage with SAR-in-diet, animals were divided into 3 experimental groups. In all groups, blood sampling was done at various time-points to determine serum pharmacokinetics (PK). Daily weight gain and food consumption were recorded. All animals were euthanized, hippocampal and serum samples were assessed for SAR concentrations at different time points and compared with the oral SAR. Our results showed that SAR, whether formulated in-solution or in-diet was stable at room temperature for 12 days (97% in solution and 86% in the diet- compared to freshly prepared drug). The rats on SAR-in-diet consumed

about 3.5g/day less compared to the regular diet (14.88±0.8 vs. 18.44±0.5 grams/day). However, the average weight gain/day was not significantly different between the groups (3.167±0.3g/day vs. 2.571±0.73g/day). Importantly, we achieved the anticipated SAR dose range (10-16mg/kg). Serum SAR concentrations did not significantly vary between the animals on SAR-in-diet and orally gavaged SAR group [at 48h, 216.25 vs. 191.59 ng/mL and, at 72h, 333.43 vs 283.17 ng/mL, respectively]. SAR concentrations in the hippocampus in diet were higher than the repeated gavaging suggesting SAR-in-diet is a better approach [4th day, 238.6±143 vs 551.8±209.3ng/g and at day 7, 271.1±62.33 vs 323.4±84.61ng/g]. The serum SAR concentrations in repeated oral dosing and SAR-in-diet showed a strong correlation from day 2 through day 7 and there were no significant differences between groups. Overall, SAR's longer stability at room temperature and comparable concentrations in the serum and the hippocampus in the animals fed on SAR-in-diet are useful for disease-modifying studies such as epilepsy. Test drugs in diet is a translational approach and no handling-stress to animals and abates variables in experiments.

Disclosures: S. Sundara Vasanthi: None. **N. Massey:** None. **L. Showman:** None. **T. Thippeswamy:** None.

Poster

086. Advances in Drug and Gene Delivery Systems

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 086.18

Topic: I.05. Biomarker, Drug Discovery, and Experimental Therapeutics

Support: NIH Grant NS103957
NIH Grant NS107281
NIH Grant NS075321

Title: Longitudinal oral administration of centrally-acting drugs to nonhuman primates

Authors: *S. M. MOERLEIN[1], S. LOFTIN[2], E. WILLIAMS[2], A. LI[2], L. TIAN[2], J. S. PERLMUTTER[2];
[1]Radiology, Washington Univ. Sch. of Med., Saint Louis, MO; [2]Neurol., Washington Univ. Sch. of Med., St. Louis, MO

Abstract: ***Objective and Rationale****:* Longitudinal drug studies in nonhuman primates pose a challenge to the neuroscientist. Parenteral drug administration via infusion is not an option, since it would require anesthesia inappropriate for lengthy studies involving multiple doses per day. Oral administration of drug is thus the preferred route, but animal subjects will not voluntarily swallow the drug formulations typically used in human subjects. Restraint of the animal for drug administration would be unethical, and moreover perturbs the scientific rigor of the study by inducing stress in the subject. ***Methods:*** We have developed a method for administration of CNS drugs to nonhuman primates by disguising the study drugs as food. Specifically we have

administered carbidopa/levodopa, pramipexole or lactose to *Macaque fasicularis* (N = 13; 6.25-9.4 kg) on a twice daily dosage regimen for up to eight weeks. Each dosage form contained a total drug weight of approximately 250 mg, corresponding to 20mg/kg levodopa and 50 mg carbidopa. Pramipexole was dosed at 0.03 mg/kg po twice daily. Formulation involved loading of the drug into a paste made using the minimum amount of honey. The resulting drug paste was placed into a one of three drug delivery packages: pitted date, pitted apricot or bored marshmallow. The weight of these food vehicles was approximately 5-6 g. The dates and apricots were sealed by simple pressing of the material together. The marshmallows were sealed with marshmallow cream after drug loading. ***Results:*** The animals responded well to these covert drug delivery platforms. Dose regimens were maintained throughout the study, although individualization of the specific drug package was modified to accommodate the preferences of the subject. In some cases, dosage forms needed to be altered during the study period to add variety and subject acceptance. It was also necessary to administer drug-free treats to neighboring non-study primates to maintain social harmony in the facility.HPLC analysis of the blood of test subjects demonstrates that plasma concentrations of levodopa of 2-4 μg/mL were reliably attained using these drug administration methods. ***Conclusions:*** This methodology is an effective means for longitudinal oral administration of anti-Parkinson drugs or placebo to nonhuman primates. The technique may be adapted to studies of alternative centrally-active agents.

Disclosures: S.M. Moerlein: None. **S. Loftin:** None. **E. Williams:** None. **A. Li:** None. **L. Tian:** None. **J.S. Perlmutter:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.01

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

Support: Carney Innovation 2020 Grant

Title: Developing genetically encoded photoconvertible voltage probes

Authors: *A. LIN[1], A. C. THOMPSON[2], C. D. AIZENMAN[3], A. S. ABDELFATTAH[5,4]; [1]Brown Univ. Grad. Dept. of Neurosci., Providence, RI; [2]Dept. of Neurosci., [3]Dept Neurosci, [4]Brown Univ., Providence, RI; [5]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Genetically encoded voltage indicators (GEVIs) enable monitoring of electrical activity of neurons with high spatiotemporal resolution. However, existing GEVIs do not allow highlighting of specific cells such that their changes in membrane potential could be imaged in a second spectral channel. Highlighting could, in principle, be achieved by the use of photoconvertible fluorescent proteins that irreversibly change their emission wavelength upon illumination with ~400 nm light. Here, we engineer a GEVI, called Ace2-mEos (Fig. 1), where

we fuse a photoconvertible fluorescent protein, mEos3.1, with a microbial opsin (Ace2). Transmembrane voltage-dependent changes in the absorption spectrum of the Ace2 reversibly modulate the degree of fluorescence quenching of the nearby mEos fluorescent protein. We optimize Ace2-mEos for maximal förster resonance energy transfer (FRET) signal overlap. Ace2-mEos was readily photoconverted from green to red when expressed in primary neuron cultures and exhibited a decrease in both the green and red fluorescence channels in response to membrane depolarization. We also use protein engineering to develop positive-going versions of Ace2-mEos with amino acid substitutions in the opsin domain. Using a field stimulation protocol in primary neuron cultures, we characterize the dynamic range of both positive and negative-going versions of Ace2-mEos. Our GEVI constructs faithfully detects single action potentials (~-15-20% for negative going version of Ace2-mEos, ~10-15% for positive going version of Ace2-mEos) and tracks electrically driven voltage oscillations at 66 Hz in primary neurons in single trial recordings.

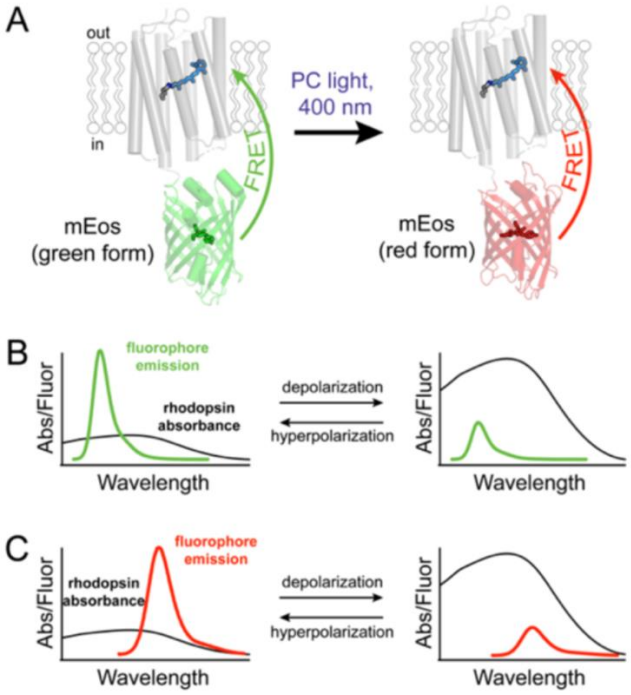


Figure 1: (A) Schematic of photo-convertible voltage sensor (Ace2-mEos). (B, C) Mechanism of action of photoconvertible voltage sensor. Overlap between opsin absorbance spectrum and the green (B) and red (C) forms of mEos cause changes in FRET efficiency upon membrane potential changes.

Disclosures: **A. Lin:** None. **A.C. Thompson:** None. **C.D. Aizenman:** None. **A.S. Abdelfattah:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.02

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

Support: VA Office of Research and Development Award # BX003431

Title: Generation of medium spiny neurons from bipolar disorder patient-derived cells

Authors: *K. E. ROHR[1], M. J. MCCARTHY[1,2];
[1]Univ. of California San Diego, San Diego, CA; [2]Veterans Affairs San Diego Healthcare Syst., San Diego, CA

Abstract: Bipolar disorder (BD) is a serious mental illness characterized by recurrent episodes of depression and mania. BD patients show alterations in reward processing in the striatum, which is predominately composed of medium spiny neurons (MSNs) that express dopamine receptors. Therefore, MSNs are critically important for motivated behavior and are the targets of antipsychotic drugs. However, to date, induced pluripotent stem cell (iPSC) models of BD have focused on glutamatergic neurons. To address this gap, we generated GABAergic MSNs from bipolar patient-derived cells using a dual SMAD inhibition protocol. The cell population was characterized by co-localizing neuronal protein expression with GABA or glutamate markers. Further, we confirmed the presence of MSN and psychiatrically relevant gene transcripts. Overall, the results indicate that this protocol yields a relatively homogeneous GABAergic medium spiny neuron population from bipolar patient-derived iPSCs. Generating these neurons provides a novel model to study bipolar disorder and complements previous work in iPSC-derived glutamatergic neurons.

Disclosures: K.E. Rohr: None. **M.J. McCarthy:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.03

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

Support: NIH R01

Title: Monitoring Neuromodulator Levels across Time and Animals with Optical Sensors

Authors: *P. MA, P. CHEN, E. TILDEN, Y. CHEN;
Dept. of Neurosci., Washington Univ. in St. Louis, St. Louis, MO

Abstract: Neuromodulator dynamics are essential for their functions. Recent development of fluorescence intensity-based optical sensors for neuromodulators has enabled us to detect

neuromodulator changes with high spatial and temporal resolution during animal behaviors. However, although fluorescence intensity can be used to measure acute and phasic changes of neuromodulators, it cannot be used to compare changes over long time scale across animals, because fluorescence intensity is dependent on sensor expression level. In contrast, fluorescence lifetime measurement is independent of sensor expression level or excitation light fluctuation, and can thus be ideal for comparison across animals and time at high spatiotemporal resolution. Here, we screened commonly used neuromodulator sensors and found that multiple sensors show changes of fluorescence lifetime. Lifetime measurement of neuromodulator biosensors can detect transient neuromodulator changes, is dose sensitive, insensitive to excitation laser power fluctuation, and shows high reproducibility within a cell. In vivo, we measured both fluorescence lifetime and intensity of neuromodulator sensors with photometry in behaving mice across sleep/wake and running/resting states, and showed that fluorescence lifetime is a much better correlate of behavior states than fluorescence intensity, especially across days and across animals. Thus, the discovery, characterization, and proof-of-principle application of lifetime measurement of neuromodulator sensors open doors to reveal neuromodulator dynamics at high resolution across animals, conditions, locations, and chronic time scales.

Disclosures: **P. Ma:** None. **P. Chen:** None. **E. Tilden:** None. **Y. Chen:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.04

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

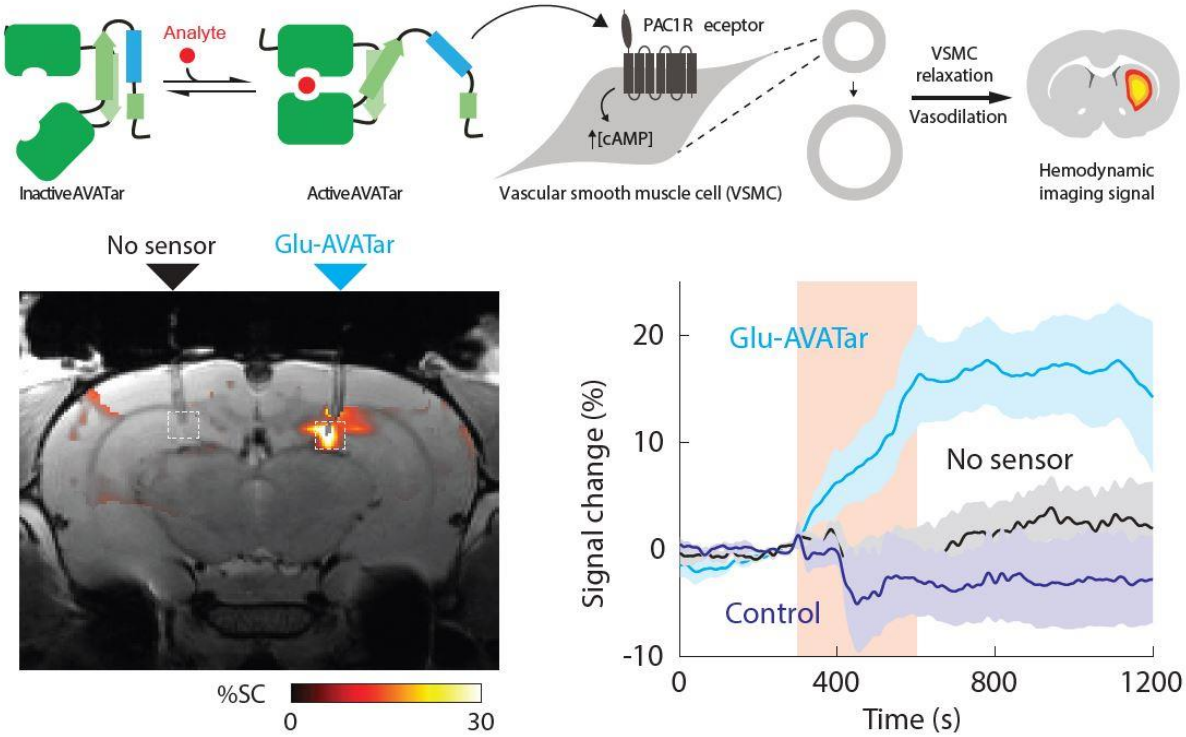
Support: Marie Skłodowska-Curie fellowship from the European Union
Deutsche Forschungsgemeinschaft
National Institutes of Health (R24 MH109081, U01 NS103470, and UF1 NS107712)

Title: Molecular imaging of extracellular glutamate in the rat and marmoset brain

Authors: *M. SCHWALM[1], R. OHLENDORF[1], S. BRICAULT[2], J. SHARMA[3], A. JASANOFF[1];
[1]MIT, Cambridge, MA; [2]MIT, Cembridge, MA; [3]Brain and Cognitive Sci., MIT, Cambridge, MA

Abstract: Monitoring extracellular glutamate dynamics provides a way to discern the engagement of excitatory activity that conducts the bulk of long-distance signaling in the mammalian brain. We recently introduced a molecular imaging approach that could permit noninvasive imaging of neurochemicals like glutamate using vasoactive sensors called AVATars. By coupling analyte detection to relaxation of vascular smooth muscle cells, these sensors induce artificial hemodynamic signals detectable by functional magnetic resonance

imaging (fMRI) or functional ultrasound. Here we describe the design and *in vivo* validation of Glu-AVATar, a novel protein-based AVATar for selective hemodynamic imaging of extracellular glutamate in the brain. We demonstrate that this probe produces robust hemodynamic responses in the presence of exogenous glutamate, while control experiments performed with vehicle stimulation or control proteins do not result in signal changes. Glu-AVATar functionality is exhibited in both rodent and primate brains, indicating potential translatability of the technology. We further demonstrate that Glu-AVATar reveals correlations between probe-infused regions of the dorsal rat hippocampus and distal regions such as the ventral hippocampus. These measurements constitute a neurochemically specific form of functional connectivity analysis that can be combined with conventional fMRI techniques to reveal correlates of excitatory signaling between different brain regions. Future AVATar-based molecular fMRI approaches will allow for more precise and less invasive methods for imaging extracellular glutamate dynamics over wide fields of view in intact brains.



Disclosures: **M. Schwalm:** None. **R. Ohlendorf:** None. **S. Bricault:** None. **J. Sharma:** None. **A. Jasanoff:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.05

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

Support: NIH grant R01 GM124038
NIH fellowship F31 CA254162
NIH fellowship F32 NS116105
NIH fellowship F32 GM123577

Title: A high-throughput multiparameter screen for accelerated development and optimization of soluble genetically encoded fluorescent biosensors

Authors: *D. KOVEAL[1], P. C. ROSEN[1], D. J. MEYER[1], C. M. DÍAZ-GARCÍA[1], Y. WANG[2], L.-H. CAI[2], P. J. CHOU[1], D. A. WEITZ[2], G. YELLEN[1];
[1]Dept. of Neurobio., Harvard Med. Sch., Boston, MA; [2]Dept. of Physics and John A. Paulson Sch. of Engin. and Applied Sci., Harvard Univ., Boston, MA

Abstract: Genetically encoded fluorescent biosensors are powerful tools used to track chemical processes in intact biological systems. However, the development and optimization of biosensors remains a challenging and labor-intensive process, primarily due to technical limitations of methods for screening candidate biosensors. Here we describe a screening modality that combines droplet microfluidics and automated fluorescence imaging to provide an order of magnitude increase in screening throughput. Moreover, unlike current techniques that are limited to screening for a single biosensor feature at a time (e.g. brightness), our method enables evaluation of multiple features (e.g. contrast, affinity, specificity) in parallel. Because biosensor features can covary, this capability is essential for rapid optimization. We use this system, called BeadScan, to generate a high-performance biosensor for lactate that can be used to quantify intracellular lactate concentrations. This biosensor, named LiLac, constitutes a significant advance in metabolite sensing and demonstrates the power of our screening approach.

Disclosures: D. Koveal: None. **P.C. Rosen:** None. **D.J. Meyer:** None. **C.M. Díaz-García:** None. **Y. Wang:** None. **L. Cai:** None. **P.J. Chou:** None. **D.A. Weitz:** None. **G. Yellen:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.06

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

Support: National Science Centre, Kraków, Poland, Grant2016/21/B/NZ7/01131

Title: Recent improvement for the analysis of acetylcholine levels in brains

Authors: *L. M. VAN HEERWAARDEN[1], H.-J. BROUWER[1], M. EYSBERG[1], M. HERIAN[2], P. ŚWIT[2], K. GOLEMBIOWSKA[2], N. J. REINHOUD[1];

[1]Antec Scientific, Alphen a/d Rijn, Netherlands; [2]Dept. of Pharmacol., Maj Inst. of Pharmacol., Kraków, Poland

Abstract: Acetylcholine is an important neurotransmitter that plays a crucial role i.e. in memory and movement. The analysis of acetylcholine levels in brain requires an analytical method that can separate and detect the low levels of acetylcholine in the presence of the high levels of its metabolite choline.
The best/fastest high-performance liquid chromatographic method was found to be ion-pairing separation on a reversed phase C18 column, when comparing with ion exchange chromatography. The methods show a reversed elution order for the acetylcholine and choline peaks. Using the ion-pairing method, the choline elutes first, it is well separated from acetylcholine and there are no late eluting disturbances in the baseline.
For the electrochemical detection of acetylcholine, the post-column on-line Immobilized Enzyme Reactor (IMER) is required to convert it to the electrochemically detectable hydrogen peroxide. A platinum electrode has high specificity for hydrogen peroxide, and with the use of an optimized electrochemical pulse this can be used to get a reproducible response.
The calibration plots for acetylcholine typically show a correlation coefficient *r* lower than 0.999 when applying the most basic 'External Calibration' method with linear regression. To improve the quality of the results, different calibration methods were tested (1, External Calibration; 2, Standard Addition Method, 3, Integrated Calibration Method in the version of Complementary Dilution Method, and 4 basic variant of Integrated Calibration Method). A comparison of results calculated in various ways allowed an effective assessment for the most suitable method for this analysis.
For the evaluation of the method, acetylcholine analyses were done based on standards as well as mice brain (in microdialysis samples from freely moving animals, before and after a dose of the psychoactive drug pyrovalerone). The method was found to be well suited for the analysis of the low basal acetylcholine levels in mouse brain.

Disclosures: L.M. Van Heerwaarden: None. **H. Brouwer:** None. **M. Eysberg:** None. **M. Herian:** None. **P. Świt:** None. **K. Golembiowska:** None. **N.J. Reinhoud:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.07

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

Support: NRF-2021R1A2B5B02002437
NIH 5R01NS112176-03

Title: Shape Optimization of 30um Carbon Fiber Microelectrode for In Vivo fast-scan cyclic voltammetry

Authors: *H. KWON[1], J. SIM[2], H.-U. CHO[2], H. SHIN[3], Y. OH[3], K. H. LEE[3], K. E. BENNET[3], I. KIM[2], D. JANG[2];
[1]Dept. of Electronic Engin., [2]Dept. of Biomed. Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [3]Dept. of Neurologic Surgery, Mayo Clin., Rochester, MN

Abstract: Carbon fiber microelectrode (CFME) with 7-10 μm is an electrode commonly used to measure neurotransmitters through fast-scan cyclic voltammetry (FSCV). Unexpected, many studies have not been conducted in vivo FSCV with thicker carbon fibers more than 30 μm. In this study, we optimize the shape of the 30 μm carbon fiber electrodes to a corn shape with an electrochemical-etching technique to obtain in vivo reliable measurement of neurotransmitter while reducing the tissue damage. In the first experiment, dopamine was measured in vivo at 30 μm electrodes without any treatment. Although the 30 μm electrode has a larger active area than general electrodes (7-10 μm), the experiment showed a very low DA response. In addition, the success rate for dopamine detection in vivo was lower than 7 μm due to the occurrence of brain tissue damage by 30 μm electrode. The corn-shaped electrodes with a length of 100-110 μm were produced by a home-made electrochemical-etching system which can easily modify the morphology by adjusting the voltage and speed of etching. The etched carbon electrode was tested in vitro with the bare electrode of same thickness to compare the dopamine sensitivity. As a result, the background current of the bare electrode was twice as large as that of etched electrode. However, considering each surface area, the background current and the faradaic current for dopamine are expressed at the similar level before and after treatment. Despite their similar dopamine sensitivity, the degree of detection of each electrode was very different at in vivo test. The etched electrode was measured to be about three times higher than the bare electrode. Based on the experimental results, it can be confirmed that 30 μm bare electrode induces damage of tissue as the insertion, adversely affecting the in vivo detection, and electrochemical-etching can reduce the effect by changing the morphology of the electrode. This is expected that electrochemically etched electrodes could show the possibility of new use of a thicker electrode with advantages of sensitivity as well as longevity and rigidity.

Disclosures: H. Kwon: None. **J. Sim:** None. **H. Cho:** None. **H. Shin:** None. **Y. Oh:** None. **K.H. Lee:** None. **K.E. Bennet:** None. **I. Kim:** None. **D. Jang:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.08

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

Support: NIH Grant R01DA048096
NIH Grant R01MH121099
NIH Grant R01MH124115

Title: Sub-second dopamine measurements with tungsten microelectrodes used in humans during deep brain stimulation electrode implantation surgery

Authors: *A. JIANG[1], C. K. JOHNSON[1,4], K. T. KISHIDA[1,2,3];
[1]Physiol. and Pharmacol., [2]Neurosurg., [3]Biomed. Engin., Wake Forest Univ. Sch. of Med., Winston Salem, NC; [4]NASA Johnson Space Ctr., Houston, TX

Abstract: While dopamine has been extensively studied directly in animals and indirectly in humans through the use of neuroimaging, very few studies have directly measured dopamine in humans. Deep brain stimulation (DBS) is an effective procedure for alleviating motor symptoms in patients with movement disorders such as Parkinson's disease and essential tremor that offers the opportunity to record from the human brain during electrode implantation. During DBS electrode implantation surgery, tungsten microelectrode recording is used to determine the best location for the implant. Here we demonstrate these same tungsten microelectrodes can be used to detect dopamine with a fast-scan cyclic voltammetry recording protocol. Previous studies have used carbon fiber microelectrodes during DBS surgery to record the release of dopamine and serotonin in the caudate or putamen while patients complete a short task. However, the use of tungsten microelectrodes to record electrochemical data can reduce barriers to data collection in humans and make investigations into dopamine's role in human movement disorders more approachable by eliminating the need of using an additional carbon fiber research probe during surgery. We made continuous electrochemical measurements at a rate of 10Hz in solutions with varying known concentrations of dopamine using a -0.6V to 1.4V to -0.6V ($400Vs^{-1}$) triangular waveform protocol. These measurements were used to train an elastic net penalized linear regression model that included the raw, first derivative, and second derivative non-background subtracted cyclic voltametric responses as independent variables. The resulting model was able to predict the change in dopamine concentrations in out-of-sample datasets collected with new electrodes that had not been included to train the model. Values in the raw, first, and second derivatives all contributed to the model; however, we found values in the second derivative were most correlated with the concentration of dopamine. Notably, the signal extraction methods also work for carbon fiber microelectrode recordings routinely used in preclinical work. These methods can be used to confirm research done in animal models with humans and can yield insights on dopamine in patients with Parkinson's disease or essential tremor.

Disclosures: A. Jiang: None. **C.K. Johnson:** None. **K.T. Kishida:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.09

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

Support: KIST intramural fund 2E31523

Title: Elucidating the mechanism of the voltage-dependent fluorescence change yields an easy to use GEVI that brightens upon neuronal activation in vivo.

Authors: L. LEONG[1], J. RHEE[2], H. KIM[1], J. SEONG[1], J. WOO[1], K. HAN[1], D. A. STORACE[3], *B. BAKER[1];
[2]Ctr. for Functional Connectomics, [1]Korea Inst. of Sci. and Technol., Seoul, Korea, Republic of; [3]Biol. Sci., Florida State Univ. Program In Neurosci., Tallahassee, FL

Abstract: ArcLight, one of the best genetically encoded voltage indicator (GEVI) currently available is composed of the *Ciona* voltage sensing domain (VSD) attached to the pH sensitive fluorescent protein (FP), Super Ecliptic pHluorin. Electrodynamic pathways that allow for charge migration from the external surface of the FP to the internal chromophore allow ArcLight and other ArcLight-derived GEVIs to optically report conformational alterations resulting from changes in membrane potential. In this study, we demonstrate that hydrophobic residues have an essential role in transferring information from the exterior of the FP to the internal chromophore. We also find significant differences in the optical signals of asparagine and glutamine mutations demonstrating remarkable precision in the orientation of the amino acid side chains on the surface of the FP. The asparagine mutant behaves similarly to the positively charge amino acids at one surface position while the corresponding glutamine mutant behaves like other negatively charged amino acids at that position suggesting that the orientation of the oxygen and nitrogen atoms in the side chain on the surface of the FP matter. When mutations were introduced at a separate position internally located near the chromophore of the FP, the glutamine mutation exhibits a fast voltage-dependent signal while the asparagine mutant slows the signal. This result strongly implicates the twisting of the chromophore in response to membrane potential transients which affects the fluorescence of the GEVI. These new findings enabled the development of a new GEVI that gets brighter upon membrane depolarization which is capable of optically reporting neuronal activity in brain slice and *in vivo*.

Disclosures: L. Leong: None. **J. Rhee:** None. **H. Kim:** None. **J. Seong:** None. **J. Woo:** None. **K. Han:** None. **D.A. Storace:** None. **B. Baker:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.10

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

Support: DFG 374031971
DFG TRR 166/A03
DFG 417451587
Prix-Louis-Jeantet

Title: Improved Channelrhodopsins for calcium manipulation

Authors: *S. YANG[1], S. GAO[2], Y. ZHOU[3], M. DING[2], Y. ZHANG[4], G. NAGEL[2];
[1]Janelia Res. Campus, Ashburn, VA; [2]Univ. of Wuerzburg, Wuerzburg, Germany; [3]Zhengzhou Univ., Zhengzhou, China; [4]Univ. Hosp. Wuerzburg, Wuerzburg, Germany

Abstract: Optogenetics transformed neuroscience after the introduction of Channelrhodopsins (ChRs) as an easily applicable tool to manipulate neuronal activity. ChRs are normally non-selective cation channels with high proton permeability. The high proton conductance may lead to cellular acidification and mediate diverse cellular signals. On the contrary, the calcium permeability and single channel conductance of ChRs are relatively low. Through introducing up to 5 mutations, we developed a set of improved ChR2 mutants with larger charge transfer per molecule and higher ion specificity. The high calcium and low proton conductive ChRs enable robust *in vivo* calcium manipulation in multiple living organisms, providing valuable tools to dissect calcium-mediated physiological responses.

Disclosures: S. Yang: None. **S. Gao:** None. **Y. Zhou:** None. **M. Ding:** None. **Y. Zhang:** None. **G. Nagel:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.11

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

Title: Designing neuronal activity-integrating sensors with high spatiotemporal resolution

Authors: *G. ZHOU[1], W. W. WAN[2], W. WANG[2];
[1]Chem. Biol., [2]Chem., Life Sci. Institute, Univ. of Michigan, Ann Arbor, MI

Abstract: Identifying the neuronal ensembles activated by a specific stimulus is instrumental for studying their roles in neuronal circuits and behaviors. Temporally-gated neuronal activity integrators have been developed to convert transient neuronal activities into permanent signals within a user-specified time window. Most of them, such as FLARE[1] and iTANGO[2], are designed with transcriptional readout, which enable signal amplification and further interrogation of the neuronal activities with versatile readout. However, these transcription-based integrators lose subcellular spatial information and provide delayed readout, which limits their applications in short-lived brain slices. The non-transcription-based integrator CaMPARI[3] can potentially solve these problems, but it loses the versatility of transcription-based integrators and can only detect calcium-related neuronal activities. To address these limitations, I have engineered a new temporally-gated neuronal activity integrator based on the protease-activatable ascorbate peroxidase. This integrator, termed PICACHU, can detect various neuronal activities stimuli, including dopamine and calcium. Taking advantage of enzymatic reactions, PICACHU can not only be used for imaging neuronal activities with high spatiotemporal resolution, but also allow proteomics characterizations of activated neuronal ensembles. I will show the design and

development of PICACHU and its potential applications.
References:1.Wang, W. *et al.* A light- A nd calcium-gated transcription factor for imaging and manipulating activated neurons. *Nat. Biotechnol.* **35**, 864–871 (2017).2.Lee, D. *et al.* Temporally precise labeling and control of neuromodulatory circuits in the mammalian brain. *Nat. Methods* **14**, 495–503 (2017).3.Fosque, B. F. *et al.* Labeling of active neural circuits in vivo with designed calcium integrators. *Science (80-.).* **347**, 755–760 (2015).

Disclosures: G. Zhou: None. **W.W. Wan:** None. **W. Wang:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.12

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

Support: Howard Hughes Medical Institute

Title: Optimization of genetically encoded voltage and calcium indicators for in vivo imaging: GENIE Project Team updates

Authors: *J. P. HASSEMAN[1], A. S. ABDELFATTAH[3,4], B. J. ARTHUR[2], J. D. COX[2], C. GUO[2], V. JAYARAMAN[2], I. KOLB[2], W. KORFF[1], A. K. LEE[2], J. LEE[2], L. L. LOOGER[5], C. LOPEZ[2], M. PACHITARIU[2], D. REEP[1], E. R. SCHREITER[2], N. P. SPRUSTON[2], K. SVOBODA[6], A. G. TEBO[2], G. C. TURNER[2], A. K. TSANG[1], G. TSEGAYE[1], T. WANG[2], Y. ZHANG[2], J. ZHENG[1];
[1]GENIE Project Team, [2]Janelia Res. Campus, HHMI, Ashburn, VA; [3]Carney Inst. for Brain Sci., [4]Dept. of Neurosci., Brown Univ., Providence, RI; [5]UC San Diego, HHMI, San Diego, CA; [6]Allen Inst., Seattle, WA

Abstract: Genetically encoded voltage indicators (GEVIs) have continued to evolve and extend functional imaging of *in vivo* neuronal populations. Recently, several improved Ace2 rhodopsin-based GEVIs have been independently reported to have improved sensitivities. Our chemigenetic indicator, Voltron2, demonstrates a 63.8% increase in 1AP sensitivity and three-fold better sub-threshold membrane potential response. Using an automated patch-clamp system, we performed a head-to-head comparison of Voltron2 and positive-going Positron (Abdelfattah, A, 2020), to the best-in-class fluorescent protein-based GEVIs. Voltron2 shows comparable responses to Ace-mNeon2 in detecting depolarized states but outperforms in sub-threshold sensitivity. For positive-going variants, pAce was the most sensitive, followed closely by a new variant ("Positron2"), which is 2X more sensitive than Positron.
The three jGCaMP8 sensors (s, m and f) represent our next-generation suite of genetically encoded calcium indicators. These sensors exhibit faster kinetics (~3 fold shorter rise time) than jGCaMP7f and higher sensitivity (2X higher 1AP SNR) than jGCaMP7s. We have created and characterized several transgenic mice using jGCaMP8m and 8s to enable stable, long-term

expression for *in vivo* imaging applications. A tetO-jGCaMP8s x *CaMKIIa*-tetTA mouse had similarly fast kinetics and sensitivity compared to AAV-infected brain regions. The *tetO*-jGCaMP8s mouse shows ~5x increased SNR compared to *tetO*-GCaMP6s in a fast visual stimulus presentation protocol. In parallel, we generated several knock-in strains at the TIGRE locus that co-express jGCaMP8s (or 8m) and tetTA in a Cre-dependent manner. These 3rd generation TIGRE lines enable targeting of genetically and anatomically-defined neuronal subpopulations and transcriptional amplification of the GCaMP indicator. Both jGCaMP8s and jGCaMP8m mice displayed fast kinetic responses. Finally, by attenuating *tetTA* translation, we were able to avoid previously reported issues associated with tetTA overexpression; offspring from pan-excitatory and pan-inhibitory crosses displayed no signs of perinatal lethality or neurodegeneration. These mouse lines will be deposited at the Jackson Laboratory.

Disclosures: **J.P. Hasseman:** None. **A.S. Abdelfattah:** None. **B.J. Arthur:** None. **J.D. Cox:** None. **C. Guo:** None. **V. Jayaraman:** None. **I. Kolb:** None. **W. Korff:** None. **A.K. Lee:** None. **J. Lee:** None. **L.L. Looger:** None. **C. Lopez:** None. **M. Pachitariu:** None. **D. Reep:** None. **E.R. Schreiter:** None. **N.P. Spruston:** None. **K. Svoboda:** None. **A.G. Tebo:** None. **G.C. Turner:** None. **A.K. Tsang:** None. **G. Tsegaye:** None. **T. Wang:** None. **Y. Zhang:** None. **J. Zheng:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.13

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

Support: NIH Grant 5TP50ActDA044121Project04Year5654

Title: Identifying dopamine neuron subtypes recruited in opioid self-administration

Authors: *S. MORISON[1], M. V. CENTENO[1], A. V. APKARIAN[2], R. AWATRAMANI[1]; [1]Northwestern Univ. Feinberg Sch. of Medicin, Chicago, IL; [2]Physiol., Northwestern Univ., Chicago, IL

Abstract: One of the well-documented roles of ventral tegmental area (VTA) dopamine (DA) neurons is reward processing in addiction. In light of the opioid crisis sweeping the nation, and the interaction between morphine and VTA neurons, we are investigating the activation pattern and projections of DA neurons following opioid administration. VTA DA neurons have more than one known function, however, and are involved in disparate circuits. In view of this, our lab has pursued dissection of DA neuron diversity, as defined by RNA expression patterns, as a potential underlying mechanism for the varied function. To gain insight into the specific DA neuron populations which are activated by opioid self-administration, we used a tamoxifen-inducible cFos in a DA-neuron-specific transgenic mouse line. We present preliminary studies identifying VTA DA neuron populations involved in this functional activation. Such studies can

aid in our understanding of distinct addiction circuitry and allow for improved medical intervention design.

Disclosures: S. Morison: None. **M.V. Centeno:** None. **A.V. Apkarian:** None. **R. Awatramani:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.14

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

Support: R21NS122055
1R21DA048252
Stanford Discovery Innovation Award
Stanford Bio-X Seed Grant
Agilent Fellowship
Stanford Bio-X Bowes Graduate Student Fellowship

Title: Non-invasive bioluminescent imaging of kinase inhibition in living animals

Authors: Y. SU[1], ***Y. WU**[1], J. WALKER[2], L. NING[1], M. WESTBERG[1], M. MONJE[1], T. KIRKLAND[2], M. LIN[1];
[1]Stanford Univ., Stanford, CA; [2]Promega Biosci. LLC, San Luis Obispo, CA

Abstract: Aberrant kinase activity drives growth and survival of many tumor types, and specific kinase inhibitors have proven to be effective cancer treatments. There is thus intense interest in developing novel inhibitors for kinases not yet effectively drugged. However, assessing kinase inhibition in vivo in animal models remains a key bottleneck in drug optimization, as current methods are time-consuming, expensive, and allow only one observation per animal subject. Drug optimization is particularly challenging for kinase targets in the brain, as achievable plasma concentrations do not predict brain concentrations. Here, we describe a kinase-modulated bioluminescent indicator (KiMBI) that responds to kinase inhibition in vivo with increased light emission. An ERK-modulated KiMBI expressed in tumor xenografts in living mice specifically reports administration of ERK pathway inhibitors, and allows kinetic measurements of target engagement in the same subjects over time. In addition, KiMBI expressed in the brain discriminates between brain-permeant and -impermeant inhibitors. The KiMBI method thus enables rapid identification of kinase inhibitors with desirable pharmacokinetic and pharmacodynamic properties *in vivo.*

Disclosures: Y. Su: None. **Y. Wu:** None. **J. Walker:** A. Employment/Salary (full or part-time):; Promega Biosciences LLC. **L. Ning:** None. **M. Westberg:** None. **M. Monje:** None. **T. Kirkland:** A. Employment/Salary (full or part-time):; Promega Biosciences LLC. **M. Lin:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.15

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

Support: NRF 2019M3C7A1031278

Title: The influence of delta beat frequency of Temporal Interfering Stimulation on dopamine release in the striatum

Authors: *Y. KWAK[1], S. LIM[1], S. LEE[2], H.-U. CHO[1], J. SIM[1], I. KIM[1], C.-H. IM[1], D. JANG[1]; [1]Dept of Biomed. Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [2]Dept Of Biomed. Engin., Univ. of Minnesota, Minneapolis, MN

Abstract: Temporal Interference stimulation (TIS) is a promising non-invasive brain stimulation method for targeting a specific region in the brain. In this study, we evaluated TIS feasibility by examining the changes in neurochemical signals depending on TIS stimulation. In the first experiment, TIS electrodes were inserted at the striatum of an anesthetized rat and stimulated with the beat frequency (0, 2, 6, 10, 20, 60, 130Hz, intensity: 400uA) and the intensity (100, 200, 300, 400, 500uA, beat frequency: 2Hz). Phasic DA response was monitored every 10 minutes with fast-scan cyclic voltammetry for one hour. TIS was applied for 5 minutes between 30 and 40 minutes after the experiment start. As a result, the phasic DA response was significantly reduced by 40% in only a 2Hz beat frequency. Also, it was observed that the DA response decreased by about 40% and 20% at the TIS intensities of 400uA and 500uA. In the second experiment, four electrodes' position and stimulation intensity on the scalp were optimized for targeting the striatum with TIS computer simulation based on MRI imaging. With 2Hz beat frequency TIS stimulation at the simulated position, it was confirmed that dopamine concentration decreased by about 12% after stimulation. In conclusion, we expect that TIS could be utilized as a non-invasive deep brain stimulation method for neurotransmitter modulation.

Disclosures: Y. Kwak: None. **S. Lim:** None. **S. Lee:** None. **H. Cho:** None. **J. Sim:** None. **I. Kim:** None. **C. Im:** None. **D. Jang:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.16

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

Support: NIH 5R01NS112176-03
the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (no. NRF-2021R1A2B5B02002437).
the National Research Foundation of Korea (NRF) grant funded by the Korean government (MSIT) (no. NRF-2022R1A6A3A13063921).

Title: Development of a three-electrode FSCV system to reduce the influence of electrode contamination for long-term basal DA measurement

Authors: *S. HWANG[1], C. PARK[1], H.-U. CHO[1], J. JANG[2], H. KWON[2], K. H. LEE[3], K. E. BENNET[3], Y. OH[3], H. SHIN[3], I. KIM[1], D. JANG[1];
[1]Dept. of Biomed. Engin., [2]Dept. of Electronic Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [3]Dept. of Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: Long-term measurement of in-vivo basal neurotransmitter concentration is important in neurological research. While previous studies with microdialysis were to measure the slow change of basal neurotransmitter levels, we recently reported a newly developed M-CSWV (Multi-Cyclic Square Wave Voltammetry) technique, which can measure the basal levels of dopamine and serotonin with a higher temporal and spatial resolution (Oh et al., 2018, Shin et al., 2021). However, it is necessary to consider the bio and chemical fouling on the electrode surface during long-term in-vivo measurement since the sensitivity of implanted electrodes decreases harshly over time. To overcome this problem, a previous study reported that the potential shifting due to electrode fouling by FSCV (Fast Scan Cyclic Voltammetry) could be mitigated by using a three-electrode system (Seaton et al., 2020). We extend this idea to the M-CSWV technique, and therefore, developed a three-electrode electrochemical system suitable for M-CSWV. The pre-amplifier system was redesigned for M-CSWV due to the change in the slew rate, frequency response, and noise level. In order to verify the system, a comparative analysis test was performed with the two-electrode electrochemical system in vitro. In addition, the fouling prevention function of the three-electrode system was confirmed with in vivo long-term measurement.

Reference

Oh, Y., Heien, M. L., Park, C., Kang, Y. M., Kim, J., Boschen, S. L., ... & Jang, D. P. (2018). Tracking tonic dopamine levels in vivo using multiple cyclic square wave voltammetry. Biosensors and Bioelectronics, 121, 174-182.
Shin, H., Goyal, A., Barnett, J. H., Rusheen, A. E., Yuen, J., Jha, R., ... & Lee, K. H. (2021). Tonic Serotonin Measurements In Vivo Using N-Shaped Multiple Cyclic Square Wave Voltammetry. Analytical Chemistry, 93(51), 16987-16994.
Seaton, B. T., Hill, D. F., Cowen, S. L., & Heien, M. L. (2020). Mitigating the effects of electrode biofouling-induced impedance for improved long-term electrochemical measurements in vivo. Analytical chemistry, 92(9), 6334-6340.

Disclosures: S. Hwang: None. **C. Park:** None. **H. Cho:** None. **J. Jang:** None. **H. Kwon:** None. **K.H. Lee:** None. **K.E. Bennet:** None. **Y. Oh:** None. **H. Shin:** None. **I. Kim:** None. **D. Jang:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.17

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

Support: NIH 5R01NS112176-03
NRF 2021R1A2B5B02002437

Title: Combination of Fourier transform electrochemical impedance spectroscopy with fast-scan cyclic voltammetry for real-time monitoring of the electrode surface

Authors: C. PARK[1], S. HWANG[1], H.-U. CHO[1], J. JANG[2], H. KWON[2], Y. KWAK[1], H. SHIN[3], Y. OH[3], K. E. BENNET[3], K. H. LEE[3], I. KIM[1], ***D. JANG**[1];
[1]Dept Of Biomed. Engin., [2]Dept Of Electronic Engin., Hanyang Univ., Seoul-City, Korea, Republic of; [3]Dept. of Neurologic Surgery, Mayo Clin., Rochester, MN

Abstract: Fast-scan cyclic voltammetry (FSCV) has been used for the real-time measurement of neurotransmitters. FSCV has the advantage of minimizing tissue damage due to the usage of carbon fiber microelectrode and high temporal resolution (10 Hz) enough to monitor the neurotransmitter release in vivo. However, the electrodes are inevitably contaminated by the biofouling effect in the brain, such as protein adsorption and redox residue of neurotransmitters. Furthermore, the biofouling on the electrode surface induces redox potential shift and loss of sensitivity in the background-subtracted voltammogram of neurotransmitters. In this study, we suggest the combination of Fourier transform electrochemical impedance spectroscopy (FTEIS) with FSCV. FTEIS uses a single step pulse (25 mV amplitude and 10 ms duration in the study) as perturbation waveform potential to obtain whole frequency information. The derivative of step pulse response is assumed as a pseudo-impulse response, containing whole frequency information. Therefore, the Fourier transform of the derivative could be used as impedance information. FTEIS step pulse data was obtained in holding potential between FSCV waveform scans. In this study, the Jackson FSCV waveform, developed to measure serotonin, was used to validate the biofouling-monitoring ability of the FTEIS-FSCV scanning serotonin. The voltammogram of the serotonin solution is expected to show significant sensitivity loss. As a result, we could see a significant correlation between the capacitance change of FTEIS and sensitivity loss caused by the serotonin. In conclusion, the combination of FSCV with FTEIS showed both real-time measurements of neurotransmitters and sub-second monitoring of electrode surface status.

Disclosures: C. Park: None. **S. Hwang:** None. **H. Cho:** None. **J. Jang:** None. **H. Kwon:** None. **Y. Kwak:** None. **H. Shin:** None. **Y. Oh:** None. **K.E. Bennet:** None. **K.H. Lee:** None. **I. Kim:** None. **D. Jang:** None.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.18

Topic: I.04. Physiological Methods

Title: Automated development and characterization of iPSC-derived 3D cerebral organoids with analysis of calcium oscillation activities and high content imaging.

Authors: *O. SIRENKO[1], Z. TONG[2], C. CRITTENDEN[2], A. LIM[3], C. CARLSON[4]; [1]Mol. Devices LLC, San Jose, CA; [2]Mol. Devices, San Jose, CA; [3]Mol. Devices, LLC, San Jose, CA; [4]Fujifilm, Madison, WI

Abstract: 3D neural organoids are a rapidly developing technology that has great potential for understanding brain development and neuronal diseases, and can also be used for testing effects of compounds and neurotoxic agents. There has been significant progress in developing reproducible methods for culturing neural organoids from induced pluripotent stem cells, and there are multiple methods being utilized to generate organoids. In this report we have used iPSC - derived 3D organoids, that were composed from different subsets of iPSC-derived neurons and astrocytes at ratios and compositions mimicking specific regions of brain (forebrain or midbrain). We describe methods for automated culture and monitoring of organoids composed from different types of iPSC-derived neural cells. Also we characterized functional neuronal activity by recording and analyzing Ca^{2+} oscillations. For detection of functional activities calcium oscillations were recorded and analyzed with peak analysis software. 3D microtissues were analyzed using confocal imaging and characterized by expression of cell-specific markers. For the functional characterization, we have tested a set of 20 compounds including neuromodulators with known mechanisms of action affecting GABA, NMDA, and dopamine targets, and also substances with known neurotoxicity effects. The observed changes in oscillation patterns were consistent with expected mechanism of actions of respective compounds. Advanced biological system of 3D neural organoids composed of different neural cells, paired with high-content imaging and complex analysis of calcium oscillations and metabolic markers demonstrates a promising, biologically-relevant system for testing the effect of pharmaceutical drugs or toxic compounds.

Disclosures: **O. Sirenko:** A. Employment/Salary (full or part-time):; full time, Molecular Devices, LLC. **Z. Tong:** A. Employment/Salary (full or part-time):; full time, Molecular Devices, LLC. **C. Crittenden:** A. Employment/Salary (full or part-time):; full time, Molecular Devices, LLC. **A. Lim:** A. Employment/Salary (full or part-time):; full time, Molecular Devices, LLC. **C. Carlson:** A. Employment/Salary (full or part-time):; full time, Fujifilm.

Poster

087. Sensors and Probes for Understanding Brain Function

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 087.19

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

Title: Brain mechanisms in cancer associated cachexia

Authors: *D. KALTENECKER[1], R. AL-MASKARI[2], M. NEGWER[2], L. HOEHER[2], F. KOFLER[3], S. ZHAO[6], M. I. TODOROV[7], J. PAETZOLD[4], B. WIESTLER[5], J. GEPPERT[1], P. MORIGNY[1], M. ROHM[1], B. MENZE[4], S. HERZIG[1], M. BERRIEL DIAZ[1], A. ERTÜRK[2]; [1]Inst. for Diabetes and Cancer, [2]Inst. for Tissue Engin. and Regenerative Med., Helmholtz Munich, Munich, Germany; [4]Dept. of Computer Sci., [5]Dept. of Diagnos. and Interventional Neuroradiology, [3]Tech. Univ. of Munich, Munich, Germany; [6]Inst. For Stroke and Dementia Res. (ISD), Muenchen, Germany; [7]Inst. for Stroke and Dementia Res., Klinikum der Univ. München, Munich, Germany

Abstract: Cancer alters diverse metabolic activities in the body. Cancer-associated cachexia (CAC) is a prominent one characterized by loss of body weight via muscle and adipose tissue wasting. It negatively affects cancer treatments and is responsible for up to 40% of cancer deaths, highlighting the urgent need for novel treatment options. While the brain contributes to cachexia development by promoting anorexia, the neuronal circuits that govern CAC establishment and progression remain poorly understood. Here, we studied changes in neuronal activity in brains of tumor-bearing mice (weight-stable vs. CAC). To this end, we performed whole-brain cFos labeling using a modified SHANEL protocol (Zhao⋯Ertürk, Cell, 2020). After light-sheet imaging of whole mouse brains, we identified cFos+ cells automatically. Subsequent registration to Allen brain atlas yielded in the identification of brain regions with significant alterations.We observed increased neuronal activity in multiple brain areas in mice transplanted with a cancer cell line that does not induce weight loss (weight-stable mice). These include the lateral hypothalamic nucleus involved in regulating feeding behavior and cortical regions, which were not implicated in metabolic activities before. Interestingly, increased cFos expression in these brain regions disappeared in cachectic animals, suggesting that their overactivation is involved in blocking CAC in weight-stale mice. Thus, our findings highlight novel brain regions that can be targeted to ameliorate weight loss in cancer as novel CAC treatment options.

Disclosures: D. Kaltenecker: None. **R. Al-Maskari:** None. **M. Negwer:** None. **L. Hoeher:** None. **F. Kofler:** None. **S. Zhao:** None. **M.I. Todorov:** None. **J. Paetzold:** None. **B. Wiestler:** None. **J. Geppert:** None. **P. Morigny:** None. **M. Rohm:** None. **B. Menze:** None. **S. Herzig:** None. **M. Berriel Diaz:** None. **A. Ertürk:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.01

Topic: I.07. Data Analysis and Statistics

Title: SimplyFire: open-source software for analyzing electrophysiological recordings

Authors: *M. MORI[1], A. ROSKO[1], J. FARNSWORTH[1], P. BROOMANDKHOSHBACHT[2], P. HAGHIGHI[1];
[1]Buck Inst. for Res. on Aging, Novato, CA; [2]Univ. of San Francisco, San Francisco, CA

Abstract: We have developed open-source software for neuroscientists to analyze electrophysiology recordings. Named SimplyFire, the software gives the users the flexibility to analyze continuous digital recordings using an interactive graphical user interface. The software features a simple plug-in structure that allows users to create and deploy various electrophysiology analysis tools. SimplyFire is prepackaged with tools commonly used to analyze electrophysiological recordings, such as noise filtering, trace averaging, mEPSC analysis, and figure exporting. We will present in detail the algorithm behind the mEPSC analysis tool, which allows the user to analyze spontaneous miniature synaptic events and accurately measure amplitude, decay constant, and rise time. The accuracy of the algorithm was tested against computer-generated traces, in which the true values of the events were known. SimpleFire is distributed under the GPLv3.0 license.

Disclosures: M. Mori: None. **A. Rosko:** None. **J. Farnsworth:** None. **P. Broomandkhoshbacht:** None. **P. Haghighi:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.02

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

Support: Important: Up to now, in order to keep independent decision making, I have only used personal founds and resources.

Title: Study using Python/Excell and chronobiosymmetry of exotic states between sleep and activation: sleep spindles, alpha activity and dendritic Ca2+ in aging and Alzheimer's disease

Authors: *J. F. GOMEZ-MOLINA;
Independent Activities of Neuro-science/Engineering and Philosophy IAN(S,E,P), Medellin, Colombia

Abstract: INTRODUCTIÓN. Sleep spindles are oscillations (9-15 Hz) of 0.4-4 sec. Based on the methods of theoretical physics and engineering, we have proposed an hypothesis (1) : cortical modules can "relax/rest" if they mimic these behavioral and EEG-group of states of the whole brain. Here we analyze computationally some electrophysiological recordings to explore new symmetries. METHODS. Reconstruction of parameters and main motifs using computer programs in Python and Excell. Group Theory. Chronobiosymmetry approach (2). RESULTS. Spindles, posterior alpha activity, and calcium levels were analyzed from previous EEG/MEG data. They are generated computationally using sinusoids, Gabor functions and probabilistic methods (Fig. 1). Waxing and waning spindle events (these features were not explicitly modeled)

emerge spontaneously from most of the simulations. CONCLUSIONS. 1. Sleep spindles are a good scenario for Chronobiosymmetry and, with EEG-alpha, they might represent "exotic" activation states. 2. Simple equivalences are not realistic. There are some difficulties that need to be solved using algebra of group theory, subgroups, substates and corrections to putative symmetry groups. It is also possible that some empirical invasive results might be incorrect or misinterpreted. 3. However, we suggest that the potential benefits of a reverse engineering, mathematical neuroethology and chronobiosymmetry perspective might overweight the reasons to ignore this path. 4. Conservation of net activity might represent a useful approach to many subsystems, in particular the role of intracellular calcium and electric activation in Alzheimer's disease and sleep. 5. Althought this method has been called a "precarious and risky path" it might be an important teaching tool and solve serious philosophical problems of delicate systems. REFERENCES. (1) Gómez and Lopera IEEE 1998, 1999 and after. (2) Gómez SfN-J 2022 A teaching-lab/research-language and non-invasive unification program for engineering, physics and neuroscience based on chronobiosymmetry: from electrons to ant swarms.

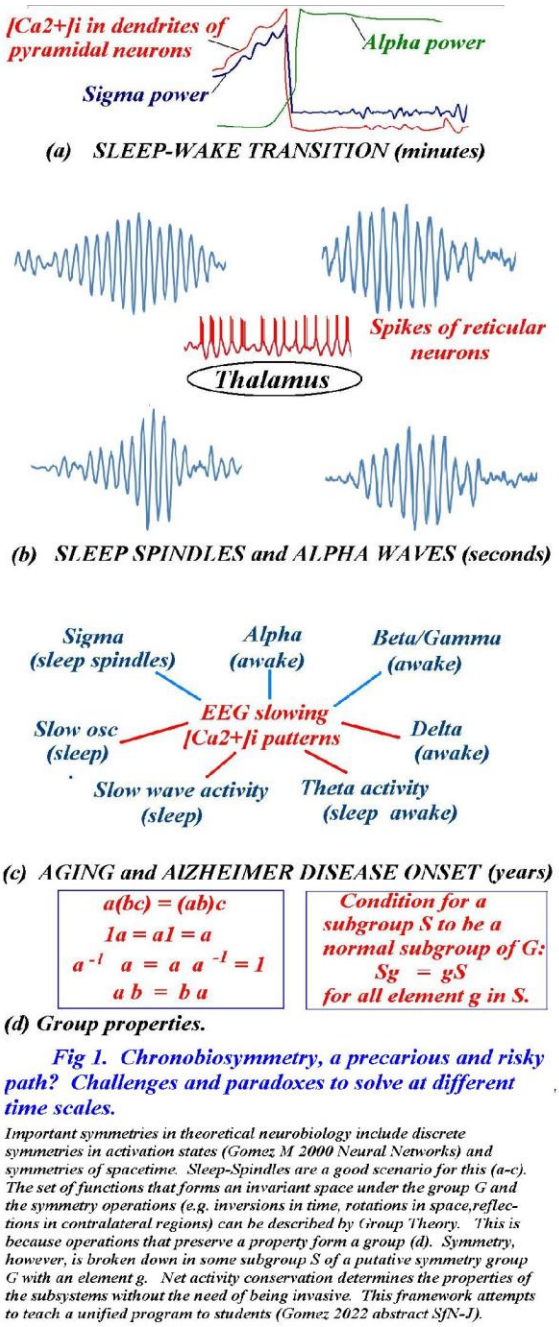


Fig 1. Chronobiosymmetry, a precarious and risky path? Challenges and paradoxes to solve at different time scales.

Important symmetries in theoretical neurobiology include discrete symmetries in activation states (Gomez M 2000 Neural Networks) and symmetries of spacetime. Sleep-Spindles are a good scenario for this (a-c). The set of functions that forms an invariant space under the group G and the symmetry operations (e.g. inversions in time, rotations in space,reflections in contralateral regions) can be described by Group Theory. This is because operations that preserve a property form a group (d). Symmetry, however, is broken down in some subgroup S of a putative symmetry group G with an element g. Net activity conservation determines the properties of the subsystems without the need of being invasive. This framework attempts to teach a unified program to students (Gomez 2022 abstract SfN-J).

Disclosures: J.F. Gomez-Molina: None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.03

Topic: I.07. Data Analysis and Statistics

Support: NCS-FO: 1835268/1834994
CBMM NSF STC award CCF-1231216

Title: Neurodecoder: an open-source package for reproducible neural population decoding

Authors: *E. M. MEYERS[1,2,3], E. LOY[1], X. FANG[3];
[1]Statistics and Data Sci., Yale Univ., New Haven, CT; [2]Ctr. for Brains, Minds and Machines, MIT, Cambridge, MA; [3]Cognitive and Information Sci. Dept., Univ. of California Merced, Merced, CA

Abstract: Neural population decoding is a powerful data analysis method for understanding how the brain processes information (Meyers and Kreiman, 2011). However, running a decoding analysis is significantly more complex than running traditional neural analyses. To make it easier to run decoding analyses, we have previously developed the Neural Decoding Toolbox (www.readout.info) in MATLAB (Meyers, 2013). While the Neural Decoding Toolbox has been successfully used by a significant number of research groups (e.g., Schmitt et al. Nature, 2017, Kamiński et al, Neuron 2020, Dotson and Yartsev, Science, 2021), there are additional ways to make running decoding analyses even simpler.
In this work we introduce the NeuroDecodeR package, which runs population decoding analyses using the R programming language. Like the Neural Decoding Toolbox, the NeuroDecodeR package is designed in a modular fashion allowing research to try different classifiers and preprocessing algorithms, and to run temporal cross-classification and generalization analyses. The NeuroDecodeR package also makes it easy to develop new methods for quantifying decoding results, to log/save results, and to run code in parallel. Advantages of using the R programming language is that it is free, and there is a rich ecosystem in R for creating reproducible data analysis documents. We have also been developing a "Shiny" web application (NeuroShiny) that allows researchers to run reproducible decoding analyses by selection options through a graphical user interface. This platform generates pdfs that contain code and plots of the results (by knitting an automatically generated R Markdown file into a pdf), which will further simplify and speed up the time it takes to analyze data.

Disclosures: E.M. Meyers: None. **E. Loy:** None. **X. Fang:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.04

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

Support: Novo Nordisk Foundation Laureate Grant NNF15OC0014186
Lundbeck Foundation R345-2020-1769
Faculty of Health and Medical Sciences, University of Copenhagen
Faculty of Science, University of Copenhagen

Title: Deep-spike: automated spike sorting of high resolution electrophysiology data is enabled by self-supervised deep learning

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Abstract: Extracellular recordings in freely moving animals are essential in understanding how single neurons contribute to the regulation of body functions, like locomotion, and how populations of neurons interact to form functional circuitries. Currently, advancements in electrophysiology probes are enabling acquisition of long duration recordings at high resolution (>50kHz) and high channel count. These probes are resulting in large volumes of data requiring specialised methods that are able to process such big data. Many existing spike sorting methods are unable to reliably handle these vast amounts of data; spike sorting and clustering is especially challenging for long recordings with high unit counts per channel.
In this work, we present a novel automatic signal processing tool capable of spike detection and sorting which is inspired from recent advancements within Self Supervised Deep Learning (SSL). SSL methods belong to the class of unsupervised deep learning methods that are used to obtain robust and meaningful vector representations of raw input data without the need of any labelled supervision (user inputs). The learned representations obtained from the SSL methods can capture intrinsic properties of the data that might be unique to different classes of data. In the case of electrophysiology data, SSL can be first used to distinguish neuronal spikes from background noise. The learned spike representations focus on spike signatures that could be unique to each unit; in essence, learning templates specific to units. These templates can further be used to detect and cluster spike events corresponding to a single unit, when recordings contain multiple units per channel.
The SSL based method will be presented as a complete framework - DeepSpike - consisting of pre-processing and post-processing options. The framework has the capability to progressively detect and cluster spikes that have high signal-to-noise ratio (SNR) to lower SNR in an automatic manner. The DeepSpike framework reports the confidence scores for each spike belonging to different units. Further, the framework also has options for the user to provide expert knowledge - by interacting and updating relevant parameters - to obtain more congruent spike sorting. Finally, the DeepSpike framework is evaluated on multiple synthetic and actual recordings, and is able to detect spikes from background noise with high specificity and cluster them into plausible units.

Disclosures: **R. Selvan:** None. **M.A. Andersen:** None. **O. Kiehn:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.05

Topic: I.07. Data Analysis and Statistics

Support: Simons Foundation
Flatiron Institute

Title: A collaborative effort to standardize, maintain, and develop spike-sorting tools through SpikeInterface

Authors: S. GARCIA[1], J. BOUSSARD[2], M. CHAPUT[3,4], F. CHAURE[5], Y. CHUANG[6], B. DICHTER[6], R. GREENE[7], M. H. HENNIG[7], C. L. HURWITZ[2], K. LEE[8], J. MAGLAND[9], L. M. PANINSKI[2], V. PREVOSTO[10], H. G. REY[11,12], J. H. SIEGLE[13], J. SOULES[9], J. SPRENGER[14], A. STEFAN[7], E. VAROL[2], C. WINDOLF[2], O. WINTER[15], ***P. YGER**[16,17], A. P. BUCCINO[13];
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Abstract: Spike sorting is an important processing step in the analysis of electrophysiology data in which single-neuron activity is extracted from extracellularly recorded voltage traces. Although substantial research and software development efforts over the last decades have generated a plethora of methods and tools, spike sorting remains an unsolved problem and lacks standardization or benchmark. The same is true for the steps surrounding spike sorting, such as loading data from many different formats, pre- and post-processing, visualization, and quality control.
SpikeInterface is an open-source Python package introduced in 2020 that aims to centralize and standardize spike sorting analysis, and is now widely adopted by the neuroscience community. The maturity and breadth of the SpikeInterface project calls for a novel collaborative and centralized approach to its development and maintenance. To this end, we organized the first Spike Sorting Hybrid Hackathon event at the Flatiron Institute in New York in June 2022, which resulted in significant progress in many areas.
At the infrastructural level, we worked on improving SpikeInterface documentation and user experience. For example, we now provide precise definition, implementation details, references, and suggested usage for many quality metrics offered by SpikeInterface. A second project aimed at creating, testing, and sharing container images for all available spike sorters (including MATLAB-based sorters) so that they can be run via Docker or Singularity without separate installation steps and licenses. Another effort was made to create efficient and intuitive visualization tools for raw data and to integrate other existing visualization ecosystems into SpikeInterface.Finally, we worked on porting spike sorting methods and related tools into SpikeInterface, including pre-processing (e.g. *destriping* and *DeepInterpolation*), motion

correction, clustering, and template-matching. A parallel effort was also made to implement benchmarking tools specifically designed for different steps of the spike sorting pipeline. The success of the hackathon demonstrates the power of collaborative and community-based development of scientific software and establishes SpikeInterface as a widely-embraced project that contains a variety of state-of-the-art and maintained tools to process electrophysiology data.

Disclosures: **S. Garcia:** None. **J. Boussard:** None. **M. Chaput:** None. **F. Chaure:** None. **Y. Chuang:** None. **B. Dichter:** None. **R. Greene:** None. **M.H. Hennig:** None. **C.L. Hurwitz:** None. **K. Lee:** None. **J. Magland:** None. **L.M. Paninski:** None. **V. Prevosto:** None. **H.G. Rey:** None. **J.H. Siegle:** None. **J. Soules:** None. **J. Sprenger:** None. **A. Stefan:** None. **E. Varol:** None. **C. Windolf:** None. **O. Winter:** None. **P. Yger:** None. **A.P. Buccino:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.06

Topic: I.07. Data Analysis and Statistics

Title: Compression strategies for large-scale high-density electrophysiology data

Authors: *A. P. BUCCINO, J. SIEGLE, D. FENG, K. SVOBODA;
Allen Inst. for Neural Dynamics, Seattle, WA

Abstract: With the rapid adoption of high-density neural probes with thousands of electrodes used to record neural activity both in vivo and in vitro, the amount of raw data produced as a field is growing at an unprecedented pace. For example, a 1 hour recording with a Neuropixels probe generates over 80 GB of raw data, and commercial in vitro devices with over 4000 electrodes recorded simultaneously produce around 600 GB/hour. Therefore, there is a pressing need for the neurophysiology community to find a compression strategy to reduce the amount of actual data that needs to be stored to disk.
Here, we present a comprehensive survey of several compression strategies for large-scale electrophysiology data. We utilized both experimental and simulated recordings from Neuropixels 1.0 (NP1) and 2.0 (NP2) and benchmarked the compression performance of both lossless and lossy approaches. For lossless compression, we reported the compression ratio (CR), compression speed and decompression speed for both general-purpose codecs (implemented by the Blosc library and saved in Zarr format) and two lossless audio codecs (FLAC and WavPack). In all cases, we also benchmarked several compression parameters, including pre-shuffling options, block sizes, and compression levels. We found that audio codecs generally achieve higher compression ratios than general-purpose codecs, with a maximum CR of 3.4 for NP1 and 2.3 for NP2. Among the codec built in to Blosc, we found that the best overall performance is achieved using the Z-standard codec. For lossy compression, we looked at two strategies: *bit truncation* and *WavPack hybrid* mode. In addition to reporting compression ratio, we used the simulated ground-truth recordings to assess whether lossy algorithms affect spike sorting results

and preserve waveform shapes. We found that bit truncation can be used to reach a CR between 5-10 without affecting spike sorting results and only slightly distorting spike waveforms. The WavPack hybrid codec can also successfully compress up to a CR of 7 without visible degradation of spike sorting results, and with an overall better preservation of waveforms. In conclusion, our results will help electrophysiology labs choose the best existing lossless and lossy compression stategies for their needs. We also establish a set of benchmarks that can be used to test future compression strategies that will allow the electrophysiology community to more efficiently handle large-scale datasets and reduce data storage costs over the long run.

Disclosures: **A.P. Buccino:** None. **J. Siegle:** None. **D. Feng:** None. **K. Svoboda:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.07

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

Support: Simons Foundation (687458, N.A.S.)
Wellcome Trust
Washington Research Foundation (D.B.)

Title: Multi-probe targeting for Neuropixels using interactive 3D visualizations and simulated electrophysiology

Authors: *D. BIRMAN[1], Y. BROWNING[2], .. INTERNATIONAL BRAIN LABORATORY[3], J. H. SIEGLE[2], N. A. STEINMETZ[1];
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Abstract: Targeting deep brain structures during electrophysiology experiments requires intensive training and expertise. Even with experience, the time constraints of typical experimental setups often make it difficult to know whether an electrode is placed precisely in a target location and this complexity scales with the number of simultaneous electrodes used in a recording. To solve this problem we have developed a virtual targeting environment for Neuropixels probes to allow experimenters to pre-plan recordings involving complex spatial geometry. Our 3D environment is intuitive and runs in a web browser, reducing the need for one-off software tools created by individual researchers. When needed, our environment can be extended to take into account specific constraints imposed by rig design and implant or probe geometry. In addition to the targeting environment we leverage a large electrophysiology dataset with over five hundred insertions to further improve accuracy. We use this dataset to adjust our coordinate system to account for differences between the mouse common coordinate framework and the *in vivo* mouse brain. These improvements reduce targeting error by up to 7% in position and angle offset. When anatomical MRI scans are available for individual mice, the common

coordinate framework model can be further warped to reflect the unique brain geometry of each subject. To help users target deep brain structures and reduce uncertainty about probe depth our tool can generate predicted electrophysiological signatures during live recordings. Combined, these advances in visualization and live recording tools lower the barrier to entry for performing complex multi-probe recordings in mice.

Disclosures: D. Birman: None. **Y. Browning:** None. **.. International Brain Laboratory:** None. **J.H. Siegle:** None. **N.A. Steinmetz:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.08

Topic: I.07. Data Analysis and Statistics

Support: HHMI Janelia Research Campus
NIH Grant U01NS115587

Title: Neuron tracking with chronic Neuropixels 2.0 recordings from mouse visual cortex

Authors: *X. YUAN[1,2], J. COLONELL[2], A. S. CHARLES[1], T. D. HARRIS[2,1];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]HHMI Janelia Res. Campus, Ashburn, VA

Abstract: Accurate identification and tracking of the same neurons across multiple days is crucial for studying how neurons change their responses over time during learning and adaptation. In extracellular electrophysiology recordings, identifying the same neurons is complicated by movement of the tissue relative to the recording sites (drift), loss of signal from some neurons (dropout), and the limited spatial information provided by electrode arrays. Neuropixels, a new class of high-density electrode arrays, offers improved spatial information.. Steinmetz et al. (2021) tracked visual cortex neurons in chronic Neuropixels recordings, with "ground truth" established by the stable visual receptive fields to check assignment of unit pairs. Their unit tracking method corrected drift across days by estimating the vertical shift between two recordings by maximizing correlation between similar units detected in a pre-sort, and applying a shift equivalent to an integer number of probe site spacings. The corrected recordings were concatenated and sorted with Kilosort 2 as a single dataset, which automatically pairs units across the two recordings. This method achieves high accuracy, but is inconvenient for N>2 datasets, potentially very slow for many datasets, or if run as is, requires running and aligning multiple pairs, and may be error prone under large activity changes. We evaluate tracking accuracy using varying amounts of information about the individual units, with the goal of creating a tracking algorithm that is robust against change and easily scaled to tracking neurons across many (N >> 3) recordings. Using the data from Steinmetz, et al (2021), we spike sort individual recordings data using Kilosort 2.5. Individual spikes from the sort are localized in 3D

using the method of Boussard et al. (2021). The position of each unit is determined from its spikes. We then use the Earth Mover's Distance (EMD) to compare unit locations across days which preserves the geometrical relation between units and is robust against loss or movement of individual units. We report tracking accuracy using only the EMD-based alignment between the identified unit locations. We further report results using various waveform characteristics, e.g., amplitude and width, to weigh the EMD alignment; and results combining the EMD tracking with similarity metrics between the multi-electrode waveform templates (also computed as an EMD). We evaluate metrics for estimating the likelihood that putative unit matches are correct, including simple metrics of unit quality, EMD similarity of the average waveform, and overlap of the matched units in feature space.

Disclosures: X. Yuan: None. **J. Colonell:** None. **A.S. Charles:** None. **T.D. Harris:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.09

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

Support: NSF GRFP
NSERC Award #PGSD3-532647-2019

Title: Recovering Corrupted Brain Data via Deep Neural Imputation

Authors: *S. TALUKDER[1], J. J. SUN[1], M. K. LEONARD[2], B. W. BRUNTON[3], Y. YUE[1]; [1]Caltech, Pasadena, CA; [2]Neurolog. Surgery, UCSF, San Francisco, CA; [3]Biol., Univ. of Washington, Seattle, WA

Abstract: Multielectrode neural recordings play an integral role for neuroscientists, neuroengineers, and clinicians studying the brain. Regardless if electrophysiology data comes from neuropixels, Utah arrays, or electrocorticograms there is almost always neural signal corruption arising from causes such as: stimulation artifacts, experimental noise, or participant movement to name a few. In the field of neuroscience, experimentalists often discard these corrupted neural signals, thereby diminishing the amount of data available for downstream neuroscientific analyses. We recently developed the *Deep Neural Imputation (DNI) Framework* to solve this problem by recovering corrupted electrophysiology data. The DNI Framework is compatible with both linear and nonlinear models, and here we explore its reconstruction and imputation capabilities with a linear nearest neighbor approach and two deep generative neural network autoencoders. All of our models are day-generalizable, meaning they can recover neural data on days in which they were not trained. Additionally, our DNI models operate in either participant-specific or joint-participant regimes. Significantly, a single joint-participant model successfully processes multiple participants even when their electrodes are in different hemispheres. Our day-generalizable and joint-participant capabilities make DNI widely

applicable to many experimental neuroscience subfields. Here we explore DNI across 12 human participants implanted with electrocorticograms who behave naturally across 100s of recording hours. To justify DNI's usefulness to the neuroscience community we present DNI's local field potential time series reconstructions, frequency based power spectral content reconstructions, and neural decoding task performance recovery.

Disclosures: S. Talukder: None. **J.J. Sun:** None. **M.K. Leonard:** None. **B.W. Brunton:** None. **Y. Yue:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.10

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

Title: A Partial Information Decomposition Analysis of Grid Cell Encoding

Authors: *A. K. FELDMAN[1,4], P. VENKATESH[5], D. J. WEBER[2,1,4], P. GROVER[3,1]; [1]Neurosci. Inst., [2]Mechanical Engin., [3]Electrical & Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA; [4]Ctr. for Neural Basis of Cognition, Pittsburgh, PA; [5]Mindscope Program, Allen Inst. For Brain Sci., Seattle, WA

Abstract: *Objective:* To better understand neural representations, we must develop methodologies to study codes as expressed by the spiking activity of neurons. We explore this question from an information-theoretic perspective to investigate the firing of grid cells in the entorhinal cortex. Unlike other spatially tuned neurons, a given grid cell will have multiple locations at which its firing rate is high in a typical rodent experimental paradigm, which may overlap with other grid cells. This suggests the neural representation of the environment constructed by grid cells may be rich with redundant and synergistic information, indicating these neurons would be good candidates for a Partial Information Decomposition (PID) analysis. PID deconstructs mutual information about some random variable (here, fine-grained location) into unique, redundant and synergistic information as represented by other random variables (outputs of each grid cell). *Methods:* We first employ a conditional-entropy method to verify the results of our PID analysis in the case of fine-grained information about location. On both fine-grained and crude information, we then utilize PID analysis on increasing cardinalities of grid cell sets, and quantify the average unique, synergistic and redundant values at each cardinality. We also perform PID analysis on Hamming coded simulated grid cells, as certain Hamming codes are known to be efficient error-correcting codes. *Results:* Using the conditional-entropy measure, we observe that, on average, there is redundancy in representing location across grid cells that appears to increase gradually, but consistently, as the number of grid cells is increased, suggesting that the encoding performed by grid cells is not pairwise independent (unlike in efficient coding). As the cardinality of the cells being conditioned on increases, the conditional entropy of fine-grained location, as represented by real grid cells, falls more gradually than in

efficiently (e.g. Hamming) coded grid cells. We show that this conditional entropy analysis is equivalent to a PID analysis on representing the fine-grained location; we observe that the conditional entropy term is the unique information, and the synergistic information is negligible. In contrast, PID analysis on crude location information (left vs. right halves of the environment) becomes completely synergistic at all analyzed grid cell cardinalities. *Conclusions:* Real grid cell encoding, from our analysis, appears not to adhere to efficiency in the Shannon theoretical sense. This work illustrates how PID analysis, applied to different messages, can provide new insights about representations in the brain.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.11

Topic: I.07. Data Analysis and Statistics

Support: NIMH R01MH11559
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The JPB Foundation

Title: A state-space random effects point process model for estimating spike rate functions

Authors: *J. CORREA MENENDEZ[1], E. N. BROWN[2,4], E. K. MILLER[3];
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[4]Dept. of Anesthesia, Critical Care, and Pain Med., Massachusetts Gen. Hosp., Boston, MA

Abstract: A common approach for analyzing spike train data in stimulus-response experiments is to estimate spike rates relative to the onset of the stimulus. These experiments typically involve collecting repeated measurements from the same subject across several trials and sessions, and performing the same experiment across multiple subjects. Accounting for these different sources of variability is important in order to accurately estimate the population response. However, current spike rate estimation methods only allow for estimating trial-level, session-level or group-level spike rates, neglecting the variability between levels. We develop a state-space random effects point-process (SSREPP) model to estimate population and subject-level spike rate functions from multi-subject, multi-session and multi-trial spike train data. The statistical model allows for including stimulus information, and for quantifying the effect of the stimulus on the spiking propensity. Our approach provides goodness-of-fit assessment and a Monte Carlo algorithm for computing confidence intervals for population and subject-level spike rate functions. Our Monte Carlo algorithm also provides a framework for performing uncertainty

quantification on model parameters. We assess the accuracy of our SSREPP model in simulation. We apply our SSREPP model to data from previously published stimulus-response experiments to characterize the effect of: 1. thalamic stimulation on cortical spiking from ten recording sessions of two non-human primates under propofol-induced unconsciousness; 2. a repetitive whisker stimulus on simultaneous recordings from individual thalamic barreloids in the rat somatosensory whisker/barrel system across 50 trials and 14 rodents; 3. ipsilateral photoinhibition of the anterior lateral motor cortex during a pole location discrimination task recorded across different numbers of trials and sessions and 7 rodents. By using a hierarchical Bayesian framework, our SSREPP model pools information across levels and can estimate population-level spike rates that describe both population-level and subject-level data.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.12

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

Support: Veritas Fund

Title: Event detection performance in human intracranial microwire recordings

Authors: J. T. WIXTED[1], ***P. N. STEINMETZ**[2];
[1]UC San Diego, UC San Diego, La Jolla, CA; [2]NeurTex Brain Res. Inst., NeurTex Brain Res. Inst., Dallas, TX

Abstract: Four spike sorting techniques have been used to identify the firing activity of single neurons in human intracranial microwire recordings. These techniques have a low level of agreement (AMIall ranging from 0.041 - 0.16 on a 0-1 scale), underscoring the need to better understand the underlying neurophysiological events.
The first step in each technique is the detection of events, which are then sorted into clusters of similar waveform shape representing the activity of putative single neurons. Although true performance is unknown, simulating events in realistic noise followed by analysis of detection performance can provide insight into neural activity that is correctly and falsely detected by different spike sorting techniques.
In this study, we simulated extracellular waveforms produced by a single neuron firing in the presence of noise colored to have a power spectrum typical of human intracranial microwire recordings (standard deviation of 5 μV in the passband from 300-3000 Hz). The firing rate was varied from 0.05 Hz to 50 events/s and the waveform amplitude was varied from 0 to 150 μV. Each detected event was classified as being either a hit or a false positive (FP) depending on whether the time of its occurrence was within 1 ms of a simulated event.
In general, for both the BML and WaveClus (WC) sorting methods, positive predictive value

(PPV) increased with increasing firing rate. PPV was higher for WC than for BML, consistent with WC using a detection threshold of 4.5 times the standard deviation of recording versus 2.8 times for BML. At the same time, the hit rate using BML was higher than WC for lower waveform amplitudes such as 20 µV. The BML technique showed an unexpected trend of decreasing PPV for higher amplitudes (>55 µV), which may reflect increasing false positives due to the event detector having a finite dwell time. The WC technique showed an unexpected decreasing hit rate and PPV as a function of firing rate for lower amplitudes (~25 µV) which may also be due to the dwell time of its event detector.
Evaluating spike sorting techniques under conditions in which ground truth is known will not only provide an indication of how well they are performing now but will also suggest ways to enhance their performance going forward.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.13

Topic: I.07. Data Analysis and Statistics

Support: FJC is supported by a Peruilh doctoral fellowship, School of Engineering, University of Buenos Aires
SS is funded by NINDS grants (U01 and UH3)
HGR is supported by seed funding from MCW

Title: A new kernel for dynamic experimental paradigms based on single neuron responses in the human brain

Authors: *F. J. CHAURE[1], B. SHOFTY[2], S. A. SHETH[2], H. G. REY[3,2];
[1]Univ. of Buenos Aires, Ciudad Autónoma de Buenos Aires, Argentina; [2]Dept. of Neurosurg., Baylor Col. of Med., Houston, TX; [3]Dept. of Neurosurg., Med. Col. of Wisconsin, Milwaukee, WI

Abstract: The advances in electrophysiology acquisition hardware and computing power allows us to revisit the way in which we design and perform experimental paradigms. This is particularly challenging when electrodes are implanted in the brain of patients with epilepsy for diagnosis purposes. The time to work with the patients is very limited and the time for analyses to search for stimuli to be used in follow up experiments should be minimized (neurons can be lost due to electrode movement, or patients might have seizures, that will prevent from running a follow up experiment).
Here we propose a new kernel that allows us to design experiments where the task will be contingent with the data that is being analyzed online as the subject performs it. First, different APIs allow us to collect online continuous data from the microelectrodes (current

implementation for Blackrock Microsystems and Ripple Neuromed). Two Matlab instances are used, one for delivering the task and another one for collecting and analyzing the data, and they exchange messages and information via a simple UDP protocol. Multicore processing allow some cores to focus on the task, others on data collection, and others on data processing. This way, when enough data has been collected, spike sorting can be launched in the background while other cores remain delivering the task and collecting new data. After a certain time, some statistical method can be used to evaluate the neural activity (e.g., whether a certain stimulus elicits a response from a certain neuron). If such a method is not fully automatic, the task will have a short break (a few seconds), the results will be displayed, and the user can decide what to do next (e.g., marking a stimulus as responsive after looking at its raster plot, which in turn will affect which stimuli will be shown in the subsequent trials).
We used this kernel to implement a dynamic screening with patients implanted with microelectrodes. After presenting 180 pictures 6 times each, the response from each putative unit to each stimulus was ranked with a custom statistical method. Then, the best 40 responses were displayed to the user. We also implemented methods to automatically prevent overclustering and also detect activity associated with putative artefacts, so these cases could be excluded from the ranking. The user could quickly mark the response candidates (while we automatically discarded a number of stimuli that were ranked the worst). Then, new stimuli were added according to the experimental design, particularly, different pictures from the same concept. In ~30 minutes, we were able to show over 300 pictures to the patient, without the need to separate the screening and follow up experiment.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.14

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

Support: NIH Grant R01MH124763

Title: Decoding Human Reward Choices from Intracranial Readings

Authors: *L. PETERS[1], J. OVERTON[1,2], M. STICKLE[4,1], K. MOXON[1], I. SAEZ[3];
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Abstract: Decision-making involves the coordinated activity of multiple brain areas and involves multiple sub-processes including reward evaluation, comparison, and choice. However, disentangling the contribution of individual human brain areas to choice is difficult due to the constraints of non-invasive neural recording methods. Here, we studied neural activity from multiple brain areas in epilepsy patients undergoing intracranial electrophysiological monitoring

(n=20). We recorded from several reward-related areas, including prefrontal and parietal cortices and deep temporal lobe structures, while patients played a risky decision-making task in which they faced a binary choice between a safe bet or a risky gamble for a higher monetary reward with the goal of building a decoding model that could predict patients' choices. We applied different decoding algorithms to time-frequency decomposed neural data from all recorded regions in each patient. First, we decomposed time-frequency data using Principal Components Analysis (PCA). We then applied Linear Dynamic System (LDS) modeling to the first 3 principal components, and sought to decode overt behavioral choices from the resulting LDS latent variables using two different strategies, namely a simple Euclidean Distance (ED) classifier and a Dynamic Time Warping (DTW) classifier which accounts for differences in the timing of neural encoding. Using a leave-one-out paradigm, we achieved 71+/-3% trial-by-trial decoding performance, showing that decoding trial-by-trial choices based on a frequency-specific, region-agnostic strategy is possible. Importantly, we achieved above chance performance in all patients in our dataset, demonstrating that our decoding strategy is robust to variation in electrode placement and behavior across patients. The effect of ED versus DTW was subject-dependent suggesting that variation in the timing of decision encoding across trials is important for some subjects. The latent neural dynamics identify separate attractors for gamble and safe bet trials that appear approximately 500ms before choice that neural trajectories visit multiple times during individual deliberation trials, consistent with alternate evaluation of choice alternatives. These results demonstrate that decoding cognitive states (in this case, choices) from multi-areal intracranial data is possible . Future studies will need to examine the accuracy of more complex (e.g. non-linear) decoders, and to expand these decoding strategies into other brain states.

Disclosures: L. Peters: None. **J. Overton:** None. **M. Stickle:** None. **K. Moxon:** None. **I. Saez:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.15

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

Title: Automatic event and unit curation for spike sorting from a time-domain waveform analysis perspective using an autoencoder

Authors: X. LI, *J. W. REDDY, V. JAIN, M. CHAMANZAR;
Electrical and Computer Engin., Carnegie Mellon Univ., Pittsburgh, PA

Abstract: Spike sorting is a widely-used analysis method to identify single-unit activity in extracellular recordings. The standard solution for assessing spike sorting is manual curation. Extracellular recordings contain signals, spike events that correspond to neural activity, as well as noise and non-spike events that might be wrongly detected by threshold crossing. The

classification of events and units as signal or noise is often subjective and further complicated by a lack of ground-truth information in the extracellular recording. This manual step is difficult to scale with the ever-increasing size and complexity of electrophysiology datasets. Therefore, an automated curation technique is highly desired. We have implemented an automatic curation method based on waveform evaluation by an autoencoder. The model learns features of extracellular spike waveforms to perform dimensionality reduction. The reconstruction error is used to classify noise and spike events. The autoencoder is trained on a simulated dataset generated by the biophysical extracellular simulator MEArec with cell models from layers 4 and 5 of the cortex by the Neocortical Microcircuit Collaboration Portal. We used the Kampff dataset with ground-truth patch-clamp recording and in-house spontaneous datasets, recorded from wild-type mice, for evaluation. The model can separate noise and spike events on the Kampff dataset with 96.6% accuracy. The model can improve current spike sorting pipelines by removing noise events before clustering. We evaluate cluster quality using the interspike interval (ISI) violation rate that measures if a putative neuron has an unrealistically short refractory period, which can indicate that it contains noise events. By filtering out noise events before sorting using MountainSort, the ISI violation rate decreased from 44.7% to 14.5%, indicating improved sorting. The autoencoder is also an alternative to traditional manual curation. Noise units were classified via reconstruction error of the template waveform. The model can also improve the quality of each unit by removing noise events after clustering. By removing noise events from spike-sorted units, variances in each unit can be reduced by 46.3%. In conclusion, we have proposed a new automatic evaluation model that improves the analysis of extracellular recordings from a time-domain perspective instead of the common frequency-domain and statistical perspective. The model is built based on simulated data and performs well on real-world experiments without further modification, proving a high level of generalizability. The presented method can be used as an add-on to current spike sorting pipelines.

Disclosures: **X. Li:** None. **J.W. Reddy:** None. **V. Jain:** None. **M. Chamanzar:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.16

Topic: I.07. Data Analysis and Statistics

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Göran Gustafsson foundation for medical research

Title: Functional imaging of signal conduction dynamics in cortical and spinal axon

Authors: *M. RADIVOJEVIC, A. ROSTEDT PUNGA;
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Abstract: Introduction | Axons are neuronal processes specialised for conduction of action-potentials. Mainly due to technical difficulties to monitor axonal conduction, axonal information processing has been neglected, and axons are classically seen as cables that conduct action-potentials in an all-or-none fashion. Later studies have challenged this view and suggested that axons have much more complex roles than previously thought. Advanced high-density microelectrode arrays (HD-MEAs) have the potential to reveal complex axonal functionalities. These arrays have not yet been fully exploited, arguably due to lack of methods for complex data analysis and visualisation.
Aims | The first part of this project aims at developing methods for automatic tracking and visualisation of signal conduction in mammalian axons. The second part aims at using these methods to compare conduction dynamics in axons of the cortex and spinal cord.
Methods | We cultured primary cortical and motor neurons from rat directly on HD-MEAs with 26,400 densely-packed microelectrodes. Array-wide recording enabled sampling electrical activity from entire neuronal networks. Spike-sorting and spike-triggered averaging techniques enabled to discern activities of individual neurons and to reconstruct spatiotemporal distribution of neuronal signals. Adaptive thresholding technique and customised greedy algorithm were used to detect three-dimensional peaks. Bayes optimal template matching was used to discern neuronal signals from the background noise. Kalman-filter-based algorithm was used to interlink propagating peaks over consecutive timespans and to reconstruct trajectories of discrete axonal paths. Skeletonization of neuronal signals was used to assemble reconstructed paths into a complex axonal arbour - referred to as ‘functional morphology’.
Results | We developed a method for tracking extracellular action-potentials along cortical and spinal axons. Reconstructed trajectories revealed information about waveforms of axonal action-potentials, local conduction velocities and times at which action-potentials arrive at axonal terminals. We next used the method to reconstruct axonal functional morphologies for 50 cortical and 50 motor neurons (total length of 1.02 and 0.8 m, respectively). We found axons with more complex morphologies in cortical neurons compared to axons of motor neurons. Motor neuron axons provided faster signal conduction and larger variance in conduction speed as compared to cortical axons.

Disclosures: M. Radivojevic: None. **A. Rostedt Punga:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.17

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

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ETH Zurich Postdoctoral Fellowship 19-2 FEL-17 (APB)
Swiss National Science Foundation under contract 205320_188910/1

Title: Exploring the feasibility of tracking axon initial segment plasticity by means of high-resolution extracellular electrophysiology

Authors: *T. GÄNSWEIN, S. S. KUMAR, A. P. BUCCINO, V. EMMENEGGER, A. HIERLEMANN;
ETH Zurich, Basel, Switzerland

Abstract: The action potential is generated in a specialized microdomain at the proximal end of the axon called the axon initial segment (AIS). Recent studies have shown that the AIS is structurally dynamic in response to changes in the network state. Changes in its length, its position along the axon, and its molecular architecture have all been reported, and were accompanied by altered intrinsic neuronal excitability. AIS plasticity is thought to contribute to the homeostatic regulation of activity in neuronal networks. However, many aspects of AIS plasticity and its functional role are still poorly understood. The impact of AIS plasticity on neuronal excitability appears to be co-modulated by other factors including neuron type, soma size, and the baseline AIS geometry. To systematically characterize AIS plasticity, an experimental approach that tracks AIS plasticity over time in individual neurons, while providing simultaneous access to morphological parameters and functional readouts at the single-neuron and the network scale, would be desirable. High-density microelectrode arrays (HD-MEAs) featuring sufficient spatiotemporal resolution could be a powerful tool for high-throughput investigations of AIS plasticity. However, reliably inferring subtle microstructural changes from long-term changes in extracellular footprints - the distribution of the extracellular electrical potentials of the neuron across the array electrodes during action potential activity - is a formidable data-analysis challenge. Here, we explored the feasibility of making such inferences by using sophisticated computational models to characterize changes in the extracellular footprint arising from structural changes of the AIS. Multi-compartment models of simplified and realistic neuronal morphologies were simulated, and the corresponding extracellular signals were generated for various high-density probe models using the LFPy package. We found systematic changes in amplitudes, half-widths, and relative latencies in the neuron's extracellular footprints as a result of structural modifications at the AIS. We trained machine learning models using these features and found them to be reliable indicators of AIS plasticity. We characterized the sensitivity of the approach and estimated the optimal electrode geometry necessary to detect and infer a specific microstructural change. Our results could facilitate high-throughput experimental studies of activity-dependent AIS plasticity and demonstrate how computational modeling could be used as a tool to augment the interpretation of extracellular electrophysiology data.

Disclosures: T. Gänswein: None. **S.S. Kumar:** None. **A.P. Buccino:** None. **V. Emmenegger:** None. **A. Hierlemann:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.18

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

Support: NSF Grant 1831962
NSF Grant 1827847

Title: Simulating Preliminary Investigations for In-Vivo Electrophysiology to Understand CNS-Related Injuries from Space Radiation

Authors: S. R. HALL[1], H. V. TRAN[1], *M. KIM[2], T. C. LE[1], H. YOON[3];
[1]Ctr. for Materials Res., [2]Physics, [3]Dept. of Engin., Norfolk State Univ., Norfolk, VA

Abstract: A unique feature of the space radiation environment is the presence of high-energy charged (HZE) particles which may pose a health risk to astronauts. One of the greatest concerns is the possibility of radiation-induced deterioration of central nervous system (CNS) functions. Past research using rodent models has revealed that radiation exposure led to unexpected alterations in behavior where executive functions were compromised which are vital for facilitating the attainment of mission success. This research aims to investigate the effects of radiation on neural probes inside mammalian brain via in-vivo electrophysiological analysis. Neural recordings are observed from NASA's Space Radiation Laboratory (NSRL) and correlated with cognitive disfunction in male and female animal models. Monte Carlo simulation for radiation transport is conducted using the Particle and Heavy Ion Transport System (PHITS) on silicon wafers for preliminary investigations to compare with ground exposure observations using a 140 kV magnesium ion beam. This research will open new paths for developing shielding and pharmaceutical countermeasures against cosmic radiation effects detected during deep space exploration.

Disclosures: S.R. Hall: None. **H.V. Tran:** None. **M. Kim:** None. **T.C. Le:** None. **H. Yoon:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.19

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

Support: NINDS R01 NS 115877-02
Minnesota Office of Higher Education Spinal Cord Injury and Traumatic Brain Injury Research Grant Program
Mayo Foundation

Title: Preliminary results of a finite element model to guide selection of waveform parameters and electrode configurations of epidural electrical stimulation to facilitate motor activity after spinal cord injury

Authors: *C. LOPEZ[1], A. THORESON[1], M. GILL[1], A. ASP[1], M. LINDE[1], D. VEITH[1], C. MILLS[1], K. FERNANDEZ[2], M. BENDEL[3], T. SCRABECK[1], J. BLOCK[1], L. LUJAN[4], K. ZHAO[1,5], P. GRAHN[1,4];
[1]Physical Med. and Rehabil., [2]Mayo Clin. Grad. Program in Biomed. Engin. and Physiol., [3]Anesthesiol., [4]Neurologic Surgery, [5]Physiol. and Biomed. Engin., Mayo Clin., Rochester, MN

Abstract: INTRODUCTION: Epidural electrical stimulation (EES) has shown great potential to elicit motor responses in individuals with spinal cord injury (SCI). However, current methods for determining electrode configuration lead to sub-optimal targeted motor responses. A computer model that identifies activation locus for targeted muscle groups may reduce optimization session time and inform new configuration strategies. This study demonstrates EES parameters that target muscle specificity result in unique regions of similar current density or electric potential, as predicted by a finite element model. **METHODS:** To generate our preliminary results, we leveraged data collected during clinical trial (NCT05095454) activities involving a participant with complete loss of sensorimotor function due to a traumatic spinal cord injury at T5 AIS-A. Following screening and enrollment, two stimulation leads were implanted in proximity to the dorsal epidural surface of the lumbosacral spinal cord enlargement. Electrode positioning was confirmed with imaging and EMG of EES-evoked potentials recorded from lower extremity muscles. An external pulse generator was used to delivered stimulation in increments of 0.5 mA using the most rostral electrode as the anode [1] and systematically varying cathode selection. The first condition consisted of the most caudal electrode [8] and additional, adjacent cathode electrodes were added in sequence. A simplified model of the human lumbosacral enlargement was created using ABAQUS to predict tissue structure energy. Conductivity values were applied to the model. Stimulation parameters that resulted in EMG-confirmed muscle activation were simulated. Current density and voltage field outputs were superimposed and thresholded to determine similar features between configurations. Current density magnitude was also averaged for each configuration and plotted against axial distance along the spinal cord. **RESULTS:** We observed the current amplitude threshold to activate medial hamstring varied from 1 mA to 4.5 mA between use of a single and seven cathode electrodes (a 350% increase). The model predicated an increase in average gray matter current density of only approximately 200%. Identifying the minimum predicted current density required to activate this muscle, we identified a common region in the gray matter for all configurations that met this criteria in the caudal portion of the model, extending approximately 20 mm. **CONCLUSION:** These preliminary results indicate the model we have developed can be used to selectively deliver EES to spinal locations that facilitate downstream functional activity.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.20

Topic: I.07. Data Analysis and Statistics

Support: NWO (STW-Perspectief P15-42 “NESTOR”STW-Perspectief P15-42 “NESTOR”)
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Title: Comparison of electrical microstimulation artifact removal methods for a visual cortical prosthesis

Authors: *F. WANG[1], X. CHEN[1,2], P. R. ROELFSEMA[1,3,4,5];
[1]Netherlands Inst. for Neurosci., Netherlands Inst. for Neurosci., Amsterdam, Netherlands; [2]Dept. of Ophthalmology, Univ. of Pittsburgh Med. Ctr., Pittsburgh, PA; [3]Dept. of Integrative Neurophysiol., VU Univ., Amsterdam, Netherlands; [4]Dept. of Psychiatry, Academic Med. Ctr., Amsterdam, Netherlands; [5]Lab. of Visual Brain Therapy, Sorbonne Université, Inst. Natl. de la Santé et de la Recherche Médicale, Paris, France

Abstract: Recent studies have started to explore the utility of visual cortical prosthesis (VCP) devices based on electrical stimulation of the visual brain and aim to pave the road to clinical application. It is advantageous to record neuronal activities while electrical stimulation is delivered to the brain. These recordings can be used for the calibration of the device and to study the relation between neuronal activity and perception. However, stimulation artifacts are an obstacle for recording neuronal activity during microstimulation in VCP, in particular if the stimulation site is close to the recording sites. Many methods have been proposed previously to suppress these stimulation artifacts, but their performance and effectiveness have not been tested in a VCP scenario. Which method best serves the needs of such a device? One challenge for the development and evaluation of artifact suppression methods is the lack of ground truth data, i.e. recordings of neuronal activity peri-stimulation without the artifacts. In this work, we built a simulated dataset based on the parameters from recordings with a high-channel count implant in the primary visual cortex of rhesus monkey, representing a VCP prototype. We added realistic artifacts to the simulated data sets and then evaluated the performance of six different software-based artifact removal methods and compared their results to the ground truth. We also improved the time performance of a multi-exponential fitting method to make it feasible in a lightweight computational application. We found that a multi-exponential fitting method and the previously published ERAASR (O'Shea & Shenoy, 2018) method performed best in retrieving information about spikes and firing rates. Different methods were better in retrieving other aspects of data like the local field potential. Hence, it is necessary to combine two or more methods if the aim is to recover individual spikes, multi-unit activity and the local field potential. In addition, our findings suggest new approaches for a fully automatic pipeline of artifact removal, which could find many applications, including a VCP.

Disclosures: **F. Wang:** None. **X. Chen:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phosphoenix BV. **P.R. Roelfsema:** E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Phosphoenix BV.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

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Program #/Poster #: 088.21

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

Support: NIH R01 DC004290-19
NIH R01 NS117753
DOD W81XWH-19-1-0637

Title: Detecting and describing oscillatory bursts with the trispectrum

Authors: *C. K. KOVACH[1], J. R. WESSEL[2];
[1]Neurosurg., [2]Neurol., Univ. of Iowa Hosp. and Clinics, Iowa City, IA

Abstract: How best to characterize electrical signals generated by the nervous system is a question of fundamental importance to electrophysiology. Tools that address the problem tend to land in two camps: those that approach it from the frequency domain and those that approach it from the time domain. Each camp emphasizes different aspects of a signal: the first gives special prominence to oscillatory phenomena, while the second emphasizes transient features whose timing is anticipated (such as an averaged evoked response) or self-evident in the raw data (as with the classical action potential). Signals with uncertain timing that are transient or weakly oscillatory and embedded in large amplitude noise fall within a relative methodological blind spot between the camps. Time-frequency decompositions (TFDs) would seem the natural tool for this middle ground; yet commonly used TFDs make assumptions about signal bandwidth and duration that bias the estimation of relevant spectrotemporal parameters, while still favoring the detection of narrowband signals. Here we solve the problem of unbiased detection and characterization of oscillatory bursts and other transient features through a general decomposition of higher-order spectra (Kovach and Howard 2019), applied to a specific subdomain of the trispectrum. The method can be understood as a form of spatiotemporal independent component analysis (ICA) which optimizes a fourth-order cost function related to kurtosis. The chosen subdomain of the trispectrum is particularly relevant for the detection and identification of amplitude modulation (AM), applicable to oscillatory bursting. The representation of this subdomain as a two dimensional function, the modulogram, reveals the bandwidth of the carrier and the spectrum of the modulating signal along separate dimensions. We demonstrate how the modulogram can be used to recover detailed properties of AM signals in both frequency and time. In addition, the nature of the trispectrum as a fourth-order cumulant

offers an objective criterion for deciding whether and where AM is present in a signal according to excess kurtosis in the output of a trispectrum-derived matched filter. The method is demonstrated through the detection of beta bursts in an exemplary open EEG dataset, for which it allows a rich and unbiased characterization of burst-related signal features in time, space and frequency.
Kovach, C. K. and M. A. Howard (2019). Decomposition of higher-order spectra for blind multiple-input deconvolution, pattern identification and separation. Signal Processing 165, 357 - 379.

Disclosures: **C.K. Kovach:** None. **J.R. Wessel:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.22

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

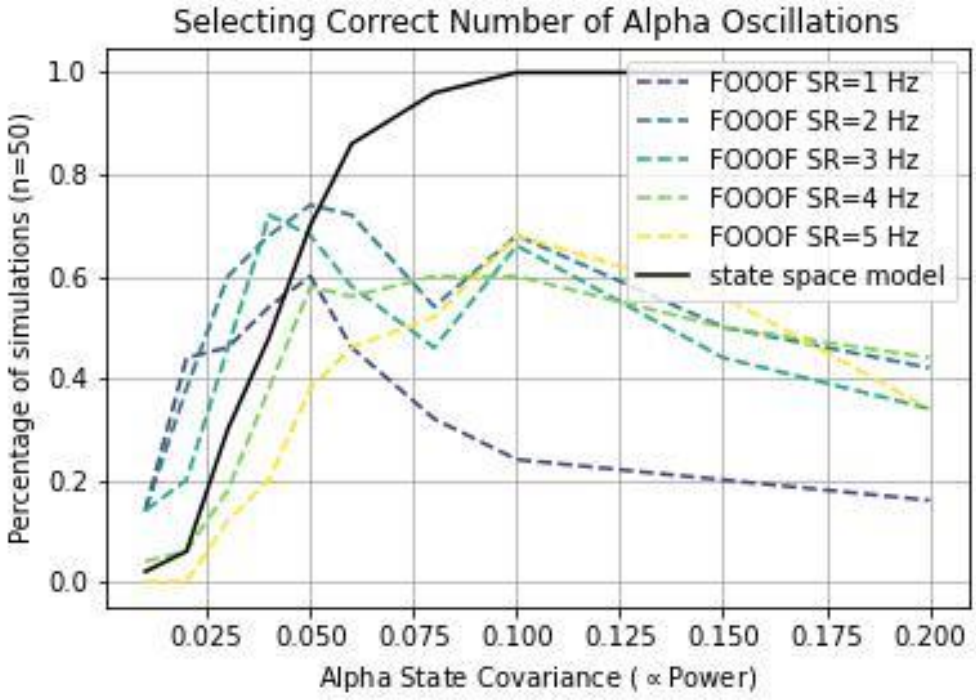
Support: NSF GFRP
NIH 1R01AG054081

Title: State space methods to identify and extract neural oscillations: a time series alternative to bandpass filtering and FOOOF

Authors: *A. M. BECK[1,2], P. L. PURDON[2,3];
[1]Electrical Engin. Computer Sci., MIT, Cambridge, MA; [2]Anesthesia, Critical Care and Pain Med., Massachusetts Gen. Hosp., Boston, MA; [3]Anaesthesia, Harvard Med. Sch., Boston, MA

Abstract: When periodic neuronal spiking is recorded at the population level, it produces an oscillatory signal that may contain many different frequencies. We can measure these population level signals using a variety of methods including electroencephalography (EEG). This oscillatory activity is typically classified into one of several canonical frequency bands and is analyzed using either narrow-band filters or spectral analysis. This method of quantifying only the power in a prescribed frequency band ignores individual variation in frequency and is susceptible to contamination by broadband noise. In particular, power will be present after narrow-band filtering even if the original signal is white or pink noise. To overcome these issues, investigators such as Donoghue, et al. have proposed frequency-domain methods such as Gaussian peak fitting (Fitting Oscillations & One-Over-F, "FOOOF"). However, these methods define an oscillation solely on the shape of the spectrum, leaving them dependent on the frequency resolution of the underlying nonparametric spectral estimate and the appropriateness of the Gaussian model. We propose a time-domain method that represents each oscillation as a latent, rotating phasor within a state space model. We fit the model's parameters and estimate the resulting time series using an expectation maximization algorithm. We then propose an iterative algorithm to fit multiple such oscillators. This algorithm requires minimal user input, does not

require arbitrary frequency band cutoffs, and is robust to inter-individual variation. We compare our method to FOOOF using power spectra with 1 to 5 Hz spectral resolution (SR) with simulated alphas of varying power. Under the best conditions, FOOOF correctly identifies the correct number of alpha oscillations in only 74% of simulations. In contrast, our method is able to reach 100% and outperforms FOOOF above 0.26 dB alpha SNR. Our method also quantifies the level of confidence in the number of oscillations identified using the log likelihood.



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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.23

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

Support: NIH Grant R01AG054081
Tiny Blue Dot Foundation

Title: Extracting common oscillatory time courses from multi-channel EEG: Oscillation Component Analysis

Authors: *P. DAS[1,2], P. L. PURDON[1,2];
[1]Massachusetts Gen. Hosp., Boston, MA; [2]Harvard Med. Sch., Boston, MA

Abstract: Non-invasive human electrophysiological recordings such as electroencephalograph (EEG) provide a unique window for studying brain function with millisecond temporal resolution. However, data from individual EEG sensors are often treated independently, without acknowledging the spatial mixing and volume conduction that are inherent to the recording modality. This practice not only involves redundant computations but also leads to sub-optimal results that can mischaracterize spatial dependencies. Numerous multi-channel filtering or blind source separation have been proposed in this context. These approaches pool information across channels to decompose multivariate EEG time series into a small number of dominant source time-courses. However, they mostly ignore the temporal (i.e. oscillatory) structure of neural data or try to represent temporal correlation via inefficient non-parametric methods. Here we propose an explicit parametric generative model where an unknown number of latent state-space *oscillation sources* are observed via an EEG sensor-array such that each sensor receives a different but unknown linear contribution from each oscillator. We then provide Oscillation Component Analysis (OCA), a Bayesian inference of the oscillation source time-courses and their spatial distributions as the sensor level mixing maps as well as an empirical Bayes model selection criterion to select the optimal number of oscillation sources. We performed extensive simulation studies to benchmark how effectively OCA can recover the oscillation time-courses, their mixing maps and identify the accurate number of oscillations under various scenarios. Finally, we apply OCA on 64 channel EEG recordings from a healthy volunteer undergoing propofol-induced general anesthesia before propofol administration (baseline) and during propofol-induced unconscious periods. OCA extracts only 10 slow (~1-4Hz) and 3 alpha (~9-13Hz) oscillation components in each case following empirical Bayes model selection. Importantly, the mixing maps corresponding to these components provide a very useful characterization of their spatial origin: they clearly delineate the posterior distribution of alpha waves in baseline versus frontal distribution of alpha waves under general anesthesia. In summary, the application of OCA on simulated and real EEG data demonstrates its capability as a principled and *physiologically relevant* dimensionality reduction tool that provides explicit distributions of oscillations over the scalp that are easier to interpret than conventional frequency-wise, cross-channel coherence.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.24

Topic: I.07. Data Analysis and Statistics

Support: NIH R01 HL071568-16

Title: Discrete event model with parallel hierarchical state observers for temporally causal segmentation of burst suppression patterns during post-cardiac arrest resuscitation

Authors: *A. WILLIAMS[1], Y. GUO[1], Z. LI[1], R. GEOCADIN[2], N. V. THAKOR[1];
[1]Biomed. Engin., Johns Hopkins Univ., Baltimore, MD; [2]Neurol., Johns Hopkins Univ. Sch. of Med., Baltimore, MD

Abstract: Over 80% of patients are unresponsive on first examination post-cardiac arrest, often leading to neurological impairment with high morbidity and mortality. Following return of spontaneous circulation during resuscitation, patient EEG must progress from isoelectric to continuous. During this transition, EEG is characterized by alternating patterns of aperiodic waveforms (bursts) and minimal activity (suppression). Features of this burst-suppression period can aid in prognosis of neurological outcome; however, current standards of feature segmentation involve time-intensive human visual analysis. Algorithms for automated segmentation have seen limited clinical translation as they are designed for post-hoc, population-based analysis, or for neurophysiological patterns specific to pharmacological agents. We present an innovative control theoretic approach to address these deficiencies, targeted to real-time use in EEG monitoring of individual patients with cerebral injury resulting from asphyxial cardiac arrest. This model treats each EEG measurement as a discrete event and determines overall system state using state observers that monitor independent attributes: entropy progression, pattern duration, voltage amplitude, and waveform phase history. The entropy progression state observer identifies the patient as in an isoelectric or continuous state, while the latter three observers deterministically identify whether the neurophysiological signal is currently presenting a burst or suppression state based on a confluence of their individual observations. Parameters of interest for each of these attributes were sourced from standard clinical practice guidelines but can be modified based on prevailing patient neurophysiological activity. Development of the system utilized EEG collected from eight male Wistar rats who underwent a 7-minute asphyxial cardiac arrest and resuscitation following an established experimental protocol. This protocol has been validated to closely reflect pathophysiological conditions and neurological functional recovery of human patients. EEG was monitored continuously using two epidural screw electrodes for up to four hours post-cardiac arrest. Two independent systems then reviewed the EEG in strict forward temporality, with overall state decision made through a weighted voting process. Subsequent versions of this software will perform review in real-time on live signals.

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Poster

088. Computational Tools and Methods for Electrophysiology Experiments

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Program #/Poster #: 088.25

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

Support: CAPES
FAPEG
CNPq

Title: Automatic sleep classification in rats with epilepsy induced by pilocarpine

Authors: C. E. G. DE OLIVEIRA[1], C. QUINTINO[1], G. H. M. SCHOORLEMMER[2], D. B. COLUGNATI[1], D. H. DA MATTA[1], ***A. P. PANSANI**[1];
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Abstract: Epilepsy and sleep interact mutually: seizures cause sleep impairment, and sleep deprivation predisposes to epileptic seizures. Increased neuronal synchronization in the NREM phase predisposes to seizures, and nocturnal seizures increase the risk for SUDEP. The development of algorithms for automatic sleep classification in animal models of epilepsy is complicated because of changes in the intensity of several EEG bands that are important for the classification of sleep. The aim of this work was to develop an automatic sleep classification model for rats with spontaneous seizures induced by pilocarpine. Six electroencephalogram (EEG) and electromyogram (EMG) recordings of male Wistar rats submitted to the Pilocarpine model were used (CEUA 28/2018). Five randomly selected recordings were used for training of a Random Forest classification model, implemented with the scikit-learn module in Python. A sixth recording was used to validate the model. The training recordings were cut in 10 s epochs, and the sleep stage for each epoch was classified by two experienced researchers. Data from unambiguously classified epochs were included for training of the model. Epoch data included the total power in the alpha (12.5-15 Hz), beta (22-30 Hz), gamma (35-45 Hz), theta (5-12 Hz) and delta (1.5-4 Hz) bands of the EEG, and the 185-235 Hz band of the EEG. Median power, power variance, and minimum and maximum values of the power of each epoch were also included for training. The sixth EEG and EMG recording was classified by the algorithm and by a third experienced researcher. In 8569 epochs, there was a sensitivity (true positive rate) of 97.5%, 98.8% and 83.2% for the awake, NREM and REM phases, respectively. The precision (correct positive prediction rate) for these phases was 98.9%, 95.3% and 97.2%. The total accuracy was 97.2%. Thus, the proposed automatic classification model appears robust and can be useful in conducting intervention experiments in specific sleep phases in rats with epilepsy induced by pilocarpine, with high sensitivity and precision

Disclosures: C.E.G. de Oliveira: None. **C. Quintino:** None. **G.H.M. Schoorlemmer:** None. **D.B. Colugnati:** None. **D.H. da Matta:** None. **A.P. Pansani:** None.

Poster

088. Computational Tools and Methods for Electrophysiology Experiments

Location: SDCC Halls B-H

Time: Saturday, November 12, 2022, 1:00 PM - 5:00 PM

Program #/Poster #: 088.26

Topic: I.07. Data Analysis and Statistics

Support: American Epilepsy Society
UCI EpiCenter NINDS T32

Title: Data workflow for multi-animal video-local field potential acquisition and seizure analysis using Open Ephys and Bonsai.

Authors: *G. TARCSAY, B. L. BOUBLIL, L. A. EWELL;
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Abstract: Chronic local field potential (LFP) and video monitoring is a common technique in neuroscience which often requires purchase of expensive hardware systems that are only suitable for low channel count, low sampling rate recordings. We aimed to develop a low-cost platform that could later be repurposed for other electrophysiological applications such as high-density single unit recording. The major challenges of chronic video-LFP acquisition are synchronization between distinct data streams and handling of large data files. Moreover, preprocessing and analyzing several days of video-LFP data is time consuming. Therefore, we developed a data workflow using the open-source Bonsai visual programming software to 1) acquire data from an Open Ephys acquisition board (LFP) and from a web-camera (video) 2) synchronize the video-LFP data 3) save data regularly to avoid potential data loss during chronic recordings and limit file sizes to facilitate later processing. Our workflow was successfully applied during 48-hour video-LFP recordings of epileptic mice (n=8 mice, two groups of four simultaneously recorded mice). In order to analyze seizure activity and to assign the behavioral state, a graphical user interface (GUI) was written in MATLAB. The GUI provides a user-friendly solution for 4) organization of the recorded video-LFP data 5) automatized seizure detection 6) convenient behavior analysis during seizure activity. In conclusion, our pipeline serves as a straightforward and easy way for chronic recordings and analysis of multi-site video-LFP data in epileptic mice. We present a low cost solution that utilizes a hardware platform that can be applied to other experimental needs.

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Poster

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Topic: I.07. Data Analysis and Statistics

Title: Neuro Stack: R shiny based web app for meta-analysis of Alzheimer’s disease RNA-Seq datasets

Authors: *A. OBLA, C. ARMOSKUS, Y. CHA, F. M. ROY, M. N. KAGALWALA, J. L. ROSS;
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Abstract: While genomic datasets are being generated at a rapid rate, the ability to leverage information from these massive datasets within a unified framework remains a challenge. This issue is more pressing in the Alzheimer's Disease (AD) space with more pronounced scarcity of such approaches, albeit seen in a few studies.
Here we present a web based app that houses various publicly available RNA-Seq AD datasets from cell culture, animal models, and human across various cell types and tissues. To explore possible AD mechanisms, we place an emphasis on neuroinflammation, which is understood as a driver of AD. This web app leverages the results from differential gene expression analysis via limma and gene set enrichment analysis (GSEA) via fgsea to explore the data through novel interactive visualization workflows.
To illustrate the utility of the app, we ran an RNA-Seq meta-analysis of various AD and neuroinflammation models that included primary microglia treated with LPS (GSE109329) and the following mouse models profiling microglial cells in-vivo: wild-type mouse treated with LPS (GSE75246), two beta-amyloid AD models (GSE158156 & GSE165306) and tau AD model (GSE123467). We were motivated to study the differences in gene expression across the various models to select apt models for testing therapeutic agents at various stages of drug development. GSEA from a curated set of microglia specific gene sets revealed key differences between culture and animal-based models. Particularly, primary microglia treated with either high dose or long duration of LPS showed positive enrichment of a neuroinflammatory gene set (West et al 2022) driven by both Interleukin-6 (IL-6) and Interferon-α (IFN-α). “IL-6 only driven” gene set was down regulated in all doses and durations. All mouse models showed positive enrichment for IL-6 and IFN-α driven gene sets. Interestingly, cholesterol metabolism gene set which is generally shown to be upregulated in AD was found to be downregulated in both LPS treated mouse and primary microglia.
Accounting for limitations seen in various AD and neuroinflammation models helps to design experiments with apt models for testing particular mechanism of action of a therapeutic agent. Our approach serves to encourage both computational and non-computational users to perform explorative analysis of Alzheimer’s data in a systematic and interactive way.

Disclosures: **A. Obla:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation. **C. Armoskus:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation. **Y. Cha:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation. **F.M. Roy:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation. **M.N. Kagalwala:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation. **J.L. Ross:** A. Employment/Salary (full or part-time):; Immuneering Corporation. E. Ownership Interest (stock, stock options, royalty, receipt of intellectual property rights/patent holder, excluding diversified mutual funds); Immuneering Corporation.